T.C.
SAKARYA UNIVERSITY
INSTITUTE OF HEALTH SCIENCE

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON BIOFILM FORMATION IN STAPHYLOCOCCI ISOLATED AS THE CAUSATIVE AGENT OF BACTERIEMIA

M.Sc. THESIS

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"This thesis was accepted unanimously by the jury below on .... / .... / 20...."

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DECLARATION

This study was conducted by T.C. Sakarya University, Institute of Health Sciences. Approved by the Ethical Committee of the Faculty of Medicine / University of Sakarya on the date 30.11.2021, This thesis is completely my work, from idea to end, I have no unethical behavior at any time, all information in the thesis is academic and scientific, all information and the comments you got are consistent with the ethical rules obtained through the sources, mention the source and include it in the list of sources, as well as work and write the thesis. I acknowledge that there has been no infringement of patents and copyrights.

Radhwan Abdulrazzaq KHALEEL

../.../....

APPRECIATION

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APPROVAL

The study proposal was approved by the Ethics Committee/Faculty of Medicine/Sakarya University on the date 30.11.2021, Clinical isolates of bacteria were obtained from Sakarya Training and Research Hospital, evidence of epidemiological studies was collected from clinical reports, tests were conducted and data were collected and results were analyzed.

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GLOSSARY

Aae **:** Autolysins/adhesins

Aap **:** Aggregation-associated proteins

AD **:** Atopic dermatitis

Agr **:** Additive gene regulator

AIP **:** Auto-inducible peptide

AMP **:** Antimicrobial proteins

AMPs **:** Antimicrobial proteins

AMR **:** Antimicrobial resistance

AT **:** Antitoxin

AtlE **:** Autolysins

CA-MRSA **:** Community-acquired methicillin-resistant *S. aureus*

can **:** Collagen binding protein

ccr **:** Cassette chromosome recombinant

CF **:** Cystic fibrosis

Clf **:** Clumping factors

CoNS **:** Coagulase-negative *staphylococci*

CoPS **:** Coagulase-positive staphylococci

CV **:** Crystal Violet solution

CWA **:** Cell wall anchoring proteins

CWP **:** Cell wall-mounted proteins

DNase **:** Deoxyribonuclease

DW **:** Distilled water

ebps **:** Elastin binding protein

eDNA **:** Extracellular DNA

Embp **:** Extracellular matrix-binding protein

EPS **:** Extracellular polymeric substances

EVs **:** Extracellular vehicles

Fib **:** Fibrinogen binding proteins

Fnb **:** Fibronectin-binding proteins

HA-MRSA **:** Healthcare associated MRSA

HBP **:** Homologous biofilm protein

Ibp **:** Laminin binding protein

ica **:** Intercellular adhesion

IE **:** Infective endocarditis

IPA **:** Intercellular polysaccharide adhesion

LA-MRSA **:** Livestock-associated MRSA

LukED **:** Leucocidin ED

MR-CoNS **:** Methicillin resistance -CoNS

MR **:** Methicillin resistance

MRSA **:** Methicillin-resistant *S. aureus*

MS **:** Methicillin sensitive

MSCRAM **:** Microbial surface component recognizing adhesive molecules

MSSA **:** Methicillin-susceptible *S. aureus*

N **:** Number

OD **:** Optical density

PBP2A **:** Penicillin-binding protein 2A

PG **:** Peptidoglycan

PNAG **:** Poly- Nacetylglucosamine

PSMs **:** Phenol-soluble proteases and modulinos

RM **:** Restriction modification system

SasG **:** S. aureus surface

SCCmec **:** Staphylococcal cassette chromosome mec

SCVs **:** Small colony variants

Sdr **:** Serine-aspartate repeat

SE **:** Staphylococcal enterotoxins

Spa **:** Surface binding protein A

T7SS **:** Type 7 secretion system

TAs **:** Teichoic acids

TB **:** Tuberculosis

TSB **:** Tryptic Soy Broth

VBNC **:** Viable but non-culturable cells

WHO **:** World Health Organization

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SUMMARY

INTRODUCTION AND AIM*:* *Staphylococcus aureus* and coagulase-negative *staphylococci* are common cause of bacteriemia and catheter-related infections and they frequently produce biofilm. Biofilm production is a serious virulence factor and it causes treatment failure especially in patients who have indwelling catheters. The aim of this study is to determine the biofilm production in staphylococci that are isolated from clinical blood samples of the bacteriemic patients and to evaluate the effect of different aerial conditions (normal, CO_2, anaerobic) on biofilm production.

MATERIALS AND METHOD: 63 clinical isolates of different types of staphylococci were enrolled in the study. The isolates were firstly identified by MaldiTOF-MS, then antimicrobial susceptibility testing was carried out by using VITEK System. The biofilm production was evaluated in the 96-well flat-bottom microplates. *E. coli* ATCC 25922 was used as positive control and uninoculated broth was used as a negative-control. The CO_2 condition was achieved in a candle jar while anaerobic gas pack was used in a closed jar to create anaerobic condition. All tests were done three times and the mean values were determined. Biofilm production were divided into four categories which were None (0), Weak (+), Moderate (++), and Strong (+++). The results were evaluated with scientific statistics software with appropriate statistics methods and the data with a p value ≤ 0.05 was accepted as significant.

RESULTS: The number of types of staphylococci is 63 samples divided as follows: *Staphylococcus aureus* 17 samples, *Staphylococcus haemolyticus* 3 samples, *Staphylococcus epidermidis* 22 samples, *Staphylococcus hominis* 16 samples, and *Staphylococcus capitis* 5 samples. The distribution of biofilm production was as follows: under normal condition and CO_2 condition 61 of the isolates (97%) produced biofilm, while under anaerobic condition, 48 of the isolates out of 63 (76%) were positive for biofilm production. Under anaerobic condition, the ability of biofilm production of the isolates was decreased ($p \leq 0.05$). There was no significant difference between normal and CO_2 conditions in terms of capacity to produce biofilm ($p > 0.05$). Under different conditions, the capacity of biofilm formation among *S. aureus* was not

affected. But there was a significant difference among coagulase negative staphylococci ($p \leq 0.05$).

DISCUSSION AND CONCLUSION: Staphylococci are highly pathogenic opportunistic pathogens and they are significant cause of hospital and community-acquired infections. They have an extraordinary ability to encase themselves in a protective environment called a biofilm, where they can survive and cause serious and hard-to-treat infections. In this study, production of biofilm in Staphylococci was determined as a serious virulence factor evaluated under different environmental conditions. Although these data are derived from relatively small number of samples, with the evaluation of the larger sample groups and other environmental and nutritional conditions, understanding of biofilm production in Staphylococci can be clarified and new alternative treatment modalities can be developed.

KEYWORDS: *Staphylococci*, *Staphylococcus aureus*, Biofilm formation, Bacteriemia, Methicillin resistance.

ÖZET

Bakteriyemin Nedeni Olarak İzole Edilen Stafilokoklarda Farklı Çevre Koşullarının Biyofilm Oluşumuna Etkisi

GİRİŞ VE AMAÇ*:* *Staphylococcus aureus* ve koagülaz-negatif *stafilokoklar* bakteriyel ve katetere bağlı enfeksiyonların yaygın nedenidir ve sıklıkla biyofilm üretirler. Biyofilm üretimi ciddi bir virülans faktörüdür ve özellikle girişimsel kateterleri olan hastalarda tedavi başarısızlığına neden olur. Bu çalışmanın amacı, yatan hastaların klinik kan örneklerinden izole edilen stafilokoklarda biyofilm üretimini belirlemek ve farklı ortam koşullarının (normal, CO_2, anaerobik) biyofilm üretimi üzerindeki etkisini değerlendirmektir.

GEREÇ VE YÖNTEM: Çalışmaya farklı türlerde 63 stafilokok izolatı dahil edildi. İzolatlar öncelikle MaldiTOF-MS ile tür düzeyinde tanımlandı, daha sonra VITEK 2 Sistemi kullanılarak antimikrobiyal duyarlılık testi yapıldı. Biyofilm üretimi the 96-kuyucuklu, düz-dip mikroplakalarda değerlendirildi. *E. coli* ATCC 25922 pozitif kontrol olarak, bakteri ekilmemiş buyon ise negatif kontrol olarak kullanıldı. CO_2'li ortam, mumlu kavanoz kullanılarak oluşturulurken, anaerobik gaz paketi içeren kapalı bir kavanozda anaerobik ortam meydana getirildi. Tüm testler üç kez yapıldı ve ortalama değerler belirlendi. Biyofilm üretimi Yok (0), Zayıf (+), Orta (++) ve Güçlü (+++) olmak üzere dört kategoriye ayrıldı. Sonuçlar uygun istatistik yöntemlere göre bilimsel istatistik yazılımı ile değerlendirildi ve p değeri ≤ 0.05 olan veriler anlamlı olarak kabul edildi.

BULGULAR: Değerlendirilen 63 stafilokokun dağılımı, *Staphylococcus aureus* 17, *Staphylococcus haemolyticus* 3, *Staphylococcus epidermidis* 22, *Staphylococcus hominis* 16 ve *Staphylococcus capitis* 5 şeklinde idi. Biyofilm üretiminin dağılımı ise, normal ve CO_2 ortamlarda izolatların 61'i (%97) biyofilm üretti. İzolatların 63'ünden 48'i (%76) anaerobik ortamda biyofilm üretimi için pozitif çıktı. Anaerobik durumda, izolatların biyofilm üretimi belirgin şekilde azaldı ($p\leq 0.05$). Biyofilm üretme durumu açısından normal ve CO_2 koşulları arasında anlamlı bir fark yoktu ($p>0.05$). Farklı ortam koşulları *S. aureus* arasında biyofilm oluşum kapasitesi etkilenmedi. Ancak koagülaz negatif stafilokoklar arasında farklı ortam koşullarında önemli bir fark saptandı ($p\leq 0.05$).

