Chapter

Factors of Nasopharynx that Favor the Colonization and Persistence of *Staphylococcus aureus*

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Abstract

Between 30 and 50% of the world population is permanently colonized in some anatomical site by *Staphylococcus aureus*, although the vast majority are asymptomatic carriers. The nose is its main niche and currently the colonization of *S. aureus* in the pharynx has become relevant due to the variety of reported carrier rates and the epidemiological importance of the dissemination of Methicillin-resistant *S. aureus* strains (MRSA) by pharyngeal carriers. For this bacterium to colonize a tissue successfully, it is necessary to establish many interactions with bacterial and host cell components such as bacterial wall teichoic acids (WTA) with the Scavenger SREC-1 host receptor and at the same time evade the defense mechanisms. On the other hand, there are host factors that will facilitate or complicate the colonization or persistence of *S. aureus* at these sites, such as physiological, genetic, immunological and microbiological factors.

Keywords: *Staphylococcus aureus*, colonization, nasopharynx, microbiota, SREC-1

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that lives in symbiosis with humans, it is an opportunistic and potentially lethal pathogen [1, 2] of great clinical importance due to the different virulence, invasiveness and resistance factors that it may possess [3]. In humans, it colonizes various tissues, forming part of the normal microbiota [3, 4], although it is also one of the principal cause of community-associated and nosocomial-associated infections; is one of the main causes of bacteremia and infective endocarditis, as well as skin, soft tissue and pleuropulmonary infections and contamination of medical devices [4]. Invasive disease is associated with a mortality rate of ≥20% [2]. Uncontrolled use of antibiotics, particularly their inappropriate and excessive use, has favored the emergence and maintenance of strains of *S. aureus* resistant to multiple antibiotics such as penicillin (penicillin-resistant *S. aureus*, PRSA), methicillin (methicillin-resistant *S. aureus*, MRSA) [5, 6] or vancomycin, strains with high rates of morbidity and mortality in many countries of the world [5]. The most studied primary reservoir site for *S. aureus* in humans is the nose, predominantly found in the anterior nasal vestibule [7]. Approximately 30% or more of the world population is colonized with *S. aureus* on the skin, mucous membranes, or in the nose [4, 5] (**Figure 1**).

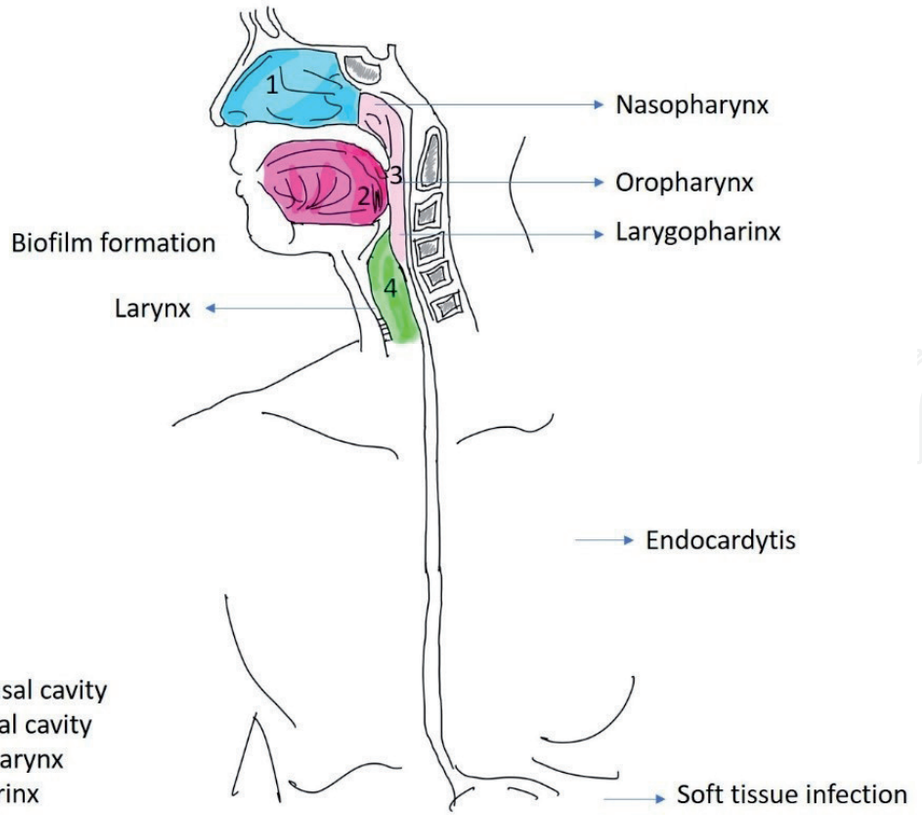

Figure 1.
S. aureus *can cause a wide range of diseases by spreading to various tissues, among the survival mechanisms being the formation of biofilms regulated by several genes such as the accessory regulatory gene* agr *and by intracellular infection mechanisms (Modified from Sasegbon and Hamdy. [49]).*

The mechanisms of colonization and persistence of *S. aureus* in the human nose have been extensively studied, however, it must be recognized that the clinical relevance of *S. aureus* carriers in the pharynx has not been sufficiently investigated [8]. This omission seems to be justified, if the nose is considered as the primary site of colonization of *S. aureus*, from there, other regions of the body are colonized by manual propagation [9]. However, in the adult population *S. aureus* can be commonly found in other parts of the body such as the armpits (8%), chest / abdomen (15%), perineum (22%), intestine (17–31%), vagina (5%) [10].

