

Biofilm formation, multidrug-resistance and clinical infections of *Staphylococcus haemolyticus*: A brief review

Formação de biofilme, multirresistência e infecções clínicas de *Staphylococcus haemolyticus*: Uma breve revisão

Formación de biopelículas, resistencia a múltiples fármacos e infecciones clínicas de *Staphylococcus haemolyticus*: Una breve revisión

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Abstract

Coagulase-negative staphylococci (CoNS) have been associated with a range of human health issues such as medical device-related infection, localized skin infection, or direct infection, are recognized as comprising the main part of human normal microbiota and associated with severe and intensive infections, causing infections in humans, especially immunocompromised patients and neonates. *S. haemolyticus* is, after *Staphylococcus epidermidis*, the second most frequently isolated CoNS from clinical cases, notably from blood infections, including sepsis. The most important factor might be the ability to acquire multiresistance against available antimicrobial agents, even glycopeptides. It is widespread in hospitals and among medical staff, resulting in being an emerging microbe causing nosocomial infections. This review discuss aspects of *S. haemolyticus* bloodstream infections associated, virulence factors, and the ability of biofilm formation on medical devices surfaces. The great adaptability and the ability to

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survive in the hospital environment, especially on medical devices, *S. haemolyticus* becomes a crucial factor in nosocomial infections caused by multiresistant strains.

Keywords: *Staphylococcus haemolyticus*; Multidrug-resistance; Biofilm; Pathogenicity; Medical device-related infection.

Resumo

Estafilococos coagulase-negativos (ECN) têm sido associados a uma série de problemas de saúde humana, como infecção relacionada a dispositivos médicos, infecção localizada da pele ou infecção direta, fazem parte da microbiota humana normal e estão associados a doenças graves e intensivas, causam infecções em humanos, especialmente em pacientes imunocomprometidos e neonatos. Os *S. haemolyticus* é, depois do *Staphylococcus epidermidis*, o segundo ECN mais frequentemente isolado de casos clínicos, principalmente em infecções da corrente sanguínea, incluindo sepse. O seu mais importante fator de virulência é a capacidade de adquirir multirresistência contra agentes antimicrobianos disponíveis, até mesmo glicopeptídeos. É difundido em hospitais e entre a equipe médica, resultando em ser um micróbio emergente causador de infecções nosocomiais. Esta revisão discute aspectos associados a infecções da corrente sanguínea por *S. haemolyticus*, fatores de virulência e a capacidade de formação de biofilme em superfícies de dispositivos médicos. Pela grande adaptabilidade e capacidade de sobrevivência no ambiente hospitalar, principalmente em dispositivos médicos, *S. haemolyticus* torna-se um fator crucial nas infecções hospitalares causadas por cepas multirresistentes.

Palavras-chave: *Staphylococcus haemolyticus*; Multirresistência; Biofilme; Patogenicidade; Infecções relacionadas a dispositivos médicos.

Resumen

Los estafilococos coagulasa negativos (ECN) se han asociado con una serie de problemas de salud humana, como infecciones relacionadas con dispositivos médicos, infecciones cutáneas localizadas o infecciones directas. Comprenden una parte importante de la microbiota humana normal y están asociados con enfermedades graves e intensivas, causan infecciones en humanos, especialmente en pacientes inmunocomprometidos y neonatos. *S. haemolyticus* es, después de *Staphylococcus epidermidis*, la segunda especie más frecuentemente aislada de casos clínicos, principalmente en infecciones de la sangre, incluida la sepsis. Su factor de virulencia más importante puede ser la capacidad de adquirir resistencia a múltiples fármacos contra los agentes antimicrobianos disponibles, incluso los glicopéptidos. Está muy extendido en los hospitales y entre el personal médico, por lo que es un microbio emergente que causa infecciones nosocomiales. Esta revisión analiza los aspectos asociados con las infecciones del torrente sanguíneo por *S. haemolyticus*, los factores de virulencia y la capacidad de formar biopelículas en las superficies de los dispositivos médicos. Debido a su gran adaptabilidad y supervivencia en el medio hospitalario, especialmente en dispositivos médicos, *S. haemolyticus* se convierte en un factor crucial en las infecciones nosocomiales causadas por cepas multirresistentes.

Palabras clave: *Staphylococcus haemolyticus*; Multirresistencia; Biopelícula; Patogenicidad; Infección relacionada con dispositivos médicos.

