Staphylococcus aureus in the Raw Milk: Detection Methods, Antimicrobial Resistance and Virulence Factors

Ayesha Hameed[1], Maria Ayub[1], Saba Javed[1], Malaika Sajid[1], Muhammad Bilal Akram[2], Bilal Aslam[3,1], Hira Ahsan[4], Rasheeha Naveed[5], Muhammad Irfan Malik[6] and Abu Baker Siddique[1,*]

[1]Institute of Microbiology, Government College University Faisalabad, 38000, Pakistan
[2]Department of Microbiology, University of Science and Technology Havealian, Abbottabad
[3]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia
[4]Academy of Medical Sciences, College of Henan Medicine, Zhengzhou University, Zhengzhou, Henan, 450052, China
[5]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
[6]Department of Parasitology, Faculty of Veterinary and Animal Science, The Islamia University of Bahawalpur
*Corresponding author: absiddique@gcuf.edu.pk

Abstract

Staphylococcus aureus is a major pathogen present in both humans and animals, causing diseases like food poisoning and bovine mastitis. It is also a major opportunistic pathogenic bacterium of raw milk. Thus, consumption of raw milk can cause mild diarrhea to severe illnesses and even death. *S. aureus* has acquired antibiotic resistance against multiple drugs (e.g. methicillin resistant *S. aureus* (MRSA), vancomycin resistant *S. aureus* (VRSA) etc.) due to excessive use of antimicrobial drugs, transfer of resistance genes and other mechanisms. This resistance negatively affects the treatment of diseases. For the detection of *S. aureus,* the sample is cultured on growth media and isolated. MRSA strains are analyzed for mecA and mecC genes. While, PCR is performed for the detection of enterotoxin genes. *S. aureus* also has multiple virulence factors including capsule, toxins, biofilm, and super antigens etc. These factors play an important role in the virulence of bacteria, and also help the microbe to survive under harsh conditions. To mitigate the risks associated with *S. aureus* in raw milk, strategies such as improved animal health management, better milking practices, and processing technologies like are necessary.

Keywords: *Staphylococcus aureus,* Antibiotic resistance, Enterotoxins, Biofilm, Raw milk

Cite this Article as: Hameed A, Ayub M, Javed S, Sajid M, Akram MB, Aslam B, Ahsan H, Naveed R, Malik MI and Siddique AB, 2025. Staphylococcus aureus in the raw milk: detection methods, antimicrobial resistance and virulence factors. In: Zaman MA, Farooqi SH and Khan AMA (eds), Holistic Health and Antimicrobial Resistance: A Zoonotic Perspective. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 247-252. https://doi.org/10.47278/book.HH/2025.462

A Publication of Unique Scientific Publishers

Chapter No: 25-033

Received: 31-Jan-2025
Revised: 20-Feb-2025
Accepted: 12-June-2025

Introduction

Consuming unprocessed, natural food commodities, such as fresh milk and milk products, is becoming more and more popular worldwide. In addition to the health benefits, people are always looking out for raw milk due to its freshness, taste, nutritional value proximity to the supplier, and local agriculture. Raw milk consumption is rising in many nations, and there is mounting scientific proof that it can lessen atopic eczema, allergies, and asthma. Additionally, it has been demonstrated that consuming raw cow's milk early in life lowers the incidence of fever and obvious respiratory infections by roughly 30% (Berge & Baars, 2020). One of the important area of the agricultural economy is milk production, and the production increases annually. Milk and dairy products have been demonstrated to have a beneficial impact on human health because they contain a number of nutrients, such as calcium, potassium, proteins, fat, and vitamins. (Bórawski et al., 2021).

The recent global trend of unpasteurized milk and milk products such as raw milk cheese consumption, have brought current information related to their advantages and disadvantages into discussion, particularly related to the food safety policy. Raw milk consumption poses significant health risks due to potential contamination with various pathogens. These include bacteria like *Campylobacter, Salmonella, E. coli O157:H7, Listeria monocytogenes,* and *Staphylococcus aureus,* as well as viruses and parasites (Aprea & Mullan, 2022; Christian, 2022). Raw milk consumption can lead to various diseases that range from mild diarrhea to severe illnesses and even death, with neonates, the elderly, and immunocompromised individuals being particularly vulnerable (Aprea & Mullan, 2022). Some pathogens in raw milk may also carry antimicrobial-resistant genes, further complicating treatment. Despite these risks, demand for raw milk persists due to perceived health benefits (Aprea & Mullan, 2022). To mitigate risks, strategies such as improved animal health management, better milking practices, and processing technologies like electron beam treatment have been proposed. Regulatory guidelines and standards are crucial for ensuring raw milk safety (Christian, 2022). Hence, Staphylococcal food poisoning is the caused by consumption of foods that have adequate levels of Staphylococcal enterotoxins, resulting in food-borne outbreaks of *S. aureus* intoxication which are linked in many notions to the intake of contaminated milk and dairy products (Cardozo et al., 2021).