S. aureus carriers have been found in the pharynx and have been reported with high variability in different populations from 4 to 64% [11], some studies mention a higher rate of carriers in the pharynx than in the nose when samples are taken in parallel [10, 12, 13].

Colonization of *S. aureus* in the nose and pharynx is a multifactorial process that involves genetic aspects of the host, virulence factors of the pathogen, and possible interactions between the microbiota of the host [14], although in principle it is thinks that colonization of the pharynx is secondary to colonization of the nose, it is likely that both processes are independent [15].

2. Human determinants

The human determinants that allow bacterial colonization can be changes at the molecular level that alter the adhesiveness, recognition and eradication properties. Epidemiological studies present little information related to molecular studies [10].

The anterior nostrils are one of the main reservoir niches of *S. aureus*, however, the colonization of the nose begins from a cutaneous site, where the bacterium plays

a role of commensal microbiota and, through contact with contaminated hands, spreads to the nose and other parts of the body [16].

Colonization of the nose begins a few days after birth [17]. Between 40 and 50% of newborns become carriers during the first eight weeks of life but decreases to 21% in the sixth month [18]. In another research, 80% of identical strains were found among mother–child pairs, and in 90% of newborns, *S. aureus* came from the maternal nose [16].

Days after birth, the hands are the main source of transmission of *S. aureus* from contaminated surfaces to the nose. Reagan et al. [19] demonstrated by means of a randomized, double-blind and placebo-controlled trial, the link between the passage from the hand to the nose of *S. aureus*, in addition they demonstrated that nasal decolonization with mupirocin decreased the carriage in the hands and nose [16].

Research in people living in the same household has found that they tend to carry genetically similar nasal strains, suggesting their horizontal transmission. Furthermore, it has been shown that the carriage of MRSA strains in various parts of the body increases the risk of nasal colonization by MRSA [16].

2.1 Overview of the nostrils

The nasal passage filters 95% of the particles with a diameter greater than 15 μm from the inspired air. The nose is extremely important in protecting the distal airways from the influence of gases, aerosols, and pathogens [20].

The anterior part of the nasal cavity (*vestibulum nasi*) is formed with stratified squamous epithelium, non-ciliated keratinized (60% of the strains of *S. aureus* originating from the nose are isolated in this part). It has also been shown that *S. aureus* can colonize and persist in ciliated nasal epithelial cells in the inner part of the nasal cavity (internal nostrils) with pseudostratified columnar ciliated epithelium [9, 14].

The epidermis and dermis are the two main layers that line the *vestibulum nasi*. The dermis is a connective tissue that contains both epidermal and lymphatic structures, and vascular ducts, nerves, nerve endings, collagen, and elastic fibers, as well as a wide variety of specialized immune cells [7].

The epidermis is made up of the basal, spiny, granular, lucid and corneal striatum [7]. These five main strata are characterized by cells in different stages of differentiation, during which the anterior nasal epithelial cells change their appearance to keratinized squamous anucleated cells called corneocytes, these cells form the stratum corneum (also called cornified layer) being the most external, they are also surrounded by a protein structure containing loricrin and involucrin (**Figure 2**) [7, 14]. The upper layers of the keratinized epithelium are constantly being replaced, which could contribute to the elimination of the attached bacteria, however, this does not happen [14].

2.1.1 Loricrin

Epithelial tissues are the main appendages that protect the internal organs of the body from environmental stress, chemical damage, and microbial infections. The stratified epithelia seen on the skin and oral mucosa are one of the most resistant and protective epithelia, as it resists severe physical and chemical forces and do so by producing a hardened structure: the cornified cell envelope (CE). Loricrin is an important component of keratins. These keratins are structural proteins and constitute approximately 85% of a fully differentiated keratinocyte (**Figure 3**) [21, 22].