1. Introduction

Staphylococcus haemolyticus is one of the most frequently coagulase negative staphylococci (CoNS) isolated from healthcare-associated infections, mainly those related to implanted medical devices. CoNS are considered as inhabitants of the human and animal microbiota, have been increasing reported as relevant nosocomial pathogens, especially due to microbial biodiversity and virulence mechanisms, involving resistance to antimicrobial agents (Heilmann et al, 2019; Al-Tamimi et al, 2020; Wolden et al, 2020). It is an increasing cause of nosocomial infections associated with indwelling medical devices, particularly affecting immunocompromised patients and neonates (Pain et al, 2019).

In recent years, CoNS has been reported as pathogenic causes of infections in human and veterinary medicine, especially in immunocompromised patients, critically ill, long-term hospitalized and in those harboring invasive medical devices such as catheters (Pereira-Ribeiro, et al 2019; Eltwisy et al, 2020). Clinicians and microbiologists are frequently confronted with determining whether retrieved CoNS are contaminants that intrude during sampling or sample processing, are regular skin or mucous membrane commensals, or are clinically relevant, because many CoNS species form part of the skin and mucous membrane microbiota, the line between innocuousness and pathogenicity may be indistinct and comes down to virulence strategies employed by the various species as well as host defense mechanisms. Although, today the CoNS are recognized as causative agents of clinically significant infections (Becker et al, 2014; Sued-Karam & Pereira-Ribeiro, 2022).

The advancement of diagnostic techniques has increased to understanding of the molecular mechanisms of pathogenicity. The identification is important to distinguish between coagulase-positive staphylococci and CoNS as a basic principle, a practice carried out in most laboratories by the slide and tube coagulase tests. Identification down to species level is important particularly for species suspected to cause infections and informs accurate clinical decision making by clinicians. Therefore, the identification of CoNS species is important in detecting exact of the causes of infection in environment nosocomial and fast identification methods are required for effective diagnoses and prompt treatment (Bouchami et al, 2011; Asante et al, 2020). Speciation with biochemical methods is gradually being replaced by molecular methods such as polymerase chain reaction (PCR) and spectrometric methods, such as matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Molecular methods such as PCR affords higher accuracy, typeability, and reproducibility and are therefore recommended in CoNS speciation. MALDI-TOF MS as an identification technique also has a useful application in toxin detection and has been shown to exhibit high accuracy and reproducibility when used to identify CoNS. Furthermore, MALDI-TOF has been reported as unable to detect rare species in close to 50% of cases (Bouchami et al, 2011; Becker et al, 2014; Pereira-Ribeiro, et al, 2019).

In several cases where rare species are suspected, sequencing of phylogenetically important target genes such as the 16S rRNA gene can prove useful. Certainly, amplification and sequencing of target housekeeping genes such as elongation factor Tu (*tuf*) gene, RNA polymerase B (*rpoB*), and 16S rDNA gene have been shown to assist in discriminating between *Staphylococcus* species and subspecies with varying levels of discriminatory power. The multiple approach to pathogen identification improves the accuracy of identification up to species level, such as *Staphylococcus haemolyticus* that has high resistance to antimicrobials, thus helping the clinician in a more effective treatment (Lamers, et al, 2012, Asante et al, 2020).

This review aims to briefly discuss aspects of *S. haemolyticus* bloodstream infections associated, their clinical relevance and virulence factors, as well as the ability of biofilm formation on medical devices surfaces.

2. Methodology

Articles were consulted to identify studies relevant of the literature review in the period of march to July of 2022, utilizing articles from the last 10 years for its writing, although articles most relevant in the last 5 years. Articles in Portuguese and English were included in this study. Databases were search from Scielo and Pubmed, Search terms included: “coagulase-negative *Staphylococcus*”, “*Staphylococcus haemolyticus*”, *Staphylococcus* biofilm and resistance”, “*Staphylococcus haemolyticus* biofilm-formation” and “*Staphylococcus haemolyticus* multiresistance”. The Table 1 shows the number of articles included and excluded for this study and the term used to search for articles.

Table 1 – Selection of the articles.