TARTIŞMA VE SONUÇ: Stafilokoklar son derece patojenik fırsatçı patojenlerdir; hastane ve toplum kaynaklı enfeksiyonların önemli nedenidir. Bu bakteriler biyofilm adı verilen koruyucu bir ortam oluşturabilecekleri, hayatta kalabilecekleri, ciddi ve tedavisi zor enfeksiyonlara neden olabilecekleri olağanüstü bir yeteneğe sahiptirler. Bu çalışmada stafilokoklarda biyofilm üretimi farklı çevresel koşullar altında değerlendirildi ve ciddi bir virülans faktörü olarak belirlendi. Bu veriler nispeten az sayıda numuneden elde edilse de daha büyük örnek gruplarının ve diğer çevresel ve beslenme koşullarının değerlendirilmesi ile stafilokoklarda biyofilm üretiminin anlaşılmasında ve yeni alternatif tedavi yöntemleri geliştirilmesinde faydalı olabilir.

ANAHTAR KELIMELER: *Stafilokok, Staphylococcus aureus*, Biofilm oluşumu, Bakteriemia, Metisilin direnci.

1. INTRODUCTION AND AIM

Staphylococci are spherical gram-positive bacteria that are typically clustered irregularly like grapes. It is adaptable to a wide variety of mediums and metabolically active, digesting carbohydrates and generating colors ranging from white to dark yellow. Some of them are natural members of the human skin and mucous membranes' normal microflora; others induce suppuration, abscess development, a variety of purulent diseases, and even lethal septicemia. Staphylococci that cause disease frequently hydrolyze blood, coagulate plasma, and create a variety of enzymes and extracellular toxins. It is caused by a heat-stable staphylococcal enterotoxin and is the most prevalent type of food poisoning. *Staphylococcus* develops fast resistance to a broad range of antimicrobial agents, offering significant treatment problems. (Riedel et al., 2019)

The genus Staphylococcus has at least 45 species. The four most often encountered clinical isolates are *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus lugdunensis,* and *Staphylococcus epidermidis*. (Riedel et al., 2019). *S. aureus* is a positive coagulase, a characteristic that sets it apart from other species. Almost everyone will contract a staph infection at some point in their lives, and the severity of the infection varies from food poisoning or mild skin infections to serious, life-threatening infections. Coagulase-negative staphylococci (CoNS) are common human bacteria that cause infection on occasion. They are frequently associated with implanted devices such as prostheses, joints, and intravascular catheters, particularly in children, the elderly, and immunocompromised individuals. *S. epidermidis* is responsible for around 75% of these CoNS infections. Other types of Staphylococcus infections include *Staphylococcus lugdunensis*, *Staphylococcus hemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus warneri*, and other less frequent Staphylococcus infections *Staphylococcus saprophyticus* is a common cause of urinary tract infection in young women; however, it is only a rare source of infections in hospitalized patients, according to the Centers for Disease Control. The treatment of additional species is crucial in veterinary medicine. (Riedel et al., 2019) Infections caused by *Staphylococcus aureus* (often referred to as Staph. aureus or *S. aureus*) are common in both humans and animals. *Staph. aureus* is a Gram-positive

pathogenic bacteria that is a prominent cause of a variety of infectious diseases in humans and animals (Adams, 2009; Schaumburg et al. 2015). This disease spectrum includes mild skin and soft tissue infections to more serious and potentially lethal circumstances, such as blood infections (bacteremia/septicemia), which can be life-threatening (Kobayashi, et al., 2015). When *S. aureus* produces extracellular polymeric substances (EPS), often known as biofilms, it helps the bacteria withstand drugs while also decreasing their effectiveness (Kaplan et al., 2018). *S. aureus* biofilms, like any other bacterial biofilm, are composed of two distinct components: water (which accounts for approximately 97 percent of the total volume) and organic matter, which comprises EPS and microcolonies (Nazir et al., 2019). A range of polymeric components, including extracellular DNA (eDNA), proteins, and polysaccharides, make up extracellular polymeric substance (EPS), which accounts for approximately 50 percent to 90 percent of the total organic matter in biofilms (Donlan,2002; Idrees, et al., 2020). The remainder, which ranges between 10 and 25 percent, is made up of minute colony-like structures (Nazir et al., 2019).

Intercellular polysaccharide adhesive (IPA) is the primary component of EPS in *S. aureus* biofilms (Reffuveille et al., 2017). The polysaccharide component of EPS has been designated as IPA in reference to its function, namely bacterial cell intercellular adhesion, and poly-(1-6) Nacetylglucosamine (PNAG) in reference to its chemical structure. IPA is cationic in nature and is involved in colonization, biofilm development, inflammation associated with biofilms, immunological evasion, antimicrobial resistance, and phagocytosis. (Nguyen et al.,2020)

The EPS of *S. aureus* contains a collection of proteins known as aggregation-associated proteins (Aap), as well as surface binding protein A (Spa), fibrinogen-binding proteins (Fib) A and B, extracellular matrix-binding protein (Embp), amyloid fibrils, and *S. aureus* surface binding protein (SasG) (Corrigan et al., 2007; Dutta et al., 2016). Through the discovery of trans-peptidases, it was discovered that other *S. aureus* proteins covalently link to the cell wall peptidoglycan (PG) (sorting). Cell wall-mounted proteins (CWP) are what these are referred to as (Lacey et al., 2017). Up to 25 distinct CWPs, categorized as a microbial surface component recognizing adhesive molecule (MSCRAM), are found in close proximity to the iron transporter (NEAT),

the three helical bundle, and the G5-E repeat proteins (Lacey et al., 2017; Foster et al., 2014). Proteins from *S. aureus* serve a variety of activities. For instance, Aap accumulation-related protein interacts with IPA and is involved in the maturation of biofilms (Reffuveille et al., 2017). The SasG and surface binding protein A proteins are involved in adhesion and infection pathogenesis (Corrigan et al., 2007). CWA proteins on the host surface promote adherence to EPS, and contact with CWA proteins on neighboring cells promotes biofilm aggregation (Foster et al., 2014). Similarly, the amyloid fibers that support *S. aureus* cells operate as a stabilizing scaffold inside the biofilm matrix, so ensuring the stability of the biofilms (Idrees et al., 2020; Taglialegna, et al., 2016). eDNA, after the EPS and IPA proteins, is the third most important component of the *S. aureus* EPS biochip, after the proteins themselves. eDNA has been implicated in a variety of processes including binding with an irreversible bond, horizontal gene transfer, biofilm integrity maintenance, antimicrobial resistance, and the immune system are all examples of such phenomena. evasion, among others (Miao et al., 2019). Additional charged (positive and negative) and hydrophobic groups are found in the additional polymeric material that makes up the biofilm. A negative charge can be found in EPS in the form of carboxyl groups and phosphates or sulfates as well as glutamic acid and aspartic acid, but a positive charge can be found in amino sugars (Neu and Lawrence, 2010). Despite the presence of both negatively and positively charged species on the EPS surface, the total charge on the EPS surface is negative, indicating that positively charged fractures may be a more appropriate target (Algburi et al., 2017; Guangyin and Youcai, 2017).

This is one of the most often encountered expressions causes of bloodstream infections brought on by medical device contamination; these bacteria are the most common cause of bacteremia. CoNS's most prevalent *S. epidermidis* species is frequently recorded as a frequent cause, particularly among neonates. *S. hominis* is the third most often isolated virus in clinical cases, and the primary causes of infection are bacteremia, septicemia, endophthalmitis, and endocarditis. the most second frequently isolated organism from human blood cultures is *S. haemolyticus* and is extremely resistant to antibiotics. *S. capitis* is found on the scalp and when cofactors such as foreign bodies or immunosuppression are present, this bacterium can cause prosthetic joint infections, prosthetic endocarditis, and bacteremia in patients with malignant

hematomas. This bacterium has also been identified as a pathogen in healthy adults. (Al-Sultany and Al-Charrakh, 2020)

The ability of CoNS to generate biofilm is an important virulence factor that enhances its adherence and colonization to synthetic materials. Biofilm produces a four-step cycle that includes attachment, aggregation, maturation, and detachment. Methicillin resistance (MR-CoNS) is a multidrug-resistant bacteria that is difficult to treat due to its ability to form biofilms, and an important factor for nosocomial infections is the emergence of antimicrobial resistance among CoNS isolates. The methicillin-resistant CoNS is also cross-resistant to all other ß-lactam antibiotics. The incidence of antibiotic resistance in staphylococci is mostly related to plasmid acquisition or transposons, rather than to other mechanisms. In staphylococci, conjugated transport of resistance determinants is usually mediated by conjugated plasmids that distribute resistance determinants between species and genera. (Al-Sultany and Al-Charrakh, 2020)

Biofilms are compact extracellular bacteria assemblies made of enzymes, nucleic acids, polysaccharides, and proteins that enable bacteria to colonize any surface irreversibly. It aids bacteria in resisting antibiotics by agglutination and the mechanism of creation of chromosomally encoded resistance genes, reduction of antibiotics and growth rate, and decreases the host's immunity. (Schilcher and Horswill, 2020; Høiby et al., 2010)

The aim of this study is to collect clinical blood samples from different types of staphylococci bacteria (*S. aureus, S. haemolyticus*, *S. epidermidis, S. hominis,* and *S. capitis*), study the biofilm formation of these bacteria, and the importance of verifying the change and the biofilm formation Properties of these bacteria in different environmental conditions (normal, CO_2, anaerobic), and antimicrobial susceptibility testing and determination of resistance to methicillin. As well as the importance of evaluating biofilm formation in staphylococci that cause bacteremia in patients, and the reason is due to contamination of medical devices, weak immunity, wrong use of antibiotics, and implanted devices such as (prosthetic limbs, joints, and catheters in blood vessels).