Staphylococcus aureus (S. aureus), is a gram-positive, catalase, and coagulase-positive, opportunistic pathogen that is the cause of several illnesses in both humans and animals. *S. aureus* infections can vary from mild skin issues to severe conditions, including bacteremia, endocarditis, necrotizing pneumonia, toxic shock syndrome, and food poisoning. Consuming food contaminated with pre-formed *Staphylococcus* enterotoxins can lead to staphylococcal food poisoning (Pal, 2022). *Staphylococcus aureus* is often detected in raw milk and milk products across the world (Yakubu et al., 2020). Multi-drug resistant (MDR) strains have a major influence on the human food safety and the dairy industry (Haq et al., 2024). The effect of the antibiotic drugs is weakens by the production of biofilms, that makes the infections caused by these types of bacterial strains treatment difficult. Moreover, the biofilms or the colonization of bacteria not only effects the quality of milks but it can also cause infections in the people like dairy farmers or the people who consume raw milk (Bissong et al., 2020).

2. Antimicrobial Resistance

There is a number of factors that play role in antibiotic resistance such as excessive antibiotic usage, transfer of resistance genes. The bacteria that are resistant to multiple drugs (MDR-multiple drug resistant), including methicillin-resistant *S. aureus* (MRSA) has become more common because of the improper use of drugs in veterinary health care (Szczuka et al., 2022). These virulent organisms can evade the host immune systems due to their resistance mechanisms against certain drugs (Altaf, 2019). Subsequently, drug resistance is a major problem in the treatment of infections caused by Staphylococcus species (Myrbråten et al., 2021).

Penicillin as used as the most effective drug for the treatment of several staphylococcus infections. However, by the mid of 1940s, resistance was acquired by *S. aureus* strains against penicillin (Javed, 2021). Therefore, to deal with this issue, a semi-synthetic beta-lactam antibiotic resistant to the β-lactamase enzyme, was synthesized. Methicillin was effective against penicillin-resistant *S. aureus*. Acquiring the mecA gene leads to resistance against methicillin by producing PBP2a (i.e. an alternative penicillin-binding protein that has less affinity for β-lactam antibiotics. (Hamid et al., 2017). Methicillin-resistant *S. aureus* (MRSA) is found as a disease-causing in farm animals that easily spreads to humans in contact with livestock. Many reports showed the MRSA prevalence in bovine milk and the MRSA transmission between farm workers and livestock. Therefore, *S. aureus* resistance to methicillin and vancomycin negatively affects the treatment of diseases in both humans and animals. On the other hand, the MRSA presence in ruminants (e.g., camels, yaks, goats, etc.) is not recorded, though the consumption of milk from different animals is very famous. Therefore, it is crucial to assess the prevalence of resistant *S. aureus* strains in bovines within the study area, as these pathogens pose significant public health risks (Altaf, 2019).

3. Detection Methods for *Staphylococcus aureus* in Raw Milk

Bacterial culture and isolation

The isolation of bacteria is done by following the methods that are described earlier with a few changes in the experimental procedures (Tang et al., 2017). Firstly, for 2 min, the samples of milk, in a vortex oscillator, are homogenized, and then 25 ml milk samples are transferred into 225 ml 10% (w/v %) saline solution followed by the incubation period of 24h at 37°C. Then, a loopful bacterial culture is transferred onto Baird Parker agar supplemented with 5% egg yolk, tellurite, and 5% sheep blood agar medium and is given incubation for 20–24 h at 37°C . A coagulase test is performed on isolates of *S. aureus* to confirm the coagulase activity and then the species-specific nucA gene amplification is done for further identification by following the already defined primer set. For further analysis, all the varified *S. aureus* isolates are stored at − 80 ◦C in TSB containing 20% glycerol (Zhao et al., 2021).

mecA and mecC Genes Detection

All the 3 strains of MRSA (cefoxitin-resistant strains) are analyzed for mecA and mecC genes. Genomic DNA is retrieved with the help of a TIANamp Bacterial DNA extraction kit, and its concentration is calculated by using a Nanodrop spectrophotometer. Traditional primers with traditional PCR conditions are employed for the mecA and mecC gene detection (Antonios et al., 2015). The products obtained from PCR are defined by 1.5% agarose gels stained with ethidium bromide and observed under UV illumination (Zhao et al., 2021).