Database	Descriptors	Numbers of results	Number of selected articles	Articles Excluded After Reading	Remained in the study
Scielo	coagulase-negative <i>Staphylococcus</i>	22	15	13	2
	<i>Staphylococcus haemolyticus</i>	3	2	1	1
Pubmed	coagulase-negative <i>Staphylococcus</i>	906	55	40	15
	<i>Staphylococcus haemolyticus</i>	148	42	23	19
	<i>Staphylococcus</i> biofilm and resistance	490	35	21	14
	<i>Staphylococcus haemolyticus</i> biofilm-formation	8	7	0	7
	<i>Staphylococcus haemolyticus</i> resistance	86	44	33	11

Source: Authors.

A narrative review of the literature was carried out on the impact of the clinical relevance of *Staphylococcus haemolyticus*, as well as the increase of the antimicrobial resistance of this species and the capacity for biofilm formation, making it difficult for the clinician to treat the infection by this species in hospitalized patients. The criterion for inclusion of articles was the verification of results and literature data consistent with the objective of the study. Articles that did not meet the study criteria, such as inadequate methodology or with little information, inconsistent data, and absence of relevant data for the research were excluded. The reference lists of all retrieved articles were checked for additional relevant references.

3. Results

3.1 *Staphylococcus haemolyticus*

Staphylococcus haemolyticus is one of the CoNS that inhabit the skin as a commensal. Studies of the skin metagenome have propped the long-known assertion that *Staphylococcus* species prefer areas of higher humidity such as the plantar foot region, axillae, and umbilicus (Asante et al, 2020). It is increasingly implicated in opportunistic infections in immunocompromised patients, particularly in hospitalized patients and with medical implants. *S. haemolyticus* has been the second most frequently pathogen of clinical nosocomial infections, particularly blood cultures of patients with sepsis following *S. epidermidis*, mostly reported in industrialized countries.

S. haemolyticus has caused skin and soft tissue infections, skin infections predominantly present as abscesses and paronychia and severe infections in various body systems including meningitis, endocarditis, prosthetic joint infections, pneumonia, bacteremia, septicemia, peritonitis, otitis media, urinary tract infections and is prevalent in the hospital environment and on the hands of healthcare workers. They are most common in elderly patients or those individuals who are immunosuppressed and tend to be broadly susceptible to antibiotic treatment (Silva et al, 2013; Eltwisy et al, 2020). A distinct

characteristic of clinical *S. haemolyticus* strains is the capacity to acquire resistance to several classes of antimicrobial agents and ability to colonize and form biofilms is regarded as the most important virulence factor of this specie (Cavanagh et al, 2014; Natsis et al, 2018; Eltwisy et al, 2020).

Moreover, there is increasing of infections related to implanted medical devices caused by this pathogen expressing multidrug-resistant (MDR) profiles, including sepsis also in newborns within Neonatal Intensive Care Units – NICUs (Pereira et al, 2014; Pereira-Ribeiro et al, 2019).

S. haemolyticus has also been seen in skin and soft tissue infections especially associated with hidradenitis suppurativa lesions (HSL). The HSL is a chronic, recurrent, and debilitating skin disease results from an inflammatory disorder of the follicular epithelium, associated or not with a bacterial infection (Lee et al, 2017). The hypothesis of the association of *S. haemolyticus* and HSL bacterial infection may be attributed to the higher colonization of *S. haemolyticus* in the axillary and inguinal regions with a higher concentration of hair follicles and associated apocrine glands (Hessam, 2016). Most infections caused by this group are characterized by subacute and chronic courses of infection with subtle clinical symptoms. However, more aggressive courses and sudden lethal outcomes in the case of inadequately managed patients with chronic foreign body related infections (FBRI) have been reported and are associated with significantly longer hospital and intensive care unit stays, higher mortality rates and higher hospital costs. Delayed diagnosis of CoNS infections contributes to increased mortality. Immunosuppression or an immature immune system represent further risk factors for foreign body-independent Staphylococci infections, including, preterm newborns are predisposed to invasive infections such as sepsis, endocarditis, meningitis and FBRI, leading to prolonged hospital stay, morbidity and mortality (Horasan, et al, 2011; Pereira et al, 2014; Heilmann et al, 2019).