2. GENERAL INFORMATION

2.1. THE GENUS STAPHYLOCOCCUS

Staphylococcus is a genus of bacteria that belongs to the family Staphylococcaceae, which also includes the genera Salinicoccus, Nosocomiicoccus, Gemella, Macrococcus, and Jeotgalicoccus. There are roughly 50 staphylococci species and approximately 30 subspecies, the majority of which are found in animals. "*S. aureus, S. saprophyticus, S. epidermidis, S. haemolyticus", S. hominis*, and *S. capitis* are the staphylococci most closely associated to human infection. Other staphylococcal species may be involved in human infection as well. (Euzeby, 2019)

Staphylococcus species are gram-positive, non-distributed cocci that appear individually or in irregular pairs in groups. Colonies are black and may be white, yellow, or orange in color. The optimal temperature for growth is between 30°C and 37°C; it has a fermentative metabolism and is a facultative anaerobe, with the exception of *Staphylococcus aureus* subsp. anaerobius and *Staphylococcus saccharolyticus*, which initially grow anaerobically but may become more tolerant of air in the subculture (James et al., 2015). With the exception of group S, Staphylococcus species are typically lactase positive and oxidase negative. Certain species create poisons that are extracellular in nature. Staphylococci can be identified by their deoxyribonuclease (DNase) production (Holt, 1994). Staphylococci are widespread in nature; their primary habitat is the human skin and mucous membranes.

2.2. *STAPHYLOCOCCUS AUREUS*

S. aureus is an opportunistic Gram-positive human pathogen that can cause a wide variety of infections ranging from localized tissue infections (lungs, skin, bones, and heart) to blood-borne sepsis. *S. aureus* and soft tissue infections are common and can manifest themselves as abscesses, abscesses, superficial infection, or even necrotizing fasciitis (Lowy, 1998; Otto, 2010). This infection is interesting not only because it is common among patients infected with *S. aureus*, but also because it can be described

as "painful" or "itchy"; this demonstrates the sensory nerve system's participation during injury. Typically, deep skin infections, such as abscesses, are described as painful. Whereas superficial infections are characterized by itching." A persistent inflammatory skin disorder known as atopic dermatitis (AD) is frequently associated with this infection (Kim, 2012).

S. aureus is particularly adept at adapting to extreme conditions, such as those present during antibiotic stress and in the host's hostile environment (for example, during an immune system attack). This pathogen's genome is highly susceptible to mutation and it is easily capable of acquiring/exchanging new environmental DNA; this allows antibiotic resistance and the acquisition of novel virulence components (Alonzo and Torres, 2014).

As such, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is resistant to the majority of drugs and exhibits increased virulence, allowing the germs to spread freely among otherwise healthy persons. Approximately 90% of *S. aureus* infections transmitted in the community are caused by skin and soft tissue infections (Deleo et al., 2010).

S. aureus is a well-known pathogen that spreads to humans via cross-contamination in hospitals and communities. *S. aureus* infection has been associated with skin lesions, pneumonia, osteomyelitis, toxic shock syndrome, nausea, severe vomiting, and diarrhea (Lowy et al., 1998). According to the Centers for Disease Control and Prevention, this year's clinical outbreaks resulted in 119,000 illnesses and more than 20,000 deaths (Kavanagh, 2019). Staphylococcal food poisoning, which occurs as a result of the consumption of tainted animal goods, is also responsible for around 241,000 illnesses each year in the America (Zeaki et al., 2019). It has been observed that MRSA strains, specially, are responsible for around 44 percent of cases and more than 20 percent of excess mortality. Furthermore, in fact, MRSA strains make up more than 20% of all *S. aureus* strains (Chung et al., 2021). Due to the disease's pathogenicity and antibiotic resistance activities, it is critical to understand the pathogen's virulence factors at the genome and transcriptional level in order to contain outbreaks produced by the pathogen (Baba et al., 2008).

Antibiotic resistance, the type 7 secretion system (T7SS), hemolysis, the generation of enterotoxins, and the antitoxin (AT) system are all associated with *S. aureus* virulence factors' adhesion and invasion of the host cell membrane. Bacterial infection penetrates the surface of the host cell through attachment to membrane glycoproteins and invasion across the host membrane caused by host tissue destruction. This is an essential first stage in the infection of the host cell by bacteria (Foster et al., 2014; Priest et al., 2012). To frustrate neutrophils in many ways, *S. aureus* has developed a unique immune evasion mechanism that allows it to produce captive polysaccharides that interfere with the host's principal defensive response (Foster, 2005; Nanra et al., 2013; van Kessel et al., 2014). The T7SS is a complicated protein system that makes a lot of virulence factors, like hemolysin and enterotoxins, as well as antibiotic resistance proteins, which may help staphylococcal abscesses populations stay alive and stay alive for a long time (Cao et al., 2016).

The hemolysin protein of *Staphylococcus aureus* is composed of three subunits: the α, β, and δ hemolysin proteins (Kielian et al., 2001). Alpha-hemolysin is involved in the formation of pores in the membrane of blood cells, whereas beta-hemolysin is involved in the lysis of membranes, which is associated with the formation of a spheroid or protoplast from blood cells. Alpha-hemolysin and beta-hemolysin are both involved in the formation of pores in the membrane of blood cells (Chung et al., 2021). Beta-hemolysin is a protein that prevents glycolytic production (Chung et al., 2021).

SCCmec (Staphylococcal cassette chromosome mec) is a methicillin-resistant staphylococcal genetic component that has been modified. mecA and ccr are the two main parts of SCCmec (Sani et al., 2014). This is from 2014: (Sani et al.) The mec gene complex is made up of the mecA gene, which makes a penicillin-binding protein 2A (PBP2A) that has a low affinity for beta-lactam antibiotics, as well as other genes that help the mec gene complex work. There are many genes called mec. MecA is one of them. This low affinity for antibiotics causes the host to become resistant to antibiotics because it stops cell wall synthesis. Because *Staphylococcus aureus* has the mecA gene, which makes it resistant to penicillin-like antibiotics, this has been shown to be the reason for this (Fogarty et al., 2015). In the SCCmec genetic material, the excision, integration, and splicing genes are encoded by the recombined ccr gene

complex, which is responsible for the integration of genetic material from the SCCmec on to the host chromosome (Huda et al., 2017). SCCmec genetic component can be classified into species I–VIII according to the mix of mec and ccr gene complexes found in each species (Kennedy et al., 2009; Zhang et al., 2009).

To get a better understanding of *S. aureus* virulence, pathophysiology, and antibiotic resistance. The genomes of *S. aureus*, as well as their functions, have been sequenced in accordance with the advancement of next-generation sequencing technology (Durand et al., 2018; Nair et al., 2011; Zubair et al., 2015). Complete genome sequences for 443 strains of *S. aureus* have been published to date (September, 2019). In the database of the GenBank, to be precise. A recent study of the entire *S. aureus* genome, using bioinformatics, revealed that it contains gene groups for the intestine, superantigen/hemolysin, immunoevasion, and potential antimicrobial resistance. The genome of *Staphylococcus aureus* MCRF184 has been sequenced and studied using bioinformatics. SCCmec pathogenesis and antibiotic resistance have been studied, but the genetic component of SCCmec has not been studied (Aswani et al., 2019). It is proposed that the proposed antimicrobial gene set, which includes a type III restriction modification system (RM), an efflux pump, an acetyltransferase, regulators, and motile elements, replace the antibiotic resistance gene SCCmec. Therefore, understanding the *S. aureus* genome is crucial for increasing our understanding of the pathogenicity and pathogenesis activities of *S. aureus* in order to better regulate this infection.

When a person gets an MRSA infection, there are three types based on where the person got it: health care-associated MRSA, community-associated MRSA, and livestock-associated (LA) MRSA (Abolghait et al., 2020). HA-MRSA is a well-known human pathogen that has been linked to hospital infections in the last few years. As a result, it is likely that clinical human MRSA infection would occur as a result of the consumption of contaminated foods, which is one of the key safety issues for human infection (Kluytmans, 2010). MRSA, for example, is commonly transmitted to humans through a variety of contaminated foods; however, the significance of MRSA in food environment survival, reproduction, and even clinical MRSA toxicity is unknown, despite the fact that the characteristics of HA-MRSA have been extensively

investigated for human infection in the laboratory setting (Sergelidis et al., 2017). MRSA infection in clinical human patients has been found in contaminated chickens on an ongoing basis, showing that clinical MRSA and human infection can spread through food, posing a threat to food safety and public health in the process (Fox et al., 2017; Hennekinne et al., 2012). MRSA infection in clinical human patients has been detected in infected chickens on an ongoing basis, indicating that clinical MRSA and human infection can spread through food, posing a threat to food safety and public health So understanding the link between clinical MRSA and the food environment is crucial for preventing food poisoning. In order to do so, in recent years, as a result of the improvement of NGS technology and the collection of *S. aureus* genome information, transcriptome analysis has been available, allowing us to acquire a deeper understanding of their genetic association with poisoning from food. Most recently, researchers found that when *S. aureus* bacteria were grown in Luria-Bertani medium, they used more amino acids and sugars from chicken breast. In this study, it was found that *S. aureus* bacteria can grow in certain foods and then cause food poisoning through the Enterotoxin pathway. After eating chicken breast that was contaminated with bacteria, the bacteria grow (Dupre et al., 2019). So, studying *S. aureus* activity when it comes into contact with raw chicken breasts could help us understand how *S. aureus* survives and spreads when it comes into contact with raw chickens, which is important for food safety reasons.