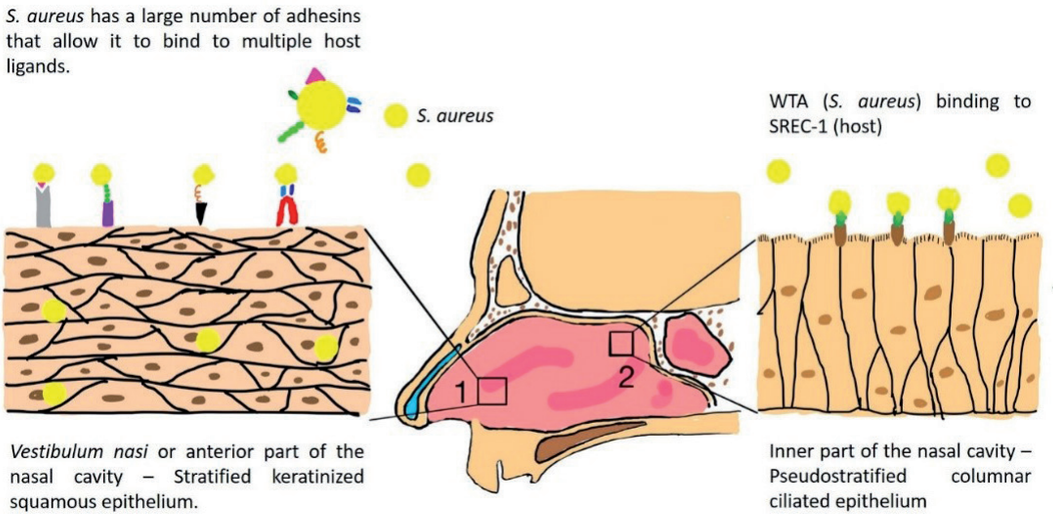

Figure 2.
Nasal colonization sites of S. aureus. *1.* Vestibulum nasi *or nasal cavity, is the ecological niche of* S. aureus *in humans.* S. aureus *Has a large amount of adhesins such as ClfB (light blue line), IcaA (strong blue line), Spa (orange line), SdrC (green dots), FnBPA (pink triangle), which can bind to various proteins of the nasal epithelial cells such as keratin and loricrin (red line), involucrin (purple line), unknown receptors (black triangle), fibronectin (gray rectangle). 2. The inner part of the nasal cavity can also be colonized by* S. aureus *and survive for a long time, at this site it binds by multiple load interactions by the teichoic acids of the bacterial wall (WTA) with the SREC-1 nasal cell receptor. (modified from Sakr et al. [16]).*

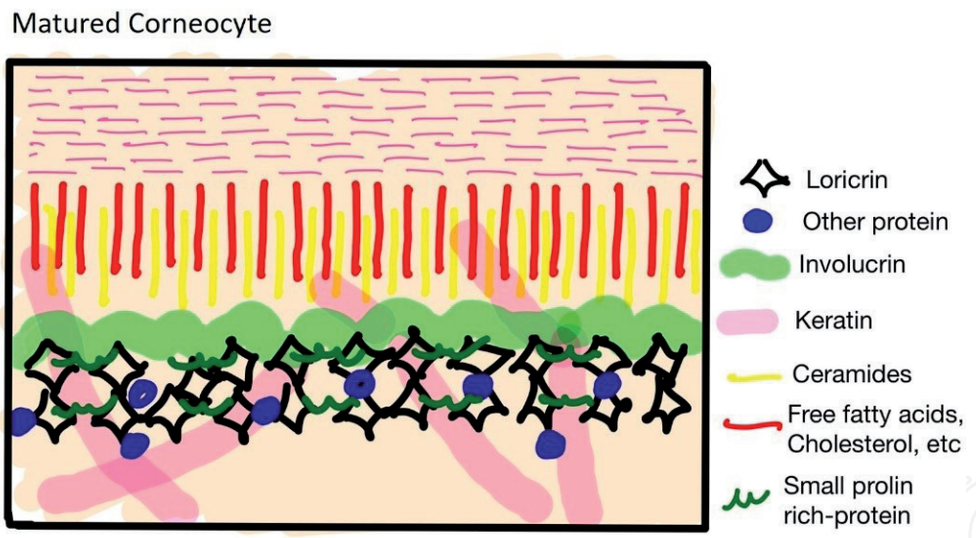

Figure 3.
Location of loricrin, involucrin, keratin and other macromolecules in mature corneocytes (modified from Ishitsuka and Roop. [21]).

Loricrin is an insoluble polypeptide with a molecular weight of 26 kDa. It has a conserved epitope and is a major cornified envelope protein seen in the cytoskeleton of the stratified parakeratinized epithelium. Being a late differentiating protein, it is introduced into the cornified envelope structure due to its cross-linking and binding property. It improves the function of the corneocyte protective barrier in differentiated keratinocytes [21].

Loricrin occupies an important part (70%) of the cornified epidermal envelope. Its concentration is reduced to approximately 30–50% in certain areas such as the palate and esophagus, while it is not expressed in many internal epithelia such as the oral mucosa. In *in vivo* studies in mammalian tissues, loricrin has a very high expression in all stratified epithelia, and is expressed even more in moist tissues of

newborns such as the epidermis, foreskin, epidermal sweat ducts, in addition to the oral and anal mucosa and the esophagus [21, 22].

In vitro analysis and studies in animal models have revealed that the main colonization target ligand of *S. aureus* is loricrin, binding with clumpling factor B (ClfB) in squamous epithelial cells [23].

2.1.2 Involucrin

Involucrin is a soluble cytosolic protein that is the precursor of the cornified envelope, its main function is to donate a glutamyl or glutamine in the crosslinking reaction catalyzed by the enzyme transglutaminase and it disappears from the soluble phase after the activation of calcium-dependent transglutaminase. 20% of its structure is glutamate and 25% glutamine. An important biochemical characteristic of involucrin is that it contains a central domain composed of 39 tandem repeats of 10 amino acids each segment, this repetitive structure is conserved in the involucrins of all higher primates, only varying the number of repeats [24]. In the cornified structure, involucrin is adjacent to the cell membrane, when the cell membrane is replaced, involucrin is the main substrate to which lipids esterify, primarily ceramides, are covalently attached to form the outer surface of the cornified envelope (**Figure 3**) [25].

The iron-regulated surface determining protein (IsdA) promotes the adhesion of *S. aureus* to squamous cells that cooperate in binding to the cornified cell envelope with the host proteins loricrin, involucrin, and cytokeratin [26].

2.1.3 Cytokeratine 10 (K10)

In the epidermis, keratinocytes travel from the basal cell layer to the postmitotic spinous suprabasal cells, and during the process, there is a significant change in the expression of basal cell keratins (K5, K14, and K15) to suprabasal epidermal keratins first to type II K1 keratin and later to type I K10 keratin. The keratin filaments are structurally composed of the K1 / K10 pair and form dense bundles, characteristic of suprabasal epidermal keratinocytes, and this gives the cells and the entire epidermis mechanical integrity (**Figure 3**). However, there are more functionalities, as K10 has been shown to specifically inhibit the proliferation and progression of the keratinocyte cell cycle and the decrease in K10 leads to an increase in keratinocyte renewal [27, 28].