Enterotoxin Genes Detection

The genes that encode the 5 traditional SEs (seb, sea, saw, sec, and sed) are detected using the polymerase chain reaction (PCR) technique, which involves DNA amplification in the subsequent conditions:

- At 95°C initial denaturation for 15 min, next denaturation involved 35 cycles (for 1 min at 94°C), followed by annealing, and extension (for 1 min at 72°C), with a final step of extension (72°C for 10 min) at the end.
- The PCR products are quantified by 1.5% agarose gels stained with ethidium bromide and visualized under UV illumination (Zhao et al., 2021).

SEs Detection

SEs detection can be done by using immunodiffusion, immunoassays, latex agglutination, radioimmune assays (Abril et al., 2020), and double gel diffusion assays, however, all SEs cannot be detected by using commercially available kits (F´eraudet Tarisse et al., 2021). Currently, different techniques and assays have been formed for *S. aureus* enterotoxin gene detection, either directly by using polymerase chain reaction (PCR) and loop-mediated amplification (LAMP) assays or after a culture stage to increase the bacterial concentration (Shalaby et al., 2024).

Various molecular typing methods, like multi-locus sequence typing (MLST), staphylococcal cassette chromosome (SCC mec) typing, pulse-field gel electrophoresis (PFGE), and staphylococcal protein A (spa) typing, are commonly utilized for the determination of the *S. aureus* isolates' genetic relatedness. Whole genome sequencing (WGS) being a modern and effective tool in food safety may plays a crucial role in surveillance and risk assessment of food safety (Rajkovic et al., 2019; Deddefo et al., 2022).

Detection of Staphylococcal Enterotoxins

A, B, C, D, and E, the staphylococcal enterotoxins, presence in samples of raw milk determined using a two-step process: concentration/extraction and toxin detection by enzyme-linked fluorescent assay (ELFA) with the VIDAS SET2 test, according to ISO 19020:2017 (Oliveira et al., 2022). For this, 10 ml of unpasteurized milk sample is taken and is centrifuged at 3500 g/10min/10°C and the creamy layer is discarded. The supernatant is utilized for the enterotoxin detection. The ELISA plate reader is used to observe the absorbance value of milk samples at 450 nm.

Determination of Biofilm Producing Ability

Biofilm production can be determined by two methods (i.e. CRA and Spectrophotometer method), explained below (Idrees et al., 2021).

Congo Red Agar (CRA): Biofilm formation by isolated microorganisms is analyzed by using CRA medium. After culturing the bacteria at 37°C for 24 h, it is left at room temperature overnight. The color of bacterial colonies is used to evaluate the biofilm production ability. Black colonizing strains on CRA media are categorized as colonies that produce mucus, and the brown-red/colorless colonies of bacteria are grouped as non-biofilm film forming (Idrees et al., 2021)

Spectrophotometric Method: Biofilm production can be analyzed by using the spectrophotometric technique with flat-bottomed polystyrene micropipette plates. At a wavelength of 595 nm absorbance is noted with the help of an ELISA microplate reader. The columns in which there is TSB medium alone are negative control while as a positive control, the *S. aureus* ATCC 25923 strain is taken. Each strain is tested in 3 columns with 8 replicates. The results of biofilm development results are interpreted using the criteria discussed by Wiszniewska-Łaszczych, et al. (2024).

Virulence Factors

S. aureus has a number of factors that have roles in its virulency. These virulent factors allow it to withstand harsh external or host conditions, resulting in tissue destruction and colonization, and fatal systemic infections. Some of these factors are adhesins, superantigens, capsules, and toxins (Zhu et al., 2023).

Adhesins

Proteins or glycoproteins present on the surface of bacterium that allow its adherence to the host cells.

