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Impacts of Staphylococcus Aureus on Dairy Animals and Humans

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Abstract

Staphylococcus aureus is a mammalian commensal and opportunistic pathogen that colonizes niches on 20-30% of people's skin, nares, and various mucosal membranes. *S. aureus* can infect people with a variety of ailments, and both methicillin-sensitive and methicillin-resistant strains are frequently responsible for nosocomial and community-acquired infections. Regardless of the fact that there are a number of studies describing staphylococcal pathogenesis in humans and dairyanimals, *S. aureus* is a major cause of infection and disease in a wide variety of animal hosts, with a substantial impact on public health and farm animals specifically dairy cow. Animal health is adversely affected by infections caused by this bacteria, and also can serve as a reservoir for the staphylococcal bacteria that can harm humans. With the domestication and/or commercialization of particular animal species, host-switching events between humans and animals as well as amonganimals have become more frequent. In most cases, changing hosts is followed by adaptation through the acquisition or loss of mobile genetic elements such as plasmids, pathogenicity islands,and phages, as well as supplementary host-specific mutations that allow the organism to disseminate into new host populations. An overview of *S. aureus* in animals will be provided in this chapter, as well as information on how this bacterial species has been and is still being transported to new host species and the critical factors thought to be involved in its adaptation to new ecological host niches. The relevance of animal hosts as a wellspring of antimicrobial resistance drivers, precautionary strategies, and management techniques also be highlighted.

Background of *Staphylococcus aureus*

The existences of *Staphylococci* are as old as the age of the earth and they were first recognized asa bacterial pathogen in the 19th century and they have been grouped under the most considerable pathogenic bacteria and cause ailment both in animals and human beings. Taxonomically, the genus *Staphylococcus* belongs to the *Staphylococcaceae* family [1]. It was investigated in 1880 bya Scottish Surgeon referred to as Alexander Ogston and he additionally gave the name *Staphylococcus* "Staphyle" in Greek meaning bunch of grapes, "Kokkos" means a berry because of the typical occurrence of the cocci in grape-like clusters in pus and cultures [2]. Later in 1884, German physician Friedrich Julius Rosenbach grew organisms in pure culture and categorized them according to their color production, which he named for the pigmented appearance in their colonies *Staphylococcus aureus* from "aurum" for gold [3].

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General cultural and biochemical characteristics of Staphylococcus aureus

All *Staphylococci* grow in clusters. *S. aureus* is an aerobic or facultative anaerobic which characterized by Gram-positive, 1µm in diameter, very thick cell wall (peptidoglycan layer), non-flagellated, non-motile round shape (cocci), non-spore-forming, capsule present in some strains, and it has grape-like clusters, singly, in pairs or short chain of 3-4 bacteria [4,5]. The key cultural characteristic features of *S. aureus* are also, free coagulase, maltose fermentation character on Purple Agar Base (PAB) media plate with 1% of maltose. *S. aureus* rapidly ferments maltose than other coagulase-positive *Staphylococci* species like *S. intermedias* and *S. hyicus* and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. The coagulase test is used to differentiate *S. aureus* (positive) from coagulase-negative *Staphylococcus*. They are also catalase-positive which is an important test for distinguishing them from *Streptococci* (catalase-negative). Moreover, *S. aureus* often causes haemolysis in blood agar due to the production of four types of haemolysins (alpha, beta, gamma, and delta) [6].

In addition to Mannitol salt agar, Baired parker agar enriched with egg yolk emulsion and potassium tellurite is selective media which mainly used for the enumeration of bacteria in the food (ISO, 2015). The main characteristic features of this bacteria on BPA plates are smooth, convex, moist, and gray to jet-black (due to reduction of tellurite in the media), frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone that occurs as a result of lecithinase enzyme produced only by *S. aureus* [6].

Growth and survival characteristics of Staphylococcus aureus

Staphylococci grow and survive under different environmental conditions [7]. It displays remarkable halotolerant in the presence of external osmotic pressure that makes these bacteria capable of colonizers of environments with low water content and high salinity, which gives thema competitive advantage over many other microorganisms [8]. These bacteria are thermotolerant and can survive at high temperatures as is shown in Table 1. Additionally, their pre-formed enterotoxins are resistant to heat treatment and many other conditions. *Staphylococci* also encounter numerous nutrient-limiting environments and can persist for extended period [9].