It has been shown that cytokeratin 10 is a receptor for ClfB of *S. aureus*, which facilitates the nasal colonization of this and other bacteria [29].

2.2 Interactions with the nasal cavity

Another ecological niche of *S. aureus* in addition to the *vestibulum nasi* is the internal part of the nasal cavity (**Figure 2**). The teichoic acids of the bacterial wall (WTA) are a principal factor for the colonization process. A study reported in an animal model that mutated strains deficient in the *tagO* and *tarK* genes that participate in WTA biosynthesis did not adhere to or colonize the nose cells of cotton rats compared to control bacteria [14].

Baur et al. [30] re-studied the adhesion to nasal cells of WTA and reported the expression of the SREC-1 receptor (from the family of Scavenger receptors type F) in the nose of cotton rats and in epithelial cell lines of the human internal nasal cavity. In addition, they found that SREC-1 interacts with WTA and verified it in cotton rats infected with a previous treatment with anti-SREC-1 antibodies, significantly decreasing colonization after 8 hours and 6 days after inoculation compared to the control group.

2.2.1 SREC-1 receptor

The key role that WTA plays in the early colonization stages of *S. aureus* has been demonstrated, however, until recently the ligand with which it binds to initiate adhesion and colonization with the host was found.

The Scavenger receptor superfamily can bind and endocyte many ligands, which causes the elimination of both exogenous and unnecessary endogenous molecules [31]. It is important to mention that the affinity for the ligands is shared by several Scavenger receptors, regardless of whether their classes (A-J) have little or no biochemical homology [31, 32].

The Scavenger 1 class F receptor (SREC-1, SCARF1 or SR-F1), is the most expressed by endothelial cells (of the Scavenger family). It is a type I transmembrane protein that weighs 86 kDa, contains some epidermal growth factors (EGF) with similarity to the extracellular region, a small transmembrane domain, and a long cytoplasmic tail rich in proline and serine [33].

The size of the cytoplasmic domains could have a role in intracellular signaling, however, this function has not been found. SREC-1 is an evolutionarily highly conserved receptor, particularly in the extracellular domain, and shows significant homology with the *Caenorhabditis elegans* Scavenger receptor CED-1, important in homeostasis and innate immunity of *C. elegans* [34].

SREC-1 was obtained from human umbilical vein endothelial cells (HUVEC), but its expression has been reported in multiple cells, including epithelial cells [30], sinusoidal endothelial cells [33, 35], dendritic cells, B-1 cells. [36] and macrophages [35, 36]. It is important to mention that almost all studies focused on the functionality of this receptor have used transfected cell lines, designed to express the receptor extracellularly in vitro, and some studies have used cells that naturally express the SREC-1 receptor in vivo [33]. Furthermore, the first reports of the expression of this receptor showed high transcriptional expression in multiple human tissues such as spleen, lung, heart, liver, and kidney and it has been corroborated in murine tissues [33, 36]. However, more studies are needed to investigate its expression and cellular distribution at the protein quantity in these tissues. So far, there is only one study that has fully characterized the cellular distribution and expression of the SREC-1 receptor in healthy and chronically ill human liver [35]; therefore, much remains to be studied to understand the activity of this receptor in human cells and tissues [33].

2.3 Individual host factors favoring nasal colonization of *S. aureus*

Some studies have found that *S. aureus* nasal carriers are more common in people infected with the Human Immunodeficiency Virus (HIV) [37] and obese patients [38], compared to healthy individuals. Nowak et al. [39] published the positive correlation between percentage of body mass and susceptibility to colonization by *S. aureus* in healthy male individuals. This high prevalence was also reported in diabetic dialyzed patients, compared with non-diabetic patients [16]. Other diseases such as granulomatosis with rheumatoid arthritis, skin and soft tissue infections [40], atopic dermatitis, and recurrent furunculosis have been associated with an increased carrier rate [16].

Contrary to what was reported by Nowak et al. [39], Liu et al. [41] found similar percentages of carriers in women and men, however, men had a higher density of bacteria. To date, it has not been confirmed that hospital workers are at increased risk of being nasal carriers of *S. aureus* compared to the rest of the population [42, 43]. The association between nasal carriers of *S. aureus* and

smoking is controversial, Olsen et al. [44], reported that healthy active smokers are protected from becoming carriers of *S. aureus*, due to the possible antibacterial activity of tobacco. However, another experimental inoculation study showed that smokers are colonized more frequently than non-smokers and that quitting smoking improves clearance of *S. aureus* nasal [45]. Other host pathologies, such as hormonal contraception [46], have also been extensively studied, and the presence of hemoglobin in nasal secretions has been reported as an additional predisposing factor [16].

Regarding host genetics, no significant heritability data has been detected for nasal colonization of *S. aureus* in twins and family studies [47, 48]. However, some polymorphisms have been found in genes involved in inflammatory processes and have been associated with the carriage of *S. aureus* in the nose, for example, the phenotype of the histocompatibility antigen HLA-DR3 could be a predisposition [16].

The host cell presents modified carbohydrates and secretes surface proteins, such as blood group antigens, which are involved in bacterial adhesion and colonization. An investigation found that people with blood group O have a 6.5 times higher risk of being carriers of *S. aureus* in the pharynx, compared to people with blood group A [9].