2.3. VIRULENCE FACTORS IN *STAPHYLOCOCCUS AUREUS*

This virus utilizes a diverse array of toxins and virulence factors to invade and spread within the host, eventually escaping the host's immune system. Molecular factors and proteins such as cytolysins, exotoxins, coagulants, and carotenoids are included in this category. Numerous virulence factors are expressed by pathogens and are regulated by a variety of pathogen regulatory systems in order to enhance their response to host defenses. The additive gene regulator (Agr) utilizes the quorum sensing locus to regulate the expression of a diverse array of virulence determinants and metabolic genes during *S. aureus* infection in order to facilitate pathogenesis, particularly during acute infections (Le and Otto, 2015; Wang and Muir, 2016). The Agr locus regulates

the expression of virulence factors that are largely employed for tissue invasion and proliferation (pore-forming toxins, proteases, and lipases, for example). The Agr locus also protects the host against pro-inflammatory virulence factors (Le and Otto, 2015; Wang and Muir, 2016). Auto-inducible peptide (AIP) expression by Agr loci and subsequent secretion into the environment is critical for cell-cell communication. This is because AIP reaches sufficiently high quantities — often during log phase development — to be'sensed' on its own. and other *S. aureus* cells of the same Agr type via the histidine kinase sensor AgrC. (Le and Otto, 2015; Novick and Geisinger, 2008; Wang and Muir, 2016). This contributes to the reinforcement of a two-component signaling chain. The Agr locus has been demonstrated to be required for the severity of skin infections in numerous animal models. In this case, Dagr *S. aureus* is utilized to target a variety of putative virulence factors that may induce pain or itching during infection.

S. aureus is a multidrug-resistant opportunistic bacterium that has the potential to cause a wide variety of illnesses, ranging from acute infections to chronic and difficult-to-treat infections. The fact that *S. aureus* lives in the nasopharynx of many people doesn't make it less likely that this colonization can cause illnesses that range from simple skin infections to more serious conditions like pneumonia or osteomyelitis. Mortality and morbidity rates are very high when it comes to people who have very bad staphylococcal infections. *S. aureus* bacteria's ability to cause many types of infection is a result of their enormous pool of virulence factors and mechanisms of infection. (Löffler and Tuchscherr, 2021)

People get sick from *S. aureus* because the pathogen attaches to and penetrates their tissues and cells with a variety of surface-bound proteins that help the pathogen get inside their bodies. Bacteria can proliferate and initiate toxin production following ingestion and adhesion. *S. aureus* is capable of transmitting a variety of chemicals, most notably toxins, that decimate and destroy host cells. Toxins help bacteria to damage and enter deeper tissues during infection, obtaining the nourishment necessary for development and defending against the immune system. However, once the acute and destructive phase has passed, the *S. aureus* bacteria want to survive within the host. Numerous poisons require this for long-term bacterial persistence in host tissues.

Thus, the immune system recognizes germs less frequently and can avoid removing phagocytes. Thus, downregulation of poisons is important for them to stay silently for lengthy periods of time within host cells and tissues. When considered as a whole, virulence factors such as toxins must be strictly controlled throughout infection, for example, through quorum sensing systems that act as feedback mechanisms for the surrounding milieu. Although staphylococcal toxins have been extensively examined, the question remains whether a "virulent" strain that secretes a large number of toxins or a persistent "silent" strain poses a greater threat to the host and at what stage of infection. 2021) (Löffler and Tuchscherr).

17 staphylococcal enterotoxins (SE) have been found recently (AYDIN). They may have biological effects on superantigens, such as generating toxic shock syndrome by activating and multiplying pyrogenic T cells, increasing lethal endotoxin shock, and inducing pro-inflammatory cytokines, among other outcomes. SEA is known to be connected with food poisoning in humans, which results in gastrointestinal illness. (Chung et al.,2021)

toxins, which are essential virulence factors in severe invasive infections, are discussed in this section, as well as their relevance and consequences. Two unique research articles discuss *S. aureus* leucocidins that efficiently produce cytotoxicity in susceptible leukocytes, with cell type and type specific effects. To obtain a better understanding of leukopenia, Hodille and colleagues investigated the effect of licosides what licosides do to the cells in bone marrow in a laboratory setting. Leukopenia has been described in a variety of clinical settings. Tromp and colleagues discovered the host cellular pathways that are activated when licosides connect to the cellular receptors of sensitive cells, which they then investigated further. Experiments are being conducted to determine the creation and release of extracellular vehicles (EVs) that contain cytotoxic components in response to diverse stress situations. The final section of Schelin's research examines the regulatory networks that control the expression of *S. aureus* toxins, such as superantigens. ClpXP is a protein that was discovered by RNA sequencing to have a function in the complex regulation of *S. aureus* infection by the authors. Löffler and Tuchscherr (Löffler and Tuchscherr, 2021)

Two research papers published in this special issue looked on the role of toxin production in the development of *S. aureus* pneumonia. Deinhardt-Emmer and colleagues found that pneumonia strains have increased invasiveness and cytotoxicity, although these virulence traits were not necessary when the pneumonia strains were produced through viral–bacterial co-infection, as previously reported. Lacoma and colleagues discovered *S. aureus* in individuals who were mechanically ventilated. No indication of pathological adaptation associated with virulence, resistance, or adequate adaptation was revealed despite comprehensive investigation. These two publications demonstrate that practically any strain of *S. aureus* is capable of generating a lung infection when the lung has already been injured, as in the case of an influenza infection that occurred prior to the infection or the use of mechanical ventilation. Löffler and Tuchscherr (Löffler and Tuchscherr, 2021)

Illness with *S. aureus* is a chronic and difficult-to-treat infection, which is yet another important clinical topic treated in this special issue of the journal. As an illustration, chronic sinusitis has been linked to the presence of *S. aureus* leucocidin ED (LukED), which has been identified as a persistent trigger that leads to the advancement of the disease. Cystic fibrosis (CF) is the second disease that has been linked to *S. aureus* infection and aggravated by it. It is reported in this paper that *S. aureus* isolates from cystic fibrosis patients had a drop in virulence and toxicity genes, which the scientists believe is a result of the bacteria's adaptation to a permanent state. Furthermore, the germs found in *S. aureus* osteomyelitis have been identified. Butrico et al. analyzed in detail how adaptation techniques mostly regulated by the additional gene regulator locus (Agr) stimulated the host's immunological response, resulting in bacterial strains that were chronically adapted to the host's immune system. Löffler and Tuchscherr (Löffler and Tuchscherr, 2021)

The seal of the *S. aureus* section, which is accompanied with two articles on preventive and therapeutic actions for the bacteria. According to Shukla and colleagues, a statistical approach for combining data on antimicrobial-induced alterations in gene expression has been developed. According to this strategy, a therapeutic regimen that is capable of not only eliminating bacteria but also drastically reducing their virulence will be developed. S. aureus toxin (Hla)-mediated disease was discovered by Joyner

and colleagues, who created a virus-based vaccination that inhibited the progression of the disease. In the future, this method may be employed as a component of a multicomponent *S. aureus* vaccine, which would be more effective. Löffler and Tuchscherr (Löffler and Tuchscherr, 2021)

2.4. COAGULASE - NEGATIVE *STAPHYLOCOCCI* (CoNS)

CoNS are responsible for the vast majority of bloodstream infections (BSTIs). CoNS species have been identified in thirty-two individuals, and several of them, particularly *Staphylococcus epidermidis* and *Staphylococcus hominis*, are important components of the resident human skin flora, according to the literature. Despite the fact that CoNS are commonly isolated in clinical microbiology laboratories, they are more often than not contaminants rather than pathogenic agents. As a result, doctors have begun to question the importance of the study. The identification of CoNS as pathogens is made even more difficult by the fact that infections induced by these bacteria express themselves in a subtle and generic manner in the clinical setting. Recent years, however, have seen an improvement in our understanding of the association between these extraordinarily aggressive microbes and a variety of illnesses, which can be attributed in part to the development of more accurate tools for their identification and characterization. The involvement of the central nervous system (CNS) in hospital bacteremia is becoming more common. It is important to note that this trend coincides with advancements in medical practice, such as the increased use of indwelling medical devices such as vascular catheters, artificial joints, vascular grafts, artificial heart valves, and peritoneal catheters, as well as an increase in the number of immunocompromised patients, among other things. Methicillin resistance is found in a large number of CoNS. In part, the rising use of broad-spectrum antibiotics, which encourages the selection of multi-resistant strains, is responsible for the concomitant increase in resistance among CoNS. Congenital neuronal syndromes (CoNS) are increasingly recognized as the primary agents of postoperative endocrine disruption (PED), prosthetic endocarditis, and original valve endocarditis, particularly right endocarditis in intravenous drug users. Also reported are CNS infections caused by the CoNS, which have been linked to various conditions such as shunts, pneumonia and

osteomyelitis as well as wound infection. When it comes to young women, *Staphylococcus saprophyticus* is a prominent cause of urinary tract infection. However, bacterial urine in hospitalized patients is a rare occurrence. (Casey et al.2007)