Table 1: Growth and survival characteristics of *S. aureus* and its enterotoxins production.

Factors	Optimal growth	Growth limit	Enterotoxin production	
			Optimal SEs	SEs limits
Temperature	35-41 °C	6-48 °C	34-40 °C	10-45 °C
Aw	0.99	0.85≥0.99	0.99	0.86≥0.99
pH	6-7	4-10	7-8	5-9.6
NaCl	0%	0-20%	0%	0-10%
Atmosphere	Aerobic	Anaerobic-Aerobic	Aerobic	Anaerobic-Aerobic

Source: [10].

Virulence factors of Staphylococcus aureus

Staphylococcus aureus can produce various virulence factors [11]. These virulence factors includeantigens (e.g. capsule and adhesins), enzymes (e.g. hemolysins, proteases, coagulase, lipase), andtoxins (e.g. enterotoxins and exfoliative toxins) which enable the organisms to be successful as a pathogen that causes a wide range of infection both in human and animals [12]. The toxins produced by *S. aureus* retain their biological activities even after the thermal processing of the food, which imposes a crucial challenge in the food industry. These toxins are also resistant to proteolytic enzymes such as pepsin, rennin, and trypsin proteases in the gastrointestinal tract [13].

Staphylococcal Enterotoxins (SEs) are also one of the toxins produced by enterotoxigenic strains of Coagulase-Positive *Staphylococci* (CPS), majorly by *S. aureus* that result in *Staphylococcal* Food Poisoning (SFP) which are pre-formed in food materials during the growth of this bacteria [14]. Inaddition to SEs, hemolysins, leukotoxins, exfoliative toxins, and Toxic Shock Syndrome Toxin (TSST-1) are also the most relevant secreted toxins. Recently, around 25 SEs (SEA-Z) have beendescribed, excluding variants and TSST-1, but new types are frequently discovered and SEA-E areconsidered "classical enterotoxins", while SEG-Z is termed "new enterotoxins" [15].

Epidemiology

Staphylococci are ubiquitous and widely spread in nature [16]. *Staphylococcus aureus* is one of the *Staphylococcal* species and commensal of human beings. Nearly 50% of the human population areasymptomatic carriers of *S. aureus* [1]. Animals can act as a reservoir for *Staphylococci* and the prevalence of *S. aureus* varies from host species to species. For instance, up to 14 and 35% of cowsand heifers are carriers of this bacteria [17]. They also colonize the skin and mucous membranes in the nostrils of humans and different animals, including cattle [18]. Additionally, birds can alsoharbor *S. aureus* as mammals [19]. They may be found in the mouth, blood, intestinal, genitourinary, and upper respiratory tracts of these hosts [20].

There are various risk factors for the distribution of the *S. aureus* in raw cow milk like age of the lactating cow, parity, stages of lactations, udder washing intervals, and drying towel [21]. The unhygienic conditions in the farm including uncleaned bedding might have exposed teat ends andfacilitated the entry of *S. aureus* into the udder of the cow [22]. Some of the silent features for thespread of these pathogens to the udder of the cows are milker's hand, towels, flies, milking equipment such as teat liners [23]. Moreover, *S. aureus* also commonly results in a chronic infection that will persist from one lactation to another despite dry cow therapy [24]. From more than 140 reported bovine mastitis-causing bacterial species, *S. aureus* stands in an average of numbers as the leading source of intra-mammary infections in dairy cows [25].

Sources of food contamination and reservoir of S. aureus

Humans and animals are reservoirs of *S. aureus* [26].The chronically infected mammary gland of an animal serves as the reservoir and source of *S. aureus* and vestibulum nasi (anterior nares) of animals and humans mainly serves as a reservoir for the spread of the pathogen [27]. The nasal cavity may also represent the primary reservoir in other species of animals (e.g. Sheep). Furthermore, *S. aureus* can survive for some time in the dairy cow environment, including bedding materials, milking equipment, and facilities (Rainard *et al.,* 2018). Additionally, meat and its products are also important reservoirs of *S. aureus* (Weldeselassie *et al.,* 2020). The contaminationof the raw milk with *S. aureus* can occur in the dairy farm which is either associated with milkersor milk handlers, especially those with poor hygienic habits such as coughing or sneezing during milking or

milk handling, the milking parlour environment or skin of dairy animals (Petróczki *et al.,* 2021).