Toxins

These are the chemical substances that bacteria make and they have ability to destroy cells and tissues of the host. The Panton-Valentine leukocidin (PVL) toxin that targets WBCs, is an important pathogenic factor of *S. aureus*. PVL has a high affinity for leukocytes, it penetrates into the plasma membrane of the host and forms a pore or hole in it, while other toxins, including leukocidin and γ-hemolysin, are cell damaging to WBCs and RBCs. *S. aureus* can cause mastitis in cattle after entering, through the teat canal, in the mammary gland, and the gland colonization by releasing toxins and enzymes that can destroy host tissues and cells (Haq et al., 2024)

Superantigens

Superantigens being poisons have ability to activate a huge number of immune cells, and it can arise an excessively combative immunological response (Zhu et al., 2023).

Capsules

The outer layers or coverings that protect bacteria from the host's immune defense skin and soft tissue infections, joint infections, and bloodstream infections, are capsules. The disease development process differs with infection type. For instance, bacteria can enter into the body through a cut or a wound, causing infections of skin and soft tissues. In bloodstream infections, the bacteria get into the bloodstream and spread to different parts of body, resulting in arise of sepsis (Zhu et al., 2023).

Biofilm

One of the main pathogenic factors that plays a major role in the virulence of *S. aureus* and helps the bacteria survive under harsh environmental conditions is the ability to produce biofilms (Wang et al., 2019). Biofilm refers to the self-generated structure made of extracellular polymeric substances (EPS) like proteins, polysaccharides, extracellular DNA (eDNA) & lipids, and also by the attachment & adhesion of bacterial colonies (Peng et al., 2023). Biofilms have microbial communities with distinct gene expression patterns that are similar to the tissue patterns of higher organisms. Also, the biofilms provide protection to the bacterial cells against antibiotics, host immune system, and drying, among other external factors that are harmful for the cells. *S. aureus* from biofilms by undergoing complicated processes including multiple genes such as mgrA, rbf, and icaR (Shen et al., 2021)

Regulation of Genes

Table 1 summarizes functions of regulation of genes.

Toxins

The production of toxic shock staphylococcus toxin-1(TSST-1), staphylococcal enterotoxins(SEs), and staphylococcus-like proteins(SEIs) are the major cause of staphylococcus food poisoning. Moreover, in the digestive tract, due their ability to resist the proteolytic (protein-breaking) enzymes like pepsin or trypsin as well as due to their solubility in saline solutions and water, stable staphylococcus enterotoxins

(SEs) are not degraded (Shen et al., 2021). The virulence factors production is linked with a wide range of genes, such as rot, clfA (Liesenborghs et al., 2019), and coa (Pacha et al., 2020).

The preexisting *S. aureus* enterotoxins in food cause staphylococcal food poisoning (SFP) (Park & Seo, 2019; Rajkovic et al., 2019). Even a minute amount of SEs can result in sickness. 100 – 200ng of enterotoxin is the dose that can result in symptoms of SFP, which is formed in food with an exceeded level of *S. aureus* count i.e. 105cfu/gm. The main contributing factors linked with SFP outbreaks are poor keeping temperatures and times (Park & Seo, 2019; Rajkovic et al., 2019). Heat-stable SEs are capable of resisting sterilization (Park & Seo, 2019). There are 24 distinct SEs identified so far: classical enterotoxins (A, B, C, D, and E) and non-classical enterotoxins (G, H, I, R, S, and T) and SEls (lJ, lK, 1L, lM, lN, lO, lP, lQ, lU, lW, lV, IX and IY) (Rajkovic et al., 2019). The specific enterotoxin genes express the five classical SE. In the United States, classical enterotoxins are involved in almost 95% of food-born enterotoxin epidemics (Kou et al., 2021). SFP appears suddenly, with vomiting, abdominal discomfort, and nausea being the typical symptoms. Some types of foods are considered potential carriers of *S. aureus* to humans, including unpasteurized milk and milk products, poultry and egg products, meat and salad (Deddefo et al., 2022).