Staphylococcus haemolyticus, a commensal bacterium but a frequent pathogen associated with catheter-associated bacteremia, is one of the most methicillin-resistant species among CoNS. One of the CoNS, *S. haemolyticus*, has a complete genome. Numerous putative virulence factors were identified, including hemolysin and the bacterial capsule. Since no publication has explored the existence of pathogenicity in this species to yet, additional research is needed to determine whether or not they exist. Several prior investigations reported the presence of enterotoxins and cytotoxins in the CoNS, which is rare for this species. Furthermore, this species has the ability to create biofilms. After employing long read sequencing from PacBio, he released the first complete sequencing of *S. haemolyticus* (Pacific Biosciences, Menlo Park, CA, USA). Alternative pathways for biofilm development were implicated because no ica operon was found, but there were homologs to the Agr regulatory system. So, *S. haemolyticus* is an important human disease, despite the fact that numerous whole genomes of these species have already been obtained, there appears to be a lack of whole-genome investigation of the virulence components in this pathogen. (Argemi et al.,2019)

Staphylococcus epidermidis has a diameter of approximately 0.5 to 1.5 μm and is arranged in clusters resembling grapes. They are facultative anaerobic organisms that can grow aerobically or via fermentation, though some strains do not ferment. After an overnight incubation period, they form grayish-white, raised, circular, smooth, shiny, translucent to slightly opaque, coherent colonies about 1-2 mm in diameter. They are insoluble on blood agar. They grow well up to a NaCl concentration of 7.5 percent, are sluggish at 10%, and die at 15%. They are either sensitive to lysostaphin or have a slight resistance to it, but are resistant to lysozyme. *S. epidermidis* is susceptible to novobiocin, which differentiates it from *Staphylococcus saprophyticus*, which is also coagulase negative but resistant to novobiocin. (Health Protection Agency, 2007)

CoNS-associated infections are most commonly caused by *S. epidermidis*, which is the most common CoNS recovered from microbiological specimens. For this species, molecular studies have yielded the greatest information on virulence factors that can be classed as pathogenicity factors. *S. epidermidis* colonizes and infects medical equipment and prosthetics mostly through the production of biofilms. The life cycle of biofilms is a complicated process that involves MSCRAM, exopolysaccharide matrix proliferation, and finally biofilm dispersal, which is facilitated primarily by phenol-soluble peptides. (Argemi et al.2019)

2.5. BIOFILM FORMATION IN STAPHYLOCOCCI AND FACTORS AFFECTING BIOFILM FORMATION

The adherence of planktonic bacteria to a surface (which may be a biological host or any inorganic surface), colonizing and formation of biofilms, maturation of biofilms, and dispersal of biofilms are all steps in the formation of biofilms (Landini et al. 2010). When free-floating *S. aureus* cells attach to an accessible surface and begin colonizing, biofilms are formed (Petrova, and Sauer, 2012) Biological and abiotic surfaces interact with the *S. aureus* cell surface in an amphiphilic manner, influencing *S. aureus* adherence (Hamadi, et al., 2005; Krasowska, and Sigler, 2014; Maikranz, et al., 2020). The cell surface of *S. aureus* attaches to hydrophobic surfaces via a network of weakly bonding macromolecules, whereas it adheres to hydrophilic surfaces via a network of fewer but stronger binding molecules (Maikranz, et al. 2020; Otto, 2018).

EPS, or extracellular polymer, grows into a biofilm when microcolonies form (Landini, et al., 2010). To break down and spread the biofilm, the bacteria in the biofilm make substances like damino acids and enzymes that break down EPS, like alginate lyase (Kostakioti, et al., 2013). They can now recolonize the same place or stick to a new place and start making biofilm again. (Donlan, 2002)

S. aureus cells that have been encapsulated and protected by biofilms exhibit distinct phenotypic characteristics as compared to planktonic cells. *S. aureus* biomembrane-associated cells are more resistant to antibiotics than their free-living counterparts, and

their cell size, proliferation, protein production, and gene expression are all different. (Otto, 2018)

It has been shown that *S. aureus* cells linked with biofilms exhibit four distinct metabolic states, namely aerobic growth, fermentability, dormancy, and even death (Archer, et al., 2011). Aside from cells' ability to resist antibiotics due to the existence of a polymeric extracellular matrix, it has been established that inert and metabolically slow developing cells can also contribute to antimicrobial resistance through their ability to accumulate antibiotic resistance (Lister, and Horswill, 2014). According to Mormer, *S. aureus* cells encased in biofilms develop at a variable rate, with some cells developing quicker than others inside the same biofilm (Moormeier, and Bayles, 2017). These cells are smaller and revert to their usual size following dispersal of the biofilm. Due to the production of thin biofilms, *S. aureus*-associated infections are challenging and difficult to cure. The host's immune system and medications are ineffective against *S. aureus*, resulting in the development of chronic infectious illnesses. (Reffuveille et al.,2017) *S. aureus* can build biofilms on a variety of medical surfaces and devices in healthcare facilities, including surgical tools, implants, and so on. If *S. aureus* on the surface of medical devices is not removed, it might cause infection in patients. (Archer et al. Biofilms linked with medical devices, particularly implantable devices, frequently necessitate their removal and replacement, posing additional surgical risks. (Reffuveille et al.,2017)

In addition, *S. aureus* generates biofilms within the cells and tissues of its host. Resistance to antibiotics has made this illness more difficult to treat, and in many cases, the removal of damaged tissue is necessary to establish a good cure. ' According to (Khatoon et al., 2018), The presence of dormant *S. aureus* cells (also known as permanent cells) within a biofilm can partially explain this biofilm-associated antimicrobial resistance. Because they remain latent throughout antimicrobial therapy and reawaken upon withdrawal, persistent infections can recur repeatedly. (Arciola et al.,2018)

The creation of microbial biofilms is encoded by genes that are unique to biofilm formation (Sauer, 2003). Among the 12 different genes that regulate biofilm formation in *S. aureus* are those that encode fibrinogen binding proteins (Fib), elastin binding

proteins (ebps), clustering factor Clf, inter - cellular adhesion ica, fibronectins-binding genes Fnb, collagen binding protein (cna), and laminin binding protein (lbp). (Nourbakhsh et al., 2016).

The genes encode a variety of surface proteins that enable *S. aureus* to stick to, penetrate into, and colonize the host, ultimately resulting in biofilm formation and pathogenicity. The fibroblast gene and the cna gene make it easier for fibrinogen binding proteins to be found on the surface. The collagen binding protein gene makes it easier for the surface to stick to itself. (Paharik et al., 2016)

The intercellular commitment genes icaABCD are involved in cell-to-cell adhesion and the establishment of biofilms (Chen, et al. 2020; Azmi, et al., 2019). Co-expression of Fnb genes in *S. aureus* has the added impact of promoting biofilm formation in the bacteria. The Fnb genes in *Staphylococcus aureus* are not involved in the adhesion process, but they do contribute to the creation of biofilms; in particular, the fnb gene acts as an invader, allowing *S. aureus* to infiltrate host cells and colonize them; this is a major finding. (O'Neill et al., 2008; Sinha et al., 2000)

CLF is clustering factor genes that produce cell wall-repairing proteins that bind with the host's fibrinogen on the cell's surface (Wolz, et al., 2002). As a result of the clustering factors A and B genes, which are expressed in the host, *S. aureus* can colonize and colonize more effectively, form biofilms, and improve the likelihood of virulence by evading immune recognition (Foster, et al. 2014; Herman-Bausier, et al., 2018). Association with scaly epithelial cells and nasal colonization are facilitated by the Sdr. The matching SDR genes encode the proteins (Sabat, et al., 2006). Elastin-related and flap-binding proteins, which are encoded by their respective genes ebps and Ibp, aid in host colonization and biofilm development. (Kot, et al., 2018)

When the biofilm has attained full maturity, it dissipates and releases cells that are ready for repopulation in the same or a different location, depending on the circumstances. It is believed that four different genes related to the Additional Gene Regulatory System (Agr) are responsible for *S. aureus* dispersion (Moormeier, and Bayles,2017). Agr biofilms are among the Agr genes that encode biofilm dispersal (Le, and Otto, 2015). The induction of different phenol-soluble proteases and modulins

(PSMs) results in agriculturally regulated biofilm dispersal (Lister, and Horswill, 2014). These proteases and PSMs work as surfactants, dispersing biofilms. (Peschel, Otto, and Saggu, 2013; Saggu et al., 2019)

It is possible for *S. aureus* to co-exist while sensing quorums and controlling gene expression through the additive gene regulator (Agr). In order to communicate with other bacteria, Agr, a genetic modification mechanism, is required. Toxins and virulence factors produced by *S. aureus* and the bacteria's interactions with the host are controlled by a unique culture system, making it a very dangerous pathogen. A pair of genes known as Agr, which are activated in response to peptide secretion, are activated in the Agr system and AIP. According to the research, the genes Agr play a role in regulating AIP expression and transmission. Activation of Agr component systems results in intracellular communication and quorum sensing when AIP accumulates in the extracellular environment. (Idrees et al., 2021)