2.4 Overview of the pharynx

The pharynx is a muscular chamber that serves the respiratory and digestive systems to receive air for the nasal cavity and food and water for the oral cavity [49]. The oropharynx consists of five layers: mucosa, submucosa, pharyngobasilar fascia, constrictor muscle, and oropharyngeal fascia [50]. The pharynx has stratified non-ciliated epithelium that secretes mucus with mucin. Specifically, the posterior wall of the oropharynx (and the soft palate) is lined by a nonkeratinized stratified squamous epithelium, supported by an underlying lamina propria and a muscular layer. In the palatal and lingual tonsil regions, there are nodules of lymphoid tissue located below the epithelium, each tonsil is in a fixed position, in other regions there are membrane-associated lymphoid tissue [MALT], found throughout the body. The structural support is mainly provided by reticular fibers composed of type III collagen. These fibers condense and combine with elastin fibers to form septa that dissect the tonsillar parenchyma [50]. *S. aureus* can bind to multiple ligands of the pharynx such as collagen, fibronectin, fibrinogen through adhesin proteins such as Cna, FnBa, FnBb, among others (**Figure 4**) [16].

The oropharynx links the mouth, nasopharynx, lower respiratory tract, and gastrointestinal tract and is always exposed to a large number of microorganisms, both exogenous and endogenous. The set of species to be studied is wide, since there are very diverse bacterial communities in both adults and the elderly. The pharynx is also a niche for pathogenic bacteria that can cause localized (pharyngitis) or disseminated disease (primarily lung disease or systemic if spread) [51].

2.4.1 Importance of the study of S. aureus *in the pharynx*

The pharynx has recently been identified as a potential colonization site for *S. aureus*, this colonization can occur in the presence or absence of nasal colonization [52]. Being a carrier of *S. aureus* in the pharynx has potentially important implications in decolonization strategies for populations at high risk of infection, it is unlikely that topical drugs aimed at eradicating nasal colonization affect transport in the throat, therefore which could be an important focus in future infections if *S. aureus* persists in the pharynx [53].

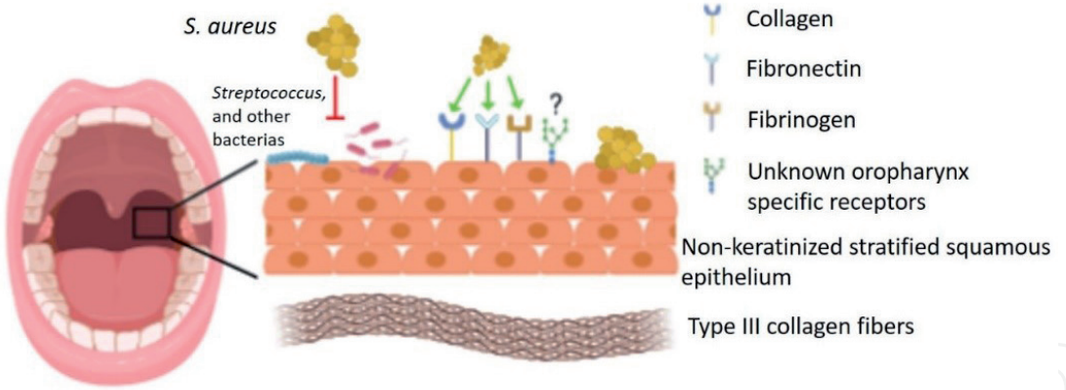

Figure 4.
Colonization of S. aureus *in the human pharynx.* S. aureus *Competes directly with bacteria that predominantly colonize the pharynx; it is known that bacteria such as* Streptococcus pneumoniae, Streptococcus mutans, Streptococcus mitis, *among others, inhibit the growth of* S. aureus. *Regarding the colonization mechanism, so far only the same ones as in the nose have been studied, for example, their binding to fibrinogen, fibronectin, collagen, among other proteins expressed in both anatomical sites, however, they have not been analyzed in depth its specific interactions with the pharynx.*

Most of the *S. aureus* detections, in particular MRSA, are only made from nasal swabs, since the pharyngeal swab is not considered a standard, Mertz et al. [54] mention that some drawbacks of pharyngeal exudate is causing discomfort to the patient and that it increases the cost to the health care system without significantly greater sensitivity. However, as mentioned above, pharyngeal colonization may be more common than nose colonization than has been published.

The rate of nasal carriers of MRSA in hospital patients has been found to be 5.9 to 15.6%, however, the rate of pharyngeal MRSA carriers is between 10 and 23.1%, which represents a greater number of carriers in the pharynx [55].

Cirkovic et al. [56] conducted a study of carriers and genetic diversity of MRSA in 195 hospitalized patients and 105 workers of a University Hospital in Serbia and reported a rate of 32.2% of exclusive MRSA carriers in the nose and another 32.2% of exclusive carriers of the pharynx, in addition of 35.5% of MRSA carriers in both sites, so the exclusion of pharyngeal exudates would result in a significant error in a substantial part of this work, since around a third are MRSA carriers exclusive to the pharynx [12, 13].

2.4.2 Interaction of S. aureus *with the microbiota*

Pathogenic bacteria can coexist with their host in two ways, as harmless microbiota microorganisms or as invading pathogens that enter healthy tissues after overcoming innate defense mechanisms or if the immune system is compromised [57]. Despite microbiology studies regarding infection mechanisms, ecological studies of pathogenic microorganisms present in the human microbiota are still lacking [58].