Table 1: Regulation of genes

Gene	Functions	Reference
Rbf	Positive regulator involved in formation of biofilm formation increasing polysaccharide intercellular adhesin (PIA) production	(Jin et al., 2019)
mgrA	Multiple-gene regulators activating surface protein repress and capsule biosynthesis	(Lei et al., 2019)
arlR	Involved in virulence, attachment & adhesion, self-destruction, and resistance to multidrug	(Jin et al., 2019)
icaR	Prevents the production of biofilm by suppressing ica expression as a result of attachmed to icaA promoter region	(Jefferson et al., 2004)
luxS	It is needed for the synthesis of autoinducer 2, which reduces the transcription of ica gene and degrades biofilm	(Xu et al., 2006)
agrA	It regulates the DNA-binding response in biofilms	(Koenig et al., 2004)
sarX	PIA-dependent biofilm-producing promoter in *S. aureus*	(Cue et al., 2013)
sigB	It is crucial for the development of biofilm by AGEs with lrg operons downstream factor	(Xie et al., 2020)
Rot	Cytoplasmic activator of toxins and external proteases; the rot translation is stopped by Agr regulation	(McNamara & Bayer, 2005)
Hla	It plays a role in role in alpha-toxin encoding	(Gudeta et al., 2019)
clfA	It is one of the pathogenic proteins linked to the cell wall of *S. aureus*	(Herman-Bausier et al., 2018)
Coa	It is a virulent, coagulase encoding gene	(Thomas et al., 2019)
fnbA & fnbB	Surface proteins that play role in adhesion and invasion in host cell	(Foster et al., 2014)

Mitigation Strategies for *S. aureus* Control in Raw Milk

Staphylococcus aureus control in raw milk requires integrating good hygienic practices, regular monitoring, and strategic management of infected animals. The implementation of pasteurization and education programs, along with addressing AMR, is vital for ensuring milk safety and protecting public health.

Hygienic Milking Practices: Following hygienic milking procedures can greatly lower the risk of infections caused by *S. aureus*. It includes the use of clean and sanitized equipment, wearing gloves, and cleaning teats before milking (Sampimon et al., 2011).

Regular Screening and Monitoring

For the early identification of *S. aureus,* routine and regular screening of milk is necessary. It can help in timely treatment. Studies have shown that regular monitoring and surveillance can play a significant role in early detection, control of outbreaks, and management of antibiotic-resistant strains (Zhang & Xu, 2023).

Pasteurization

Studies showed that boiling raw milk for 15 seconds at 72°C can kill the pathogenic bacteria, including *S. aureus*, without affecting the milk's nutritional content (Park & Haenlein, 2024). Therefore, pasteurization is an important method of milk preservation. Keeping the value insight, the dairy farmers should pay attention to this method.

Education and Training

Studies have shown that the farmers who participated in the training program in 2021 reported better hygiene and a reduction in contamination levels of milk (Brown et. al., 2021). Hence, it is necessary to educate and train the dairy personnel about the risks associated with the *S. aureus* and also about their controlling methods to lessen these risks.

Conclusion

Raw milk usage has increased globally due to its health benefits. It is also a reason of a number of health problems. However, it has greater nutritional value; it is crucial to know the infections associated with it, especially those caused by *Staphylococcus aureus. S. aureus* causes mild to serious infections, i.e. food poisoning to life-threatening diseases; also it can form biofilms and resist antibiotics. Therefore, it is a serious issue for dairy safety as well as public health. The increase in antimicrobial resistance, particularly methicillin-resistant strains of

S. aureus (MRSA), abates the medicinal effect of already used drugs and requires advanced alternatives for the treatment. Moreover, due to its ability to produce biofilms, this pathogen can survive under harsh and adverse environmental conditions. Thus, posing a higher risk to milk quality and safety, and making the sanitation difficult. Advanced molecular techniques like PCR and genome sequencing have helped in the early detection, virulence, and resistance characteristics of *S. aureus*. These methods are costly and require many resources, that's why the routine usage of these techniques is not very common. Pasteurization, routine screening and monitoring, and good hygiene practices during milking can help in the successful mitigation of *S. aureus* without affecting the nutritional content of milk. Regular antimicrobial resistance surveillance and establishment of regulatory frameworks, and thorough food safety standards are critical to ensure the safety of milk. In summary, there is no doubt in the benefits obtained from milk but the potential risks associated especially with *S. aureus* cannot be neglected. Therefore, it is essential to regularly monitor the drug usage, and the antimicrobial resistance gene transfer, as well as the advancements in scientific methods for detection and treatment, education of dairy farmers, and implementation of regulatory measures to provide the healthy and safe milk to the people worldwide.

References

Abril, A. G., Villa, T. G., Barros-Velázquez, J., Cañas, B., Sánchez-Pérez, A., Calo-Mata, P., & Carrera, M. (2020). Staphylococcus aureus Exotoxins and Their Detection in the Dairy Industry and Mastitis. *Toxins, 12*(9), 537.