As a result of AMR, many classic antimicrobial agents have lost their effectiveness, posing a serious threat to human life (partially or completely). The World Health Organization (WHO) said in 2019 that antibiotic resistance kills 700,000 people each year and that drug resistance to tuberculosis (TB) drugs is responsible for 230,000 deaths directly related to TB medication. (World Health Organization, 2019) Antimicrobial resistance, in addition to being a substantial cause of illness and mortality in both humans and animals, leads in huge economic losses for the world economy as a result of its widespread prevalence. (Shrestha et al., 2018)

As with any other biofilm forming bacteria, *S. aureus* creates a biofilm to impede or block the diffusion of antimicrobial drugs, preventing them from reaching the bacteria within. Several factors, such as temperature swings (too hot or too cold), nutritional restriction, and dehydration, may initiate the formation of biofilms. If you're interested in learning more about the effects of stress on the human body (Fadeeva et al., 2016; Rizzato et al., 2019). Antibiotic-resistant *S. aureus* bacteria have embraced biofilm as a valuable weapon because of its ability to encapsulate cells and protect them from antibiotics that normally kill planktonic bacteria. (Ou, et al., 2020) Biofilm-coated bacteria are up to 10-1000 times more resistant to antibiotics than their planktonic counterparts. (Reffuveille et al., 2017)

The commensal bacterium *S. aureus*, which infects up to 30% of the world's population, usually does no harm. (Sakr et al., 2018). However, these common niches might occasionally serve as the primary source of *S. aureus* infection. (Otto, 2018) As a result of the pathogen's ability to form biofilms on both biotic and abiotic surfaces, *S. aureus* infections are difficult to treat. This is especially true for methicillin-resistant strains (MRSA). It has been found that (Rasmussen et al. in 2011; Amyes in 2005). Those who have atopic dermatitis, for example, are more susceptible to *S. aureus* colonization and subsequent biofilm formation because of their skin condition. (Sonesson et al., 2017). *S. aureus* cells can persist in the presence of low concentrations of antibacterial agents because antibacterial medications become less permeable through biofilms. It's (Donlan, 2000) Other factors, such as gene evolution and genetic material exchange, genome plasticity, and the host's innate immune system, all contribute to the development and emergence of antimicrobial resistance, in addition to the primary role of bacterial biofilms. (Otto, 2018; Verma et al., 2019)

In addition, *S. aureus* biofilms on nonliving surfaces, like in hospitals and food businesses, can be dangerous to public health and safety. Medicinal equipment must be sterilized and free of pathogens to be used in the field. Medical devices and equipment can be made of many different materials, like glass, steel, and more. If the devices and equipment aren't properly sterilized, they can be a major source of infection in hospitals. (Manzur et al., 2008) Because *S. aureus* has become resistant to surface cleaners and disinfectants, it is hard to clean these surfaces properly. (Marques et al., 2007)

A wide variety of bacteria, including *S. aureus*, may be present in biofilms associated with the food sector, making them more resistant to disinfectants and surface cleaners. According to the researchers (Galié and coworkers, 2018), Antimicrobial resistance to disinfectants causes food spoiling, microbial contamination of newly delivered goods, corrosion of storage surfaces as well as an elevated risk for infectious disease transmission. As cited in (Chmielewski and Frank, 2003)

Infections caused by *S. aureus* produce significant, life-threatening illnesses in humans and animals and provide medical scientists with extremely complex clinical consequences. (Idrees et al., 2021)

CoNS adhesion and biofilm development Bacteria proliferate largely in biofilms in clinical settings. Several types of biofilms are colonies of bacteria that are multicellular and organized. They are encased in a matrix of extracellular polymeric components that form on a surface and protect the bacteria. Bacteria in biofilms can fight off a lot of things, like antimicrobial drugs and the body's own defenses, which can be very bad. Given that CoNS's ability to form biofilms is a fundamental component of their viability, the mechanisms that drive biofilm formation have attracted greater attention in recent decades, as previously indicated. Biofilm formation by CoNS is a complicated and multi-step process that can be roughly divided into three phases: adhesion or attachment to the surface, maturation into a complex multicellular structure, and cell dispersal into the surrounding environment. Adhesion or attachment to the surface, maturation into a complex multicellular structure, and cell dispersal into the surrounding environment are the three phases of biofilm formation by CoNS. The initial attachment to host proteins and tissues, as well as the development of biofilms, are both facilitated by surface-bound adhesives. In addition to proteins that have been covalently and non-covalently linked, these adhesives include non-protein components. It is necessary to have an LPXTG component that has been recognized by a sorting enzyme in order to stabilize the peptidoglycan protein in the case of covalently anchored proteins (CWA). There are two separate CWA protein families in *S. epidermidis* based on the presence of shared unique domains: a MSCRAM family, which comprises members of the Sdr and Additionally, a second family of G5-E repeat proteins, which includes the aggregation-associated protein, has been identified (Aap). The inclusion of two folded IgG-like domains, which can be involved in cross-linking via the 'dock, lock, and latch' process, separates the MSCRAM family from other families. This technique permits a critical adhesive static compound to maintain a tight connection even when subjected to fluid shear stresses, which typically occur in the vicinity of static medical devices. Additionally, there are unclassified CWA proteins that may play a role in biofilm formation, such as homologous biofilm protein (HBP). AtlE and Aae are autolysins/adhesins that are not covalently linked to the cell wall. Finally, the group of non-proteinaceous molecules includes teichoic acids (TAs) and intercellular polysaccharide adhesion molecules (IPA). (França et al., 2021)

2.6. INHIBITION AND INACTIVATION OF BIOFILM IN STAPHYLOCOCCI AND ANTI-BIOFILM DRUGS

Biofilms are a significant contributor to antibiotic resistance and treatment difficulties associated with Staphylococci infections. (Agostinho et al., 2009). As a result, it is critical to attack these biofilms in order to further limit the likelihood of antimicrobial resistance by the use of proper antibiotic methods. Antibiotic methods fall into two categories: those that restrict or prevent the creation of new biofilms and those that promote or eradicate existing biofilms. (Bhattacharya et al., 2015)

Numerous antibiotic agents, including antibiotics and other antimicrobial molecule (natural or synthetic), have been reported to inhibit and inactivate Staphylococci biofilms. Numerous publications have been identified that contained information about antibacterial drugs with antibiotic activity. Antibiotics such as rifampin, which is used to treat tuberculosis and other bacterial infections, have been shown to inhibit and disrupt *S. aureus* biofilms. (Holstege et al., 2014; CED Rees et al., 2015). Also, when rifampin was used with another antibiotic, ciprofloxacin, against a biofilm of *S. aureus*, it was found to have anti-biofilm properties against the bacteria. It says: (Wells et al, 2018)

When people get MRSA or MSSA, an antibacterial drug called vancomycin is used to treat them. Vancomycin has been shown to be effective against the *S. aureus* bacteria. Those are the words from Bhattacharya et al. in 2015. Anti-*S. aureus* biofilm agents, such as vancomycin and rifampin, can be used in combination with each other to treat MRSA infections. (Salem and other people, 2011) Hu et al. (Hu et al., 2019) talked about how the antibiotics azithromycin and clindamycin could stop and break up a *S. aureus* biofilm. The antibiotic azithromycin works because it can stop bacteria from communicating with each other. (Sharma et al., 2019)

Antibiotics' mechanisms of inhibition or inactivation of *S. aureus* are unknown and have not been reported to date. As a result, antibiotic mechanisms remain an unexplored area. In order to come up with a good treatment for *S. aureus* biofilm infections. Antibiotics aren't the only thing that can help fight off and get rid of bacteria. Natural compounds like amino acids, antimicrobial proteins (AMPs),

polyether ionophores, essential oils, and *S. aureus* biofilms have also been used. Antibiotic drugs derived from amino acids have been developed in several forms, including (1) as single amino acids, (2) as mixes of various amino acids, and (3) as amino acid-antibiotic combinations. Examples include the usage of D-amino acids as individual amino acids, equal amounts of D-amino acids as a combination, and grouping D-amino acids with antibiotics to suppress and disperse S. aureus biofilm (Sanchez et al., 2014). According to Warraich et al. (Warraich et al., 2021), they carried out a similar study in which they utilized a combination of lysineas and well as the antibiotic ciprofloxacin, which is not only effective but also safe inhibited the production of new biofilms but also disturbed the biofilm that was already present in the *S. aureus* strain. Antibiotics based on D-amino acids are capable of inhibiting the formation of biofilm-associated proteins and disrupting eDNA. (Hochbaum et al., 2011) investigated the role of D-amino acids in inhibiting the formation of biomembrane-associated proteins in *S. aureus* experimentally. Similarly, using confocal microscopy, Warraich et al. (Warraich et al., 2021) D-amino acids have been shown to be antibiotics because they can kill eDNA that is stuck in the EPS matrix of a *S. aureus* biofilm. People make proteins when amino acids are linked together in a chain. Antimicrobial properties have been reported for a variety of proteins. As a part of the research into these antimicrobial proteins (AMPs), scientists have also looked into their antibiotic properties, either alone or in combination. Antibiotics such as vancomycin and gentamicin, as well as linezolid and daptomycin, were employed in combination with the bactericidal P128 protein to suppress the production of *S. aureus* biofilms by Nair et al. (Nair et al., 2016). AMPs work as antibiotics by (i) inhibiting gene expression that codes for biofilm-associated proteins and (ii) binding to eDNA. (Algburi et al., 2017)

Additionally, the researchers were investigating the antibiotic activity of naturally occurring polyether ions. Hickey et al. conducted one such study. (Hickey et al., 2018). *S. aureus* was successfully eradicated using four different types of polyether ionophores: lasaloside, monensin, naracin, and salinomycin. Although polyether ionophores have been shown to have antibiotic activity, their exact mechanism of action remains unknown.