Vehicle of transmission

Carriers of *S. aureus* majorly act as vehicles of transmission and can move the bacteria from theirnose to other body parts with their hands, sometimes leading to infection [27]. *S. aureus* can be found in different body sites like the skin and gastrointestinal tract. People who are hospitalized or work in a hospital are more likely to be carriers. It is reported that 10 to 35% and 20 to 75% ofhumans are persistent and intermittent carriers of *S. aureus*, respectively [26]. *S. aureus* is usuallytransferred by direct contact with a colonized individual as well as skin-to-skin with excretions (saliva or aerosols released through sneezing and coughing) which contain these bacteria [22]. Thecontaminated objects and surfaces might also play a role in the transmission of the *S. aureus* because it has the capability of surviving on dry stainless steel and it can easily be transferred fromsponges to stainless steel surfaces and subsequently to food products (E.g. milking equipment to milk). Furthermore, food handlers also serve as determinant factors for the transmission of this bacteria to the food under unhygienic conditions [27]. It can also be spread by non-pasteurized dairy products such as milk [28].

Pathogenesis and clinical symptoms

The pathogenesis of diseases caused by S. aureus is based on the direct infection of the organismsor ingestion of the intoxicated food by its preformed enterotoxins. The process of S. aureus infection contains five stages. They are colonization, local infection, systemic dissemination and/or sepsis, metastatic infections, and toxicosis. The organism is in a carrier state in the anterior nares and can remain without causing infections for weeks or months. The colonization proceeds to infection under certain predisposing factors such as prolonged hospitalization, immune suppression, surgeries, use of invasive medical devices and chronic metabolic diseases [29]. Localized skin abscess develops when the organism is inoculated into the skin from a site of carriage. This can further spread and result in various clinical manifestations of localized infectionssuch as carbuncle, cellulitis, impetigo bullosa or wound infection[30]. In the case of SFP, the ingestion of food contaminated with preformed S. aureus enterotoxins and stimulation of vagus- nerve finally results in some symptoms like retching, abdominal cramps and diarrhea [31].

Staphylococcal foodborne disease

Staphylococcus aureus is rated as the third most important cause of foodborne disease in the world.The first foodborne disease involving *Staphylococci* was investigated in Michigan (USA) in 1884by Vaughan and Sternberg and by Denys in 1894 with contaminated cheeses and meat respectively[32]. This event was due to the consumption of cheese contaminated by *Staphylococci*. Because, dairy and dairy products are protein-rich food that are vehicles of amino acids and low-molecular-weight peptides which support the survival and growth of *S. aureus* [33]. The highest risk of *S. aureus* is its ability to produce thermostable enterotoxins and microbial count in the milk of greaterthan 5log10 CFU/ml is evidence of serious faults in milk production hygiene [34].

In foods, enterotoxigenic strains of *S. aureus* can produce heat-stable and protease-resistant *Staphylococcal* Enterotoxins (SEs), causing one of the most common foodborne intoxications [27], *Staphylococcal enterotoxins* are secreted toxins of 20 to 30 kD that interfere with intestinal function and cause *Staphylococcal* food poisoning, which is one of the most common FBDs worldwide with high occurrence next to salmonellosis*. Staphylococcal food poisoning* is highly stable, resists most proteolytic enzymes, and keeps its activity in the digestive tract after ingestion.

It is also a self-limiting disease that usually resolves within 24 to 48 hours of onset and is usuallyassociated with vomiting [12].

Besides, *S*. *aureus*, other *Staphylococcus* species, including Coagulase Positive *Staphylococci* (CPS) such as *S. intermedius* and Coagulase Negative *Staphylococci* (CNS) (*S. epidermis* and *S. haemolyticus)* can also produce exotoxins. However, nearly all cases of SFP are attributed to *S. aureus-produced toxins* [35]. The toxins produced by *S. aureus* belong to a wide family of pyrogenic toxins superantigens. Pyrogenic toxins provoke superantigenic activity [15]. The superantigenic activity of SEs helps to encourage transcytosis, which enables the toxin to enter thebloodstream, thereby allowing it to interact with antigen-presenting cells and T-cells, contributingto the activity of superantigens. The typical clinical symptoms of SFP include nausea, retching/vomiting, and abdominal cramps often accompanied by diarrhea [36].