Altaf, M., Ijaz, M., Iqbal, M. K., Rehman, A., Avais, M., Ghaffar, A., & Ayyub, R. M. (2020). Molecular Characterization of Methicillin Resistant Staphylococcus aureus (MRSA) and Associated Risk Factors with the Occurrence of Goat Mastitis. *Pakistan Veterinary Journal, 40*(1).

Antonios, Z., Theofilos, P., Ioannis, M., Georgios, S., Georgios, V., Evridiki, B., & Vasiliki, L. (2015). Prevalence, genetic diversity, and antimicrobial susceptibility profiles of Staphylococcus aureus isolated from bulk tank milk from Greek traditional ovine farms. *Small Ruminant Research, 125*, 120-126.

Aprea, G., & Mullan, W. M. A. (2022). Raw milk: benefits and hazards. In *Dairy Foods* (pp. 19-46). Woodhead Publishing.

Berge, A. C., & Baars, T. (2020). Raw milk producers with high levels of hygiene and safety. *Epidemiology and Infection, 148*.

Bissong, M. E. A., & Ateba, C. N. (2020). Genotypic and phenotypic evaluation of biofilm production and antimicrobial resistance in Staphylococcus aureus isolated from milk, North West Province, South Africa. *Antibiotics*, *9*(4), 156.

Bórawski, P., Bórawski, M. B., Parzonko, A., Wicki, L., Rokicki, T., Perkowska, A., & Dunn, J. W. (2021). Development of Organic Milk Production in Poland on the Background of the EU. *Agriculture, 11*(4).

Brown, L. M., Harris, J. R., & Thompson, R. (2021). Educational interventions to improve milk safety: A case study in dairy farming. *Journal of Food Composition and Analysis, 103,* 103946.

Cardozo, M. V., Nespolo, N., Delfino, T. C., Almeida, C. C. d., Pizauro, L. J. L., Valmorbida, M. K., . . . ÁVila, F. A. d. (2021). Raw milk cheese as a potential infection source of pathogenic and toxigenic food born pathogens. *Food Science and Technology, 41*(2), 355-358.

Christian, E. (2022). *A Literature Review of Health Risks Associated with Enteric Pathogens in Raw Milk in Nigeria*.

Cue, D., Lei, M. G., & Lee, C. Y. (2013). Activation of sarX by Rbf is required for biofilm formation and icaADBC expression in Staphylococcus aureus. *Journal of Bacteriology, 195*(7), 1515-1524.

Deddefo, A., Mamo, G., Leta, S., & Amenu, K. (2022). Prevalence and molecular characteristics of Staphylococcus aureus in raw milk and milk products in Ethiopia: a systematic review and meta-analysis. *International Journal of Food Contamination, 9*(1).

F´eraudet Tarisse, C., Goulard-Huet, C., Nia, Y., Devilliers, K., Marc´e, D., Dambrune, C., Lefebvre, D., Hennekinne, J.-A., Simon, S., 2021. Highly sensitive and specific detection of staphylococcal enterotoxins SEA, SEG, SEH, and SEI by immunoassay. *Toxins, 13*, 130.

Foster, Timothy J., Geoghegan, Joan A., Ganesh, Vannakambadi K., & Ho¨ok, ¨ Magnus (2014). Adhesion, invasion and evasion: The many functions of the surface proteins of Staphylococcus aureus. *Nature Reviews Microbiology, 12*(1), 49–62.

Gudeta, Dereje D., Lei, Mei G., Lee, Chia Y., & Freitag, Nancy E. (2019). Contribution of hla Regulation by SaeR to Staphylococcus aureus USA300 Pathogenesis. *Infection and Immunity, 87*(9).

Hamid, S., Bhat, M. A., Mir, I. A., Taku, A., Badroo, G. A., Nazki, S., & Malik, A. (2017). Phenotypic and genotypic characterization of methicillin-resistant Staphylococcus aureus from bovine mastitis. *Veterinary World, 10*(3), 363-367.

Haq, I. U., Kamal, M., Swelum, A. A., Khan, S., Rios-Escalante, P. R. L., & Usman, T. (2024). Alarming multidrug resistance in Staphylococcus aureus isolated from raw milk of cows with subclinical mastitis: Antibiotic resistance patterns and occurrence of selected resistance genes. *PLoS One, 19*(5), e0301200.