The use of essential oils and plant extracts in the development of future efficient antibiotic medicines has recently been studied in addition to the antibiotic techniques previously discussed. Bazargani and Rohloff (Bazargani and Rohloff, 2016) reported on the use of various essential oils and plant extracts for the inhibition and dissemination of *S. aureus*. As Monteiro-Neto et al. demonstrated, antibiotics can be utilized in conjunction with essential oils and plant extracts to treat *S. aureus* biofilms. (Monteiro-Neto and colleagues, 2020) When cuminaldehyde was combined with ciprofloxacin, it was found to have a synergistic effect on the antibacterial activity of *S. aureus* biofilms. Plant extracts and essential oils are able to target *S. aureus* biofilms by disrupting bacterial quorum sensing. (Nazzaro et al., 2020; Sharma et al., 2019).

Antibiotic molecules are small molecules that exhibit antimicrobial activity (as opposed to the drugs mentioned previously). Unlike drugs (the majority of which are also antibacterial), these molecules are only antibiotic. (Rabin et al., 2018). Among these are imidazole molecules, indole, pyrazole, carbazole, pyrrole, oxazolidinone and furanone, phenazine derivatives, cinnamide, and quinoline, among others. (Parrino and colleagues, 2019) The molecular mechanism by which these small molecules exert antibiotic activity is not completely understood. The use of enzymes and heavy metal nanoparticles, such as silver and zinc, to limit the growth of *S. aureus* has also been studied. EDNA has been found to be degraded in *S. aureus* biofilms by DNase I and Dispersin B while exogenous sugars have been degraded by an enzyme called a-amylase. (Sharma et al., 2019)

Additionally, nanoparticles have been used to inhibit and spread disease *S. aureus* biofilms, either alone or in combination with other antibacterial agents. Fontecha-Umaa et al. are good examples of this. They conducted their own experiments with silver and zinc oxide particles, as opposed to Hamida et al., who collaborated (Hamida et al., 2020) Ampicillin and silver nanoparticles have been shown to be effective in the treatment of *S. aureus* biofilm. Numerous studies have demonstrated the antibiotic activity of nanoparticles. However, its mechanism of action is unknown. The biomembrane agents listed above each have a unique mechanism of action for inhibiting or dispersing *S. aureus*. Impeding adhesion, quorum sensing, and eDNA, proteins, lipopolysaccharides, and exopolysaccharides, as well as N-acyl homo-serine

lactones signaling, are all examples of these mechanisms. Researchers (Parrino et al., 2019; Roy et al., 2018).

Resistance to antibiotics in the CoNS population There has been an increase in the number of MR-CoNS infections with resistant CoNS strains, as well as resistance to newer antibiotics, which has limited current treatment options. According to one study, 80% of hospital-isolated *Staphylococcus epidermidis* has a methicillin resistance greater than eight different antibiotics, and this is just one of many examples of antibiotic resistance in bacteria. CoNS isolates with decreased lipopeptide sensitivity have been reported across many species, in contrast to previous studies in which only a few isolates and species showed increased tolerance to antibiotics such as vancomycin and teicoplanin. Surprisingly, thirty years ago, resistant isolates of *S. epidermidis* and *S. haemolyticus* were discovered. *S. epidermidis*-resistant *Staphylococcus warneri* and *Staphylococcus capitis* have also been added, resulting in the spread of the disease in neonatal units, where these bacteria are frequently found. Even though the majority of CoNS isolates are resistant to vancomycin, it is still frequently used in combination with other antibiotics like cefazolin, rifampicin and fosfomycin. Fortunately, rifampicin is also frequently prescribed for the treatment of staphylococcal infections. Using this drug alone can lead to a lot of resistance quickly, so it should only be taken with other antibiotics, not by itself. Vancomycin or levofloxacin, for example, can be used to treat this infection, as well as rifampicin. Bacteria that are resistant to carbapenems have risen dramatically and alarmingly in the last few years. This includes ciprofloxacin, cephalosporins, clindamycin, and erythromycin. Most of the time, resistance to tetracycline is caused by the spread of mobile resistance genes. These genes cause tetracyclines to be removed from ribosomal binding sites and antimicrobial factors to be moved into the extracellular space by drug efflux pumps. Antibiotics like glycopeptides have been used for a long time, but bacteria have already become resistant to them. Linezolid is an antibiotic from a newer class (oxazolidinones). However, staphylococci, including CoNS, have shown that linezolid doesn't work on them. A newer antibiotic called daptomycin can also help fight MR-CoNS better than vancomycin, but it isn't as good. The fact that daptomycin is a new antibiotic doesn't mean other antibiotics, like rifampicin, should be used in addition to it. (França et al., 2021)

3. MATERIAL AND METHODS

3.1. CLINICAL BACTERIAL ISOLATES

After isolation (87) clinical blood samples of Staphylococci bacteria of different patients collected from Sakarya Research and Training Hospital (Sakarya – Turkey), were exposed to MaldiTOF-MS "Array-assisted laser absorption ionization flight mass spectrometry, bioMérieux, Franc" for identification. The isolates also evaluated with "VITEK System 2 ® (bioMérieux, Marcy l'Etoile, France)" for antimicrobial susceptibility testing. An antimicrobial susceptibility test was performed to determine its resistance to methicillin, which includes different types of staphylococci "*S. aureus, S. haemolyticus, S. epidermidis, S. hominis*, and *S. capitis*" that were previously identified. Following that, the effect of several environmental conditions (normal, CO_2, anaerobic) on the biofilm analysis of these clinical isolates will be discussed, followed by an explanation of the findings.

3.2. CLINICAL ISOLATES RECORDING

87 clinical bacterial isolates were grown on a culture medium called blood agar, and then placed in an incubator at (37) degrees Celsius for 24 hours, The next day I noticed the growth of bacteria on the dishes and the growth of bacteria was 100% in the culture medium for all the dishes. Then took the best (63) isolates of clinical bacteria, because their colonies were pure, according to the physical properties of the colonies on agar media. The isolates according to the types of staphylococci were as follows, "*S. aureus, S. haemolyticus, S. epidermidis, S. hominis*, and *S. capitis"*.

3.3. DETECTION OF BIOFILM FORMATION

Microtiter plate was used to detect biofilm formation and bacterial adhesion, which is considered the gold standard in evaluation. Then, a culture medium was prepared from Tryptic Soy Broth (TSB) (500 cc of distilled water with 15 g of TSB solid) which was

put into distilled water and mixed, then placed in an autoclave for sterilization, and clinical bacterial isolates were brought, from which 63 clinical samples were previously grown , a small portion of the transparent bacterial colonies were taken with a stick and placed in a glass tube containing distilled water to dilute them, then 42 µl of this diluted bacterial suspension was taken and placed in 810 µl of TSB culture medium, this means using a rate of 1/20 to dilute the bacterial suspension (20 TSB + 1 bacterial diluent), drip 200 µl of (TSB + bacterial diluent) into 63 wells of a 96-well microtiter, in well 64 *E. coli* bacteria were placed as a positive control because they are a strong biofilm producer according to (Cremet et al., 2000; Naves et al., 2008), selecting only TSB in well 65 as a negative control, and the microtiter plate was incubated at 37 °C for growth within 24 h, three microtiter plates were used in different environmental conditions (first under normal condition, The second is carbon dioxide (CO_2), and the third is anaerobic). The first was placed in the incubator (normal condition), the second was placed in the carbon dioxide (CO_2) inside the incubator, and the third was placed in the anaerobic condition within the anaerobic container system inside the incubator, which is called (BD GasPak^{TM} EZ Anaerobic container system from company Becton, Dickinson). The contents of the wells after the incubation period were dried, washed three times with 200 µl of distilled water (DW), the microtiter plates were shaken well and stirred to remove unwanted bacteria, the bacteria adhered to the wells were fixed for 15 min in 200 µl of 99% methanol, and after Degassing, for 5 minutes, 200 l of 1 percent crystal violet dye was added to each well. The wells were then washed and dried out of water., then 200 µl of glacial acetic acid was applied using a 33% concentration (v/v) to dissolve the bound dye. Using an Automated Immunoassay System (ELISA) reader, which is called (TRITURUS It is the immunoassay system from company Grifols), the optical density (OD) of each well was estimated to be 570 nm, under all conditions (normal, CO_2, anaerobic), all measurements were performed three times and, by absorbance measurement, were obtained Crystal violet dye for each biofilm, biofilm formation ability was compared with positive and negative controls. Strains without biofilm formation have an optical density (OD) lower than the negative control, weak strains have optical density up to twofold, moderate strains have optical density between twofold and fourfold, strong

strains have an optical density greater than fourfold. (O'Toole, 2011; Vatan et al., 2018)

3.4. STATISTICAL ANALYSIS

For each test, the mean and median values were obtained after three operations were performed on the data. The data were analyzed with the help of the "Statistical Package for the Social Sciences (SPSS) version 26.0 software (IBM Corporation)." On the basis of classifying staphylococci strains into four groups as follows: none (0), weak (+), moderate (++), and strong (+++), we sought to compare the effect of different environmental conditions (normal condition, CO_2 condition, and anaerobic condition) on the formation of biofilm in staphylococci. For the purpose of comparing groups, the non-parametric form of the analysis of variance (Kruskal-Wallis Test), the non-parametric version of the independent sample t-test, the Mann Whitney-U test, and variance analysis were employed, respectively. The total isolates were compared using graphs, and the differences between isolate groups were compared using P values less than 0.05, which was considered statistically significant.