In vitro and in vivo studies that simulate colonization by *S. aureus*, as well as analysis of microbiomes and metagenomes reveal that the nose presents an intermediate level of bacterial diversity compared to the human oral cavity and intestine, but they present greater diversity than the vagina, which has less diversity. A percentage of the human population carries several bacterial species in the nose, such as *Finegoldia magna*, *Dolosigranulum pigrum* and *Salmonella* spp., are negatively correlated with colonization by *S. aureus* [41, 59]. It is not known how the nasal microbiota can prevent *S. aureus* colonization. Understanding this mechanism can help to understand why about 30% of the human population is persistently colonized by *S. aureus*, another 30% is highly resistant to nasal colonization by *S. aureus*, and the remainder are considered intermittent carriers [58].

2.4.3 Nasal microbiota

The nasal cavity of humans harbors a diverse bacterial community that is in principle stable at the gender level [41, 59, 60], but may vary between individuals and with season [61] In the same way, other places in the human body that are exposed to the environment are the skin and the oropharynx [61–63]. The dynamics of the nasal microbiota have not yet been analyzed. The analysis of the nasal microbiota is performed by amplification and sequencing of the 16S rRNA gene, that in many cases it is not possible to distinguish between species, therefore should implement shotgun metagenome sequencing techniques [58].

Many species of nasal bacteria are anaerobic [64], indicating that part of the nasal epithelium is barely exposed to air in the nasal cavity. Many species cannot be cultivated in vitro, they require special growth conditions [58].

Bacteria in the nose belong mainly to three phyla (Actinobacteria, Firmicutes, and Proteobacteria) and 80% humans or more are colonized with *Corynebacterium* spp., *Propionibacterium* spp., and *Staphylococcus* spp. [62, 65, 66]. Other genera are found less frequently [41].

Based on the abundance of characteristic species in the human nose, seven types of community status (CST) have been defined. (CST), each of which represents a nasal bacterial community dominated by *S. aureus* (CST1), Enterobacteriaceae (*Escherichia* spp., *Proteus* spp., *Klebsiella* spp. and others; CST2), *S. epidermidis* (CST3), *Propionibacterium* spp. (CST4), *Corynebacterium* spp. (CST5), *Moraxella* spp. (CST6) or *Dolosigranulum* spp. (CST7) [41]. *S. aureus* was found in several CSTs, although much less frequently than in CST1 [58].

During the first years of life, the microbiota of the respiratory tract develops [67, 68]. The oral microbiota is similar to that of the skin, but is less similar to that of the oral cavity. So the nose could be an intermediate step between these two niches [62]. The microbiota of the sebaceous and moist areas of the skin is more similar to that of the nose than to the dry areas of the skin [61]. *Streptococcus spp.* is abundant in the oral cavity, but it is found in low numbers in the nose [69]. Species of Coagulase-negative *Staphylococcus* (SCN), such as *Staphylococcus capitis*, *Staphylococcus warneri*, *Staphylococcus hominis*, and *Staphylococcus lugdunensis*, are prevalent on the skin, but they are only found in the nasal cavity of some people, with the exception of *S. epidermidis*, which colonizes both the nose and the skin of most people [58].

2.4.3.1 Factors influencing composition of the nasal microbiota

Bacterial communities in the nose undergo seasonal variations, mainly during the change from winter to spring [70]. The environment plays an important role in the composition of the nasal microbiota as factors such as humidity, temperature, dust or pollen influence [41, 70]. Furthermore, smoking has been shown to prevent nasal colonization by *S. aureus* [44], although it is still under discussion [45]. Some studies have shown that the gender and genetic composition of the host have a moderate influence on the colonization of *S. aureus*, however the type of nasal microbiota seems to be an important factor for the colonization of this bacterium [41, 48]. Furthermore, nasal colonization by *S. aureus* hardly varies between humans of different ethnic and geographic origins [70]. The various regions of the nose, from the anterior vestibule to the sphenoethmoidal recess in the posterior nasal cavity, are lined with mucus, thus differing in the composition of the microbiota between individuals [60].

Metagenomic studies have shown the importance of the role of bacteriophages in the dynamics of the microbiota of the skin and intestine [71]. In the human nose

the abundance and diversity of bacteriophages have not yet been analyzed, they can alter the nasal microbiota. Bacteriophages are one of the main mechanisms for horizontal transfer of virulence and antibiotic resistance genes between staphylococci and other bacteria, which can contribute to the appearance of new strains [72].

2.4.3.2 Competition for nutrients

Unlike the gastrointestinal microbiota, bacteria in the nasal cavity do not interact with food in the diet and can only acquire nutrients that are excreted by cells, so nasal secretions contain few nutrients [73] and are has hypothesized that bacteria in the nasal microbiota compete for scarce nutrients. Nasal secretions contain NaCl in physiological concentrations (~ 150 mM) and low levels of potassium, magnesium, and phosphate. Carbohydrates, amino acids, and other nutrients are found in nasal secretions in much lower amounts than those found in plasma [58]. There is a synthetic nasal medium (SNM), containing nutrients in the same amounts as in nasal fluid, allowing *S. aureus* to develop, but most SCNs cannot grow under these conditions or grow very slowly [73]. This would indicate that most of the SCNs present in the nose are only transitory and are not a permanent colonization site for these bacteria. Many of the genes involved in the nutrient absorption systems and anabolic metabolic pathways in *S. aureus* are highly expressed in NMS or in the nose [73]. *S. aureus* is successful when it competes with other nasal microorganisms as it depends on its ability to grow in low amounts of nutrients. Nasal bacteria do not always compete with other bacteria for nutrients, in certain cases they can also cooperate with others to obtain specific nutrients, such as *S. aureus* and *Corynebacterium accolens* that seem to have a mutualistic relationship [60].