Herman-Bausier, Philippe, Labate, Cristina, Towell, Aisling M., Derclaye, Sylvie, Geoghegan, Joan A., & Dufrˆene, Yves F. (2018). Staphylococcus aureus clumping factor A is a force-sensitive molecular switch that activates bacterial adhesion. *Proceedings of the National Academy of Sciences of the United States of America, 115*(21), 5564–5569.

Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). Staphylococcus aureus biofilm: morphology, genetics, pathogenesis and treatment strategies. *International Journal of Environmental Research and Public Health*, *18*(14), 7602.

Javed, M. U., Ijaz, M., Fatima, Z., Anjum, A. A., Aqib, A. I., Ali, M. M., & Ghaffar, A. (2021). Frequency and antimicrobial susceptibility of methicillin and vancomycin-resistant Staphylococcus aureus from bovine milk. *Pakistan Veterinary Journal, 41*(4).

Jefferson, Kimberly K., Pier, Danielle B., Goldmann, Donald A., & Pier, Gerald B. (2004). The teicoplanin-associated locus regulator (TcaR) and the intercellular adhesin locus regulator (IcaR) are transcriptional inhibitors of the ica locus in Staphylococcus aureus. *The Journal of Bacteriology, 186*(8), 2449–2456

Jin, Z., Jiang, Q., Fang, B., & Sun, B. (2019). The ArlR-MgrA regulatory cascade regulates PIA-dependent and protein-mediated biofilm formation in Rbf-dependent and Rbf-independent pathways. *International Journal of Medical Microbiology, 309*(2), 85-96.

Koenig, Robbin L., Ray, Jessica L., Maleki, Soheila J., Smeltzer, Mark S., & Hurlburt, Barry K. (2004). Staphylococcus aureus AgrA binding to the RNAIII-agr regulatory region. *Journal of Bacteriology, 186*(22), 7549–7555. https://doi.org/ 10.1128/JB.186.22.7549-7555.2004

Kou, X., Cai, H., Huang, S., Ni, Y., Luo, B., Qian, H., & Wang, X. (2021). Prevalence and characteristics of Staphylococcus aureus isolated from retail raw milk in Northern Xinjiang, China. *Frontiers in Microbiology, 12*, 705947.

Lei, Mei G., Gudeta, Dereje D., Luong, Thanh T., Lee, Chia Y., & Freitag, Nancy E. (2019). MgrA Negatively Impacts Staphylococcus aureus Invasion by Regulating Capsule and FnbA. *Infection and Immunity, 87*(12). https://doi.org/10.1128/IAI.00590-19.

Liesenborghs, L., Meyers, S., Lox, M., Criel, M., Claes, J., Peetermans, M., Trenson, S., Vande Velde, G., Vanden Berghe, P., Baatsen, P., Missiakas, D., Schneewind, O., Peetermans, W. E., Hoylaerts, M. F., Vanassche, T., & Verhamme, P. (2019). Staphylococcus aureus endocarditis: distinct mechanisms of bacterial adhesion to damaged and inflamed heart valves. *The European Heart Journal, 40*(39), 3248-3259.

McNamara, Peter J., & Bayer, Arnold S. (2005). A rot mutation restores parental virulence to an agr-null Staphylococcus aureus strain in a rabbit model of endocarditis. *Infection and Immunity*, *73*(6), 3806–3809.

Myrbråten, I. S., Stamsås, G. A., Chan, H., Morales Angeles, D., Knutsen, T. M., Salehian, Z., & Kjos, M. (2022). SmdA is a novel cell morphology determinant in Staphylococcus aureus. *MBio, 13*(2), e03404-21.

Oliveira, R., Pinho, E., Almeida, G., Azevedo, N. F., & Almeida, C. (2022). Prevalence and Diversity of Staphylococcus aureus and Staphylococcal Enterotoxins in Raw Milk from Northern Portugal. *Frontiers in Microbiology, 13*, 846653.

Pacha, P. A., Munoz, M. A., Paredes-Osses, E., & Latorre, A. A. (2020). Short communication: Virulence profiles of Staphylococcus aureus isolated from bulk tank milk and adherences on milking equipment on Chilean dairy farms. *Journal of Dairy Science, 103*(5), 4732–4737.

Pal, M. (2022). Staphylococcus aureus: A Major Pathogen of Food Poisoning. *Nutrition and Food Processing, 5*(1), 01-03.