4. RESULTS

4.1. CLINICAL BACTERIAI ISOLATES

The isolates are pre-identified and confirmed according to, MaldiTOF-MS "Matrixassisted laser esorption ionization-time of flight mass spectrometry, bioMérieux, France" for isolate identification also "VITEK 2 ® system (bioMérieux, Marcy l'Etoile, France)" used for antimicrobial susceptibility testing. which belong to the types of staphylococci "*S. aureus, S. haemolyticus, S. epidermidis, S. hominis,* and *S. capitis*". Also conducted a test to find out whether these types of staphylococci are positive or negative for methicillin-resistans, and compared them with the production of biofilms in different conditions (normal, CO2, anaerobic). Table 1

All clinical isolates (87) samples were cultured, taken from patients of Sakarya Research and Training Hospital located in Sakarya, Turkey. On blood agar at 37 °C in the incubator for 24 hours, it gave 100% positive results. After incubating the cultures overnight, single and pure colonies were obtained, while visualizing the physical characteristics (shape and appearance) of the isolated colonies on blood agar. The best 63 samples were taken from 87 samples, and all experiments were on 63 clinical samples. Figure 1

Figure 1: *Staphylococcus aureus* on blood agar

The number of types of staphylococci is 63 samples divided as follows: *Staphylococcus aureus* 17 samples, *Staphylococcus haemolyticus* 3 samples, *Staphylococcus epidermidis* 22 samples, *Staphylococcus hominis* 16 samples, and *Staphylococcus capitis* 5 samples. Table 1

methicillin resistance (MR) among the isolates, (63) samples of staphylococcal strains were 40(63%) methicillin resistant and 23(37%) methicillin sensitive. The distribution of biofilm production was as follows: under normal condition and CO_2 condition 61 of the isolates (97%) produced biofilm, while under anaerobic condition, 48 of the isolates out of 63 (76%) were positive for biofilm production. [Weak (+), moderate (++), and strong (+++)] a biofilm production was positive. [None (0)] biofilm production was negative. Table 1

Table 1: Identification of staphylococcus isolates, biofilm production in different condition and methicillin resistance

NO.	Clinical Isolate	Methicillin Resistance	Biofilm production in different conditions		
			Normal	CO_2	Anaerobic
S1	*S. aureus*	Negative (-)	Weak (+)	Strong (+++)	Moderate (++)
S2	*S. aureus*	Negative (-)	Weak (+)	Weak (+)	Weak (+)
S3	*S. aureus*	Negative (-)	Moderate (++)	Weak (+)	Weak (+)
S4	*S. aureus*	Negative (-)	Weak (+)	Moderate (++)	Weak (+)
S5	*S. aureus*	Positive (+)	Strong (+++)	Strong (+++)	Strong (+++)
S6	*S. aureus*	Positive (+)	Moderate (++)	Weak (+)	Strong (+++)
S7	*S. aureus*	Positive (+)	Weak (+)	Weak (+)	Weak (+)
S8	*S. aureus*	Negative (-)	Weak (+)	Weak (+)	Weak (+)
S9	*S. aureus*	Negative (-)	Weak (+)	Moderate (++)	Moderate (++)
S10	*S. aureus*	Negative (-)	Weak (+)	Weak (+)	Weak (+)
S11	*S. aureus*	Negative (-)	Weak (+)	Moderate (++)	Moderate (++)
S12	*S. aureus*	Negative (-)	Strong (+++)	Strong (+++)	Moderate (++)
S13	*S. aureus*	Positive (+)	Moderate (++)	Weak (+)	Moderate (++)
S14	*S. aureus*	Negative (-)	Weak (+)	Weak (+)	None (0)
S15	*S. aureus*	Negative (-)	Moderate (++)	Moderate (++)	Strong (+++)
S16	*S. aureus*	Negative (-)	Strong (+++)	Strong (+++)	Strong (+++)
S17	*S. aureus*	Negative (-)	Moderate (++)	Moderate (++)	Moderate (++)
S18	*S. haemolyticus*	Positive (+)	Moderate (++)	Moderate (++)	Moderate (++)
S19	*S. haemolyticus*	Positive (+)	None (0)	Weak (+)	Weak (+)
S20	*S. haemolyticus*	Negative (-)	Moderate (++)	Moderate (++)	Weak (+)
S21	*S. epidermidis*	Positive (+)	Strong (+++)	Strong (+++)	Weak (+)
S22	*S. epidermidis*	Positive (+)	Weak (+)	Weak (+)	None (0)
S23	*S. epidermidis*	Positive (+)	Moderate (++)	Weak (+)	Weak (+)
S24	*S. epidermidis*	Positive (+)	Moderate (++)	Weak (+)	Moderate (++)
S25	*S. epidermidis*	Positive (+)	Weak (+)	Moderate (++)	Moderate (++)
S26	*S. epidermidis*	Positive (+)	Strong (+++)	Strong (+++)	Strong (+++)
S27	*S. epidermidis*	Negative (-)	Moderate (++)	Moderate (++)	Weak (+)
S28	*S. epidermidis*	Negative (-)	Weak (+)	Moderate (++)	None (0)
S29	*S. epidermidis*	Positive (+)	Moderate (++)	Moderate (++)	Weak (+)
S30	*S. epidermidis*	Positive (+)	Strong (+++)	Strong (+++)	Strong (+++)
S31	*S. epidermidis*	Positive (+)	Weak (+)	Moderate (++)	Weak (+)
S32	*S. epidermidis*	Positive (+)	Strong (+++)	Moderate (++)	Weak (+)
S33	*S. epidermidis*	Negative (-)	Moderate (++)	Moderate (++)	Weak (+)
S34	*S. epidermidis*	Positive (+)	Strong (+++)	Strong (+++)	Strong (+++)
S35	*S. epidermidis*	Positive (+)	Strong (+++)	Strong (+++)	Weak (+)
S36	*S. epidermidis*	Negative (-)	Weak (+)	Weak (+)	None (0)
S37	*S. epidermidis*	Positive (+)	Strong (+++)	Strong (+++)	None (0)
S38	*S. epidermidis*	Negative (-)	Moderate (++)	Strong (+++)	Moderate (++)
S39	*S. epidermidis*	Positive (+)	Weak (+)	Weak (+)	None (0)
S40	*S. epidermidis*	Positive (+)	Weak (+)	None (0)	None (0)
S41	*S. epidermidis*	Positive (+)	Weak (+)	Weak (+)	None (0)
S42	*S. epidermidis*	Positive (+)	Moderate (++)	Weak (+)	None (0)
S43	*S. hominis*	Positive (+)	Strong (+++)	Strong (+++)	Weak (+)
S44	*S. hominis*	Positive (+)	Weak (+)	Moderate (++)	Weak (+)
S45	*S. hominis*	Positive (+)	Moderate (++)	Strong (+++)	Weak (+)
S46	*S. hominis*	Negative (-)	Moderate (++)	Strong (+++)	None (0)

S47	*S. hominis*	Positive (+)	Strong (+++)	Strong (+++)	Moderate (++)
S48	*S. hominis*	Positive (+)	Strong (+++)	Moderate (++)	Weak (+)
S49	*S. hominis*	Positive (+)	Strong (+++)	Moderate (++)	Weak (+)
S50	*S. hominis*	Positive (+)	Strong (+++)	Moderate (++)	Weak (+)
S51	*S. hominis*	Positive (+)	Weak (+)	Weak (+)	None (0)
S52	*S. hominis*	Positive (+)	Moderate (++)	Moderate (++)	Weak (+)
S53	*S. hominis*	Positive (+)	Weak (+)	Strong (+++)	None (0)
S54	*S. hominis*	Negative (-)	None (0)	None (0)	None (0)
S55	*S. hominis*	Positive (+)	Moderate (++)	Strong (+++)	Weak (+)
S56	*S. hominis*	Positive (+)	Strong (+++)	Moderate (++)	Weak (+)
S57	*S. hominis*	Positive (+)	Strong (+++)	Weak (+)	Moderate (++)
S58	*S. hominis*	Negative (-)	Moderate (++)	Strong (+++)	Moderate (++)
S59	*S. capitis*	Negative (-)	Weak (+)	Moderate (++)	None (0)
S60	*S. capitis*	Positive (+)	Strong (+++)	Moderate (++)	Moderate (++)
S61	*S. capitis*	Positive (+)	Moderate (++)	Moderate (++)	Weak (+)
S62	*S. capitis*	Positive (+)	Weak (+)	Weak (+)	None (0)
S63	*S. capitis*	Positive (+)	Strong (+++)	Moderate (++)	Weak (+)

4.2. BIOFILM FORMATION ASSAY

4.2.1. Biofilm producing isolates

In three different growth media, the biofilm production of *staphylococcus species* was studied in different environmental conditions (normal, CO_2, anaerobic). (Figure 2,3,4) It was determined whether the isolates were more or less effective than the positive and negative controls by measuring the amount of crystal violet dye that was recovered from each biofilm dependent on its OD (Figure 2,3,4). Biofilm production and non-production were divided into four categories [None (0), Weak (+), Moderate (++), and Strong (+++)]. Table 2