S. haemolyticus has a feature the ability to form biofilms, which play an essential role in the establishment of infections. The produced exopolysaccharides can inhibit the growth of other bacteria and decrease their ability to form biofilms. This species has gained an increased clinical significance due to its genome plasticity, which allowed a great adaptation and development of resistance to different antibiotics, including methicillin and its ability to survive in the hospital environment. The remarkable *S. haemolyticus* ability of to acquire antibiotic resistance, especially to oxacillin, limits the available therapeutic options for catheter-related infections caused by *S. haemolyticus* methicillin-resistant isolates and may predispose to sepsis and increase patient's morbidity and mortality (Pereira et al, 2014; Ahmed et al, 2019; Pereira-Ribeiro et al, 2019; Eltwisy et al, 2020).

Further than virulence factor acquisition, staphylococci have evolved additional mechanisms to sustain within the host and even to modulate the course of an infection. Similar to *S. aureus*, *S. haemolyticus* are able to switch from a more aggressive style of infection to an intracellularly adapted lifestyle, which causes a diminished inflammatory response and facilitates chronic and relapsing infections (Tuchscher et al 2010).

3.2 *Staphylococcus haemolyticus* multidrug-resistant

The increasing number of *S. haemolyticus* in hospital-acquired infections (HAIs) is closely related to their antimicrobial resistance and the ability to survive in a hospital environment. Among all the CoNS studied, this specie is known to be resistant to most antibiotics, including cephalosporins, penicillins, macrolides, tetracyclines, quinolones, and aminoglycosides (Manoharan et al, 2020).

It is well known that the mechanism of methicillin resistance determines the resistance to all β -lactam antibiotics: penicillin, cephalosporins, carbapenems and monobactams. Methicillin resistance in staphylococci is because of the expression of a modified penicillin-binding protein, PBP2a, which is encoded by *mecA* or *mecC* genes. Research showed that most

isolates are cefoxitin resistant and also some strains are negative for the *mecA* gene, wherein resistance might have occurred due to other mechanisms such as *mecC* or hyperproduction of penicillinase, although methicillin-resistant *S. haemolyticus* isolates to be negative for the *mecA* gene. Analysis of *mecA* gene sequences in GenBank reference strains of *S. aureus*, *S. haemolyticus* and *S. epidermidis* showed 99.95% similarity, which proves the theory of the interspecies transfer of *mecA* gene (Pereira et al, 2014; Barros et al, 2015; Czekaj et al, 2015; Sued et al, 2017; Pereira-Ribeiro et al, 2019; Manoharan et al, 2020).

Horizontal gene transfer is a major source for acquisition of antibiotic resistance genes in staphylococci, facilitated by phage transduction and plasmid conjugation. Plasmids can be reservoirs and vectors for antibiotic resistance and virulence, even in the absence of selective pressure. Horizontal transfer of plasmids can occur not only between staphylococcal strains of the same species but also between species, with *S. haemolyticus* believed to act as reservoirs for antibiotic resistance genes (Mores et al, 2021).

The growing of infections for MDR bacteria strains, associated with the lack of new antimicrobial development is a global concern. It is stipulated that by 2050 antimicrobial resistance will be responsible for a global economic impact of \$ 100 trillion and approximately 10 million annual deaths (O'Neill, 2016). The increased outbreaks of antibiotic-resistant CoNS clones have been reported in ICUs and likely to become a serious problem in the future given the limited alternative therapeutic options (Rogers et al, 2009; Becker et al, 2014;)

S. haemolyticus could present a high number of enzymes that contribute to antibiotic-resistant and pathogenicity. This pathogen was the first CoNS to express decreased susceptibility to teicoplanin and vancomycin (Rogers et al, 2009). Epidemiological studies observed the teicoplanin, linezolid and oxacillin-resistant in different samples isolated from clinical sources in nosocomial environments. Treatment options for CoNS are limited because many of them are methicillin resistant. Thus, the glycopeptides (particularly vancomycin) are relied upon, especially in infections caused by the *S. haemolyticus* strains. Susceptibility patterns of this specie should be considered to inform both empiric and specific treatment (Rodríguez-Aranda et al, 2009; Pereira-Ribeiro et al, 2019).

The heteroresistance to vancomycin has also been reported in *S. haemolyticus* and is frequently associated with catheter-related bloodstream infections. Vancomycin heteroresistance in CoNS might impair the clinical response to vancomycin therapy (Bakthavatchalam et al, 2021).