Diagnosis

The diagnosis of *S. aureus* depends on the diseases they cause and clinical symptoms. Because it results in a variety of infections and food poisoning or intoxication. In the case of *Staphylococcal*food poisoning, the typical clinical signs of SFPs such as vomiting, diarrhea which starts within ahalf-hour after the consumption of food may suggest *S. aureus* intoxication tentatively. But, for confirmation, microbiological, immunological, and molecular examinations or a combination of these techniques can be carried out [26]. The bacteriological diagnosis of *S. aureus* includes morphological characteristics under a microscope after Gram staining, catalase test, coagulase test,colony characteristics on different selective culture media like Mannitol salt agar, purple agar base,and Baired parker agar media with addition of required supplement [37].

The immunological tests are also one of the major diagnostic tools of *S. aureus.* The most frequently used tests are Enzyme-Linked Immunosorbent Assays (ELISA), Reverse Passive Latex Agglutination (RPLA), and Radioimmunological Assay (RIA). But, Enzyme-Linked Immunosorbent Assays (ELISA) are commonly used. However, it is very difficult and expensive to prepare a highly-purified antibody against the SEs, which are required for the immunological test. The gel-diffusion methods have been used primarily for the detection of enterotoxin produced by *Staphylococcal* strains, although the RPLA method is used for testing strains for low production of enterotoxin. The RIA method was used for testing for enterotoxin in foods until the ELISA andRPLA were available [38].

The molecular diagnostic methods also one of the new diagnostic tests used to detect the differentmicroorganism within a short period of times. The molecular assay includes the extraction of *S. aureus* DNA either directly from food or a cultured broth and then testing for the presence of enterotoxin genes. Polymerase chain reaction PCR and real-time PCR are the majorly recommended molecular detection methods of *Staphylococcus aureus* enterotoxin genes [36].

Public Health and Economic Significance of *Staphylococcus aureus*

Public health significance of *Staphylococcus aureus*

Staphylococcus aureus is a major pathogen of public health concern and a growing burden for thehealthcare system all over the globe Approximately about 30% of the human population is colonized with *S. aureus* [30]. It causes a wide range of serious diseases in humans like bacteremia,skin and soft tissue infections, and Infectious Endocarditis (IE), osteoarticular, pleuropulmonary, and food poisoning as well as life-threatening postsurgical infections [39].

The highest incidence rate of infection with *S. aureus* occurs at extreme life (old age), immunocompromised individuals, and Acquired Immunodeficiency Syndrome (AIDS) or defects in neutrophil function, diabetes and loss of normal skin barriers are core predisposing factors of an individual. These bacteria alone have been found to cause hospitalization rates as high as 14% and the fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons [38]. For instance, the *S. aureus* bacteremia can cause mortality rates of around 20-30% [40].

The emergence of Methicillin-Resistant *S. Aureus* (MRSA) strain has also become a pathogen of increasing importance in the hospital community and also in livestock in addition to SEs [41]. Specifically, the new strain of *S. aureus*, Livestock-Associated Methicillin-Resistant *S. Aureus* (LA-MRSA), is recognized as an emerging novel pathogen that causes human infections Different figures were provided by various nations regarding the annual mortality rate due to AMR with 22000, 25000 and 12500 extra deaths in the United States, Europe, and France respectively [29].

The economic significance of *Staphylococcus aureus*

Staphylococcus aureus has major effects on the economy of the world directly or indirectly. It results in huge financial losses in dairy farms associated with mortality, culling of infected dairy cows, spoiling of the milk, lower shelf life, decreased yield of milk products, cost of treatment, and decreased milk quality (change in milk composition, and palatability [42]. There is also loss ofthe milk due to drug residue [43]. The infection caused by this species of bacteria is estimated to be present in up to 90% of dairy farms and is responsible for 35% of the economic losses in the dairy industry. For instance, annual losses due to *Staphylococcal* mastitis are estimated to be 35 billion US dollars in the world [44]. It also causes high hospitalization costs for drug resistance likeMRSA [45]. Furthermore, this species of bacteria also cause damage to food through the production of different enterotoxins [11].