The main carbohydrate in nasal fluids is glucose and it is found in low concentrations (between 35 μM and 1 mM, with an average value of ~370 μM) [73] which depends on the nutritional status of the person. The colonized by *S. aureus* is higher in diabetic people, perhaps due to high concentrations of nasal glucose. Sialic acids, which line the membrane of eukaryotic cells, can be an important source of energy for bacteria in the nasal cavity [58]. Many bacteria can metabolize sialic acids how *S. aureus* and some SCNs including *S. intermedius, S. lugdunensis*, and *S. saprophyticus*, but not *S. epidermidis*, can absorb and use sialic acid [74].

S. aureus is the only one of the staphylococci that can degrade the main phosphatidylcholine group that is released from eukaryotic cells, extracellular glycerophosphocholine (GroPC). *S. aureus* can grow with GroPC as the sole carbon source so it can survive in limited nutrient conditions [58].

Nasal fluids contain several of the 20 amino acids necessary for protein biosynthesis in concentrations between 10 μM and 250 μM; however, several such as methionine, tyrosine, aspartate, asparagine, glutamine, and isoleucine are found in very low concentrations [73] so they need to be synthesized by nasal bacteria. This was verified since a mutant of *S. aureus* that presents a deficiency in the synthesis of methionine was isolated and this affected its growth in the cotton rat nasal colonization model [14, 73]. In certain sites of the nose, the concentrations of amino acids and peptides may be higher, *S. aureus* and other nasal bacteria secrete proteases that can degrade host proteins, which are found in high concentrations in nasal fluids, such as albumin, lactoferrin, mucins, cytokeratin 10, and hemoglobin [75]. *S. aureus* produces approximately ten extracellular proteases, which makes it different from most other nasal bacteria that do not produce extracellular proteases or produce only a few [76].

Nasal discharge, in addition to being low in nutrients, is also poor in essential metal ions such as zinc, manganese, iron, and other host proteins such as calprotectin and lactoferrin, which sequester these ions from the nasal cavity to prevent

bacterial growth [75]. Because of this, the microbiota needs specific mechanisms to compete with the host's defense, known as "nutritional immunity" [58].

When the growth of nasal bacteria slows due to a lack of nutrients, some bacteria produce antimicrobial substances to inhibit competing bacteria, these antimicrobial substances are normally ribosomally synthesized and post-translationally modified peptides (RiPP) or nonribosomal peptide-synthetase (NRPS) enzymes. Bacteria that produce these molecules are protected from specific immunity. Antimicrobial RiPPs (called bacteriocins) sometimes have a limited range of activity and are produced against specific groups of nasal bacteria. Most bacteriocins in nasal bacteria show changes such as thiazole heterocycles, lanthionine bridges (lantibiotics), and oxazole (microkines) or pyridine rings (thiopeptides) [77]. There are not many reports of bacteriocins in bacterial species isolated from the human nose [78].

Nasal strains of *Staphylococcus* spp. were studied for antimicrobial substances and found to be produced with a high frequency (86%) and a wide diversity of activities against groups of nasal bacteria. Due to this, bacteriocins can play a very important role in the formation of the nasal microbiota. Most members of Firmicutes and Proteobacteria are unaffected by the inhibitory activities of staphylococci, except *Dolosigranulum pigrum* and *Moraxella catarrhalis*. However, some bacteria of the phylum Actinobacteria, such as *Corynebacterium pseudodiphtheriticum* and *Micrococcus luteus*, were inhibited by staphylococcal bacteriocins [79]. These susceptible bacteria may be the main competitors of nasal staphylococci [58]. Most of the staphylococcal genes used in bacteriocin biosynthesis are found in mobile genetic elements forming part of plasmids or on the bacterial chromosome, which undergo extensive genetic rearrangements and horizontal gene transfer [79].

Bacteriocins have a wide variation in amino acid sequence, which can cause changes in the spectrum of activity [80, 81]. Due to this, the evolutionary process could have increased and changed the type of nasal bacteria, causing changes in the composition of the microbiota. This is the case with thousands of genes that produce secondary metabolites, many of which are potential antimicrobial substances. In investigations of the human metagenome of different surfaces of the human body, it was found that antimicrobial peptides can play an important role in the maintenance of the human microbiota [81]. In an investigation of nasal strains, *S. epidermidis* strains were found to be the main antimicrobial-producing bacteria, while *S. aureus* rarely produces these substances. The production of various antimicrobials is favored by stressful conditions during colonization, such as iron limitation or the presence of hydrogen peroxide (H_2O_2), indicating that many antimicrobials are tightly regulated [79].