The role of this antimicrobial resistance is linked to genome plasticity and the widespread use of antimicrobial in nosocomial and non-nosocomial environments that stimulate many acquisitions of mobile genetic elements, beneficial mutations in DNA, rearrangements in surface-associated gene insertion sequences in its genome. Promoting the development of MDR samples persisting in the hospital environment (Takeuchi et al, 2005; Rodríguez-Aranda et al, 2009; Rogers et al, 2009; Pain et al, 2019).

Clonal diversity in CoNS have not been given much attention as compared with *S. aureus*. Pulsed-field gel electrophoresis (PFGE) has shown that CoNS species, including, *S. haemolyticus*, are not as clonally diverse as *S. aureus*, which is characterized by a wide genomic diversity *SCCmec* typing is important for characterizing MR-CoNS clones in epidemiological studies (Vanderhaeghen et al, 1992; Asante et al, 2020). *S. haemolyticus* was one of the first CoNS with a complete genome published by Takeuchi et al. in 2005. The increase of MDR *S. haemolyticus* clinical clones to become an emerging problem in nosocomial environments. The surveillance, the monitor of the bacteria profile isolated in the hospitals and molecular typing could be the keys to control the spread and evolution of these microorganisms (Rodríguez-Aranda et al, 2009; Pain et al, 2019).

The common antibiotics used for the treatment of the infections caused by CoNS are penicillin, oxacillin, ciprofloxacin, clindamycin, erythromycin, gentamicin, and vancomycin. Linezolid is an oxazolidinone group of antibiotics

with activity against Gram-positive bacteria. It is used for the treatment of serious infections caused by Gram-positive bacteria resistant to other antibiotics, Linezolid is approved as an alternative drug to be given for catheter-related bloodstream infections. In earlier studies, linezolid-resistant staphylococci have been reported increasingly all over the world (Grupta et al, 2020). Several potential resistance genes were found in *S. haemolyticus* genome, such as *norA*, *norB*, *van*, *cfr*, *lmrB* (efflux pumps), *blaZ* (β -lactamase), *fusB* (fusidic acid resistance EF-G-binding protein), *ermA* (rRNA adenine N-6-methyltransferase) and AAC(6')-Ie-APH(2'')-Ia (bifunctional aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase) and most of the identified antibiotic resistance genes were associated with transposon elements, suggesting that the genes can be spread to other strains or species (Kim; Jang, 2017).

The glycopeptides resistance was reported since 1990 and has been considered an emergent problem. The teicoplanin resistance has become more common reported than vancomycin resistance in CoNS strains. This resistance is attributed to teicoplanin resistant operon (*tcaRAB*) formed by *tcaR*, *tcaA* and *tcaB* genes; the deletion or mutation of the genomic region of *tcaRAB* locus could promote to increase the level of teicoplanin resistance. Some *S. haemolyticus* strains present the capacity to inactivate *tcaA* gene increasing the teicoplanin resistance (Veach et al, 1990; Bakthavatchalam et al, 2017).

S. haemolyticus infections are often difficult to treat because of MDR and different pathogenicity. The resistance to oxacillin and aminoglycosides in this specie have been proposed as surrogate markers for invasiveness, while the absence of these traits indicates a commensal flora. Vancomycin has been considered the antibiotic of first choice in treating severe infections caused by methicillin-resistant CoNS. However, the increased use of vancomycin has resulted in the development of vancomycin heteroresistance in this specie, due to multiple and complex molecular mechanisms, phenotype instability, variable vancomycin selective pressure and the lack of specific genetic markers for reliable detection. Vancomycin heteroresistant strains causing bloodstream infections is a growing and unrecognized clinical concern in intensive care patients and its clinical impact are well studied in *S. aureus* and has also been reported in *S. haemolyticus*, frequently associated with catheter-related bloodstream infections (Szabo et al, 2009; Bathavatchalam et al, 2021).