Treatment

In human

There is no effective long-term decolonization therapy for *S. aureus* carriers. Even with the use ofantibiotics, *S. aureus* can only be removed from the nose over a few weeks, but relapses are common within several months [46]. The treatment of *S. aureus* in human patients is based on thedisease it causes. In the case of *Staphylococcal* food intoxication, the treatment aim is to replace fluids, salt, and minerals that are lost by vomiting or diarrhoea. Because, antimicrobial treatment is not recommended in case of SFP due the additional release of Staphylococcal toxins after bacterial cell death, leading to septic shock [47]. However, infections caused by *S. aureus* in humanbeings are treated with various antimicrobials with some limitations like AMR [48]. Some studiesshowed that 90% of *S. aureus* isolated from carrier patients are resistant to penicillin due to β-lactamase enzyme production or changes like penicillin binding protein (PBPs). However, β- lactam clavulanic acid such as Co-amoxiclav is used to treat *S. aureus* β-lactamase producing strain. In the case of infection caused by methicillin-resistant strains of *S. aureus,* teicoplanin andvancomycin are used [49].

In animals

Staphylococcus aureus is a common cause of mastitis in dairy cows and a primary reason for antibiotic use on farm animals [50]. Antibiotic treatment does not control diseases in animals but it may shorten the duration of infection. *S. aureus* is associated with both clinical and subclinical mastitis, both of which frequently result in persistent and recurrent infections with a low cure rateafter antibiotic therapy [17]. The cure rates for lactational *S. aureus* treatments are low, but dry cowtherapy is typically more effective. In particular, cloxacillin is extensively used on dairy farms andcure rates for dry cow treatment of *S. aureus* infections with cloxacillin were reported to range upto 98% and pirlimycin is also effective against *S. aureus* infection in cows [23].

Prevention and Control

Prevention

The elimination of *S. aureus* from the environment is not possible. Because, they are ubiquitous [16]. Prevention of *Staphylococcal* infections/intoxication requires different strategies to interrupt various modes of transmission, including proper food handling and processingprotocols, sufficient cleaning and disinfection of equipment, protection of cross-contamination inthe home, and a kitchen [36]. Dry cow therapy and optimizing hygienic practices in the farm to fork chain is essential to minimizing or enhancing the quality of the food to protect public health from foodborne pathogens and public awareness regarding food handling [51]. The prevention of further dissemination of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) with a zoonotic potential also needs concerted action of veterinary infection control specialists and clinicians by obtaining necessary information regarding the prevalence of MRSA infection before implementingstrategies for infection control in veterinary medical practice [52].

Control

The control of *S. aureus* from entering into animal origin food, especially milk through different strategic programs includes improvements in personal hygiene practices among healthcare workers and food handlers, decontamination of equipment, surfaces, and clothing, judicious use of antibiotics, proper cooking and storage of foods, and screening program [38]. Training those who prepare food at home, prohibiting individuals with abscesses or other skin lesions from handling food; refrigeration at 4°C or lower of all foods to prevent bacterial multiplication and theformation of toxins. Foods must be kept at room temperature for as little time as possible. The veterinary service should be supervising dairy installations, the correct operation of refrigeration units and their use immediately after milking, and refrigerated transport of the milk to pasteurization plants [53]. Proper use of antibiotics for treating animals and following the principleof Hazard Analysis and Critical Control Points (HACCP) [54].

The lack of effectiveness of the current strategies (principally based on antiseptic teat dipping aftermilking and antibiotic therapy during the dry period) to suppress *S. aureus* has been

promoted in the sense of vaccine preparation against *S. aureus,* which is a reasonable/alternative approach forthe control of these microorganisms associated with mastitis [55]. Routine hygiene procedures of milking such as washing the udder using a disinfectant, use of separate towel, and teat dipping areeffectively decreasing mastitis which finally may result in contamination of the milk at different food chains and culling of MRSA suspected cows from the herd [23].

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