Most staphylococcal bacteriocins are inactive for *S. aureus*. But, *S. lugdunensis* can synthesize an antimicrobial compound called lugdunin that inhibits and kills *S. aureus*. Lugdunin is encoded by the bacterial genome and is the first identified antimicrobial NRP produced by a human commensal bacterium, and represents a new class of cyclic peptide antibiotics containing thiazolidine. The lugdunin production operon is present in almost all nasal strains of *S. lugdunensis*, allowing this bacterium to compete with and kill *S. aureus*. People colonized by *S. lugdunensis* have a six times lower risk of being carriers of *S. aureus* than people who are not colonized [82]. Similarly, SCNs that produce certain bacteriocins can prevent *S. aureus* from colonizing the skin in patients with atopic dermatitis [83]. These results show the importance of the production of antimicrobial compounds by commensal bacteria that can prevent colonization by pathogenic bacteria such as *S. aureus* [58]. Another enzyme called lysostafin that is produced by *Staphylococcus simulans* could degrade the cell wall of multiple species of staphylococci, including *S. aureus*, by hydrolyzing the pentaglycine bonds that bind peptidoglycan [84].

Other bacteria in the nose use indirect methods to inhibit the growth of competing bacteria, such as *S. pneumoniae*, which is found mainly in the throat and rarely in the nose, but colonization by *S. pneumoniae* prevents colonization of the nose by *S. aureus* [58]. Possibly, this inhibition may be due to the release of hydrogen peroxide, a metabolite produced by *S. pneumoniae* that produces the generation of free radicals that damage DNA, and which also activates the prophages contained in the genome of *S. aureus* strains, which that causes the lysis of bacteria [85]. Also, *S. pneumoniae* can interfere with *S. aureus* in other ways, such as by inducing cross-reactive antibodies that prevent *S. aureus* colonization [86]. Viridans group streptococci (*S. mitis*, *S. sanguis*, *S. oralis*, *S. mutans*, and *S. sobrinus*) have also been found to prevent MRSA colonization of the pharynx in newborns, due to bacteriocin activity of peroxidase type [87].

2.5 Pharyngeal microbiota

In the nasal, oral and pharyngeal human cavities live hundreds of microbial species, including between 25 and 40 families archeas, bacteria, amoebae and fungi, as evidenced in a wide range of cultures. The number of newly discovered species has increased considerably due to the discovery of noncultivable species [88]. A data published in the Human Microbiome Project (HMP), 5 main bacterial phyla have been identified in the pharynx: Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria and Actinobacteria. Interestingly, the pharyngeal microbiome is distinguished from other parts of the body (intestines, skin and vagina) by having more Bacteroidetes. The proportion of bacterial phyla in the pharyngeal microbiome comprises 27% of Bacteroidetes and only 10% of Proteobacteria, compared to the salivary microbiome, whose proportion corresponds to 9% of Bacteroidetes and 51% of Proteobacteria. These two phyla are mentioned because they are the main pathogens in human infections, for example, periodontitis is caused by Bacteroidetes and the most common Gram-negative pathogens (*Acinetobacter*, *Moraxella*, *Pseudomonas*, *Haemophilus*, *Klebsiella*, and *Legionella* spp) [89]. However, the two genera that dominate the micro-ecosystem of the pharynx are *Streptococcus* and *Prevotella* [90].

2.5.1 The pharyngeal microecosystem

The most common bacterial genera in the human pharynx are *Prevotella*, *Capnocytophaga*, *Campylobacter*, *Veillonella*, *Streptococcus*, *Neisseria*, *Haemophilus*, which represent 9.72% to 1.26% of the bacteria in the normal microbiome, according to HMP data. [89, 90]. As there are few studies of the pharyngeal microbiome, many interactions between the components of the microecosystem are not clear, however, the interactions of microorganisms with factors of the local environment are characteristic of the microbiome. From the above, it can be assumed that the pharyngeal microbiome may share common characteristics of other human microbiomes [89].

2.5.2 Potential roles of the pharyngeal microbiome

Animals have developed strategies that allow them to evade the invasion of microbial pathogens and humans are no exception. Therefore, the one inhabited by commensal microorganisms that participate as defenders has a fundamental action to comply with these strategies. However, the role of the pharyngeal microbiome in respiratory tract infections (RTIs) is not fully understood, there is evidence to suggest a protective effect, like the gut microbiome [89].

The pharynx microbiome plays a crucial role in lining the mucosa of the respiratory tract by protecting against infections by airborne pathogens, in addition to the immune mechanisms of the host, particularly against emerging infectious agents [89, 91].

Homeostasis of the pharyngeal microbiome is necessary to prevent infections caused by native bacterial species, which allows the abundant development of each species. Many pathogenic species can adapt well to the pharyngeal ecosystem and become established in the resident microbiome, rendering the host asymptomatic (such as *S. aureus*, *H. influenza*, and *Mycoplasma pneumoniae*) [92]. In epidemiological studies it has been suggested that the proportion of resident pathogens varies seasonally, as does the incidence of RTIs attributed to them [93].

3. Conclusions

S. aureus is an important clinical pathogen for humans that has developed the ability to bind to various components of the extracellular matrix of a wide range of cells and has generated mechanisms that allow its survival and persistence in adverse conditions such as the formation of biofilms. and intracellular infection, which overwhelmingly evades the host's immune response in various human tissues.

On the other hand, it has also been possible to integrate with other important bacterial communities of the nose and skin to form part of the normal microbiome of these sites, but it can also survive in other tissues where it is not considered a normal microorganism, as is the case of the pharynx or intestines.

Although there are many studies of the colonization mechanisms and interactions of *S. aureus* in the nose, there is little information on the processes and interactions that it performs in the pharynx. Therefore, additional studies of the pharynx as a site of colonization of *S. aureus* are required.

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Conflict of interest

The authors declare no conflict of interest.

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