3.3 Adherence and/or biofilm formation in medical devices

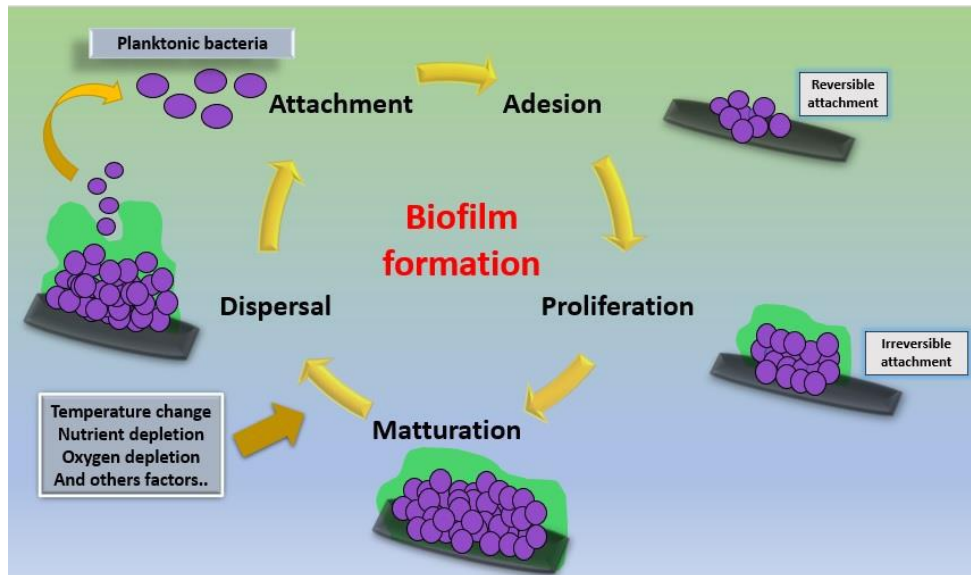
Staphylococcus haemolyticus is one of the most frequently CoNS isolated from healthcare-associated infections, mainly those related to implanted medical devices, whose the colonization and infections by resistant strains are associated to contamination during the placement of medical devices in hospital environments and breaking of aseptic barriers, causing morbidity and mortality in patients. The ability to adhere and colonize implanted biomaterials in addition to biofilm formation is considered the main virulence factors of this specie. It is an increasing cause of nosocomial infections associated with indwelling medical devices, particularly affecting immunocompromised patients and premature babies (Rodríguez-Aranda et al, 2009; Rogers et al, 2009; Natsis et al, 2018; Argemi et al, 2019; Pereira-Ribeiro et al, 2019; Eltwisy et al, 2020; Wolden et al, 2020).

The ability to colonize the surfaces of medical devices and biofilm formation is the critical *S. haemolyticus* pathogenicity factor (Sued et al, 2017; Argemi et al, 2019; Pereira-Ribeiro et al, 2019). A great number of different institutions make use of biotic and abiotic medical devices in health care and related services, including inpatient and outpatient care, diagnostic or therapeutic services, laboratory services, medicinal drugs, nursing care, assisted living and microbiological research (Aronson et al, 2020).

Biofilm-associated infections are persistent and difficult to treat. The biofilms represent a perfect structure to protect the microorganisms to the host immune response, the action of antibiotics, and to contributing to environmental agents. The steps of biofilm formation start with the attachment of bacteria to the biotic or abiotic surface, following to the proliferation and expression of intercellular adhesion traits leading to the formation of multilayered cell clusters; maturation of the biofilm

and dissolution and dissemination with the blood stream leading to infection in other regions on the body, where after initial attachment of staphylococci, they multiply and grow into multilayered cell clusters requiring intercellular adhesion. Intercellular adhesion is mediated by biopolymers, such as polysaccharides and proteins (**Figure 1**) (Heilmann et al, 2019; Pereira-Ribeiro et al, 2022).

Figure 1: General steps of *Staphylococcus sp.* biofilm formation.



Source: Authors.

Biofilm formation also plays a role in the transmission of infection by *S. haemolyticus*, that is the second most frequently isolated CoNS that colonize different parts of human bodies, causing serious infections in patients with implanted devices associated with nosocomial infections and related to biofilm formation and high levels of antimicrobial resistance. In most chronic infections, bacteria exist in biofilm communities rather than in a planktonic form to adapt in the direction of unfavorable conditions. A broad spectrum of microorganisms, including most pathogens, will form biofilm by attaching to biotic or abiotic sites to prolong their survival. Biofilms may be formed on abiotic surfaces of medical devices or on biotic surfaces, such as host factor coated foreign material or host tissue. Within the biofilm, bacteria are protected against the patients' immune system and antibiotic therapy, thus explaining why biofilm-associated infections are extremely difficult to eradicate (Costerton et al, 2005, Pereira et al, 2014; Feßler et al, 2017).