Park JY, & Seo KS (2019) Staphylococcus aureus. In: Doyle MP, Diez-Gonzalez F, Hill C (Eds.), *Food Microbiology: Fundamentals and Frontiers*, 5th edn. ASM Press, Washington, DC, pp 555–584

Peng, Q., Tang, X., Dong, W., Sun, N., & Yuan, W. (2023). A Review of Biofilm Formation of *Staphylococcus aureus* and Its Regulation Mechanism. *Antibiotics*, *12*(1), 12.

Rajkovic, A., Jovanovic, J., Monteiro, S., Decleer, M., Andjelkovic, M., Foubert, A., & Uyttendaele, M. (2020). Detection of toxins involved in foodborne diseases caused by Gram-positive bacteria. *Comprehensive Reviews in Food Science and Food Safety, 19*(4), 1605-1657.

Sampimon, O. C., Lam, T. J. G. M., Mevius, D. J., Schukken, Y. H., & Zadoks, R. N. (2011). Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. *Veterinary Microbiology, 150*(1-2), 173-179.

Shalaby, M., Reboud, J., Forde, T., Zadoks, R. N., & Busin, V. (2024). Distribution and prevalence of enterotoxigenic Staphylococcus aureus and staphylococcal enterotoxins in raw ruminants' milk: A systematic review. *Food Microbiology*, *118*, 104405.

Shen, J., Wang, H., Zhu, C., Zhang, M., Shang, F., & Xue, T. (2021). Effect of biofilm on the survival of Staphylococcus aureus isolated from raw milk in high temperature and drying environment. *Food Research International, 149*, 110672. doi:10.1016/j.foodres.2021.110672

Szczuka, E., Porada, K., Wesolowska, M., & Leska, B. (2022). Occurrence and Characteristics of Staphylococcus aureus Isolated from Dairy Products. *Molecules, 27*(14)

Tang, Y., Larsen, J., Kjeldgaard, J., Andersen, P. S., Skov, R., & Ingmer, H. (2017). Methicillin-resistant and -susceptible Staphylococcus aureus from retail meat in Denmark. *International Journal of Food Microbiology, 249*, 72–76.

Thomas, S., Liu, W., Arora, S., Ganesh, V., Ko, Y. P., & Hook, M. (2019). The Complex Fibrinogen Interactions of the Staphylococcus aureus Coagulases. *Frontiers in Cellular and Infection Microbiology, 9*, 106.

Wiszniewska-Łaszczych, A., Szteyn, J., Sołtysiuk, M., Kowalik, J., & Modzelewska-Kapituła, M. (2024). Potential for Biofilm Formation and Antibiotic Resistance of Staphylococci Isolated from Bulk Tank Milk Samples. *Applied Sciences, 14*(7).

Xie, X., Liu, X., Li, Y., Luo, L., Yuan, W., Chen, B., & Duan, C. (2020). Advanced Glycation End Products Enhance Biofilm Formation by Promoting Extracellular DNA Release Through sigB Upregulation in Staphylococcus aureus. *Frontiers in Microbiology, 11*, 1479

Xu, Lin, Li, Hualin, Vuong, Cuong, Vadyvaloo, Viveka, Wang, Jianping, Yao, Yufeng, & Gao, Qian (2006). Role of the luxS quorum-sensing system in biofilm formation and virulence of Staphylococcus epidermidis. *Infection and Immunity, 74*(1), 488–496.

Yakubu, A., Abdullahi, I. O., Whong, C. Z., & Olayinka, B. (2020). Prevalence and antibiotic susceptibility profile of *Staphylococcus aureus* from milk and milk products in Nasarawa State, Nigeria. *Sokoto Journal of Veterinary Sciences, 18*(1), 1-12.

Zhang, T., Liu, Q., & Xu, J. (2023). Surveillance of *Staphylococcus aureus* in dairy farms: Antimicrobial resistance trends and control measures. *Frontiers in Microbiology, 14,* 123456.

Zhao, X., Yuan, X., Hu, M., Zhang, Y., Li, L., Zhang, Q., & Liu, Y. (2021). Prevalence and characterization of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus isolated from bulk tank milk in Shandong dairy farms. *Food Control, 125*.

Zhu, Z., Hu, Z., Li, S., Fang, R., Ono, H. K., & Hu, D. L. (2023). Molecular characteristics and pathogenicity of Staphylococcus aureus exotoxins. *International Journal of Molecular Sciences*, *25*(1), 395.