S. haemolyticus has a potential to adhere and form biofilms on tissues, implants and catheters. A biofilm can be produced by a single species or by multiple species, including fungi, bacteria and virus, embedded in a secreted extracellular matrix. This complex biofilm matrix acts as a protective layer allowing bacteria to be more resistant to antibiotics and disinfectants (Chandra et al, 2001; Pais-Correia et al, 2010; Yong et al, 2019).

The contamination for CoNS in medical devices is a reality, especially in patients with the catheter use. Studies report this contamination as an emergent problem in hospital environments, due to a multi-drug resistance (MDR) profile of clinical samples isolated and low drug alternatives to antimicrobial therapy, however infectious diseases involving biofilms are difficult to treat (Rogers et al, 2009; Asaad et al, 2016; Argemi, et al 2019). Some of the mechanisms involved in the resistance of bacteria in biofilms to antimicrobials differ from the mechanisms responsible for the resistance of planktonic bacteria to the same antimicrobials. Biofilm resistance to antimicrobials is multi factorial and there are several resistance mechanisms that act together to provide increased biofilm resistance. Biofilm growth of staphylococci also increased horizontal transfer of plasmid-

carried antibiotic resistance and heritable antibiotic resistance through spontaneous mutation (Savage et al, 2013; Yong et al, 2019).

As part of the early biofilm maturation process, the bacteria also start to produce extracellular polymeric substance (EPS), which eventually forms the biofilm matrix. EPS mainly consists of polysaccharides, proteins, teichoic acids, and eDNA. In polysaccharide-dependent biofilms, the main EPS component is polysaccharide intercellular adhesin (PIA). The production of PIA is dependent of the intracellular adhesion operon (*icaADBC*). The *ica* locus, constituted by *icaR* (regulador/repressor) and *icaADBC* (biosynthetic) genes, is essential to biofilm formation and is induced by anaerobiosis through the staphylococcal respiratory response regulator (*SrrAB*) but they can also be regulated by other environmental factors, such as glucose, ethanol, osmolarity, temperature and some antimicrobials, such as tetracycline. These genes are responsible for polysaccharide intercellular adhesin (PIA) expression, also known as poly-N-acetylglucosamine (PNAG), essential for cell–cell adhesion. When associated to surface proteins, they form and fix the biofilm (Schilcher; Horswill, 2020; Silva-Santana et al, 2020).

Staphylococcus haemolyticus is commonly identified as one of the CoNS that takes virulence genes, such as, *icaADBC*, *aap*, *atl*, *fbp*, *bap*, *fmb*, *sesB* are involved in biofilm formation. Data showed a correlation with the presence of *icaA*, *aap*, *atl* and *fbp* genes colonizing abiotic surfaces that the process of biofilm formation is complex and may not be related to the *icaA* gene (Pereira-Ribeiro, 2019; Yong et al, 2019).

The adhesion on biotic surfaces is associated to express of a variety of surface proteins, being fibronectin-binding protein (FnBP) one of the most important. The capacity to express this protein is associated with the presence of *fmbA* and *fmbB* genes. The presence of this protein is crucial to adhesion on biotic medical devices, tissues, invasion, and internalization on the host cell (Eltwisy et al, 2020).

A study demonstrated the correlation of the concomitant presence of resistance gene and high number of virulence genes associated to biofilm formation and adhesion surface protein in different clones of *S. haemolyticus* isolated from catheter-related bloodstream infections in neonates and adults, suggesting the correlation of the biofilm production and adhesion profile with the antimicrobial resistance (Pereira-Ribeiro, 2019).

4. Conclusion

S. haemolyticus has a great ability to survive in the hospital environment, especially on medical devices, becomes a crucial factor in nosocomial infections caused by multiresistant staphylococci. This pathogen can exchange resistance genes and enhance the biofilm forming ability for spreading of infections. The important function of *S. haemolyticus* as reservoirs for antibiotic resistance and virulence traits is still an underestimated issue that requires more scientific attention. This may also have practical consequences for hospital hygiene by including resistant and virulent CoNS isolates into future infection control and surveillance measures. Further studies are, however, required to understand the genetic constitution of *Staphylococcus haemolyticus* in contributing to biofilm formation. More research is needed to clarify the mechanisms of resistance and biofilm formation of this specie, as well as the recognition of health professionals, especially in the correct hand washing, handling of medical devices and inappropriate use of antimicrobials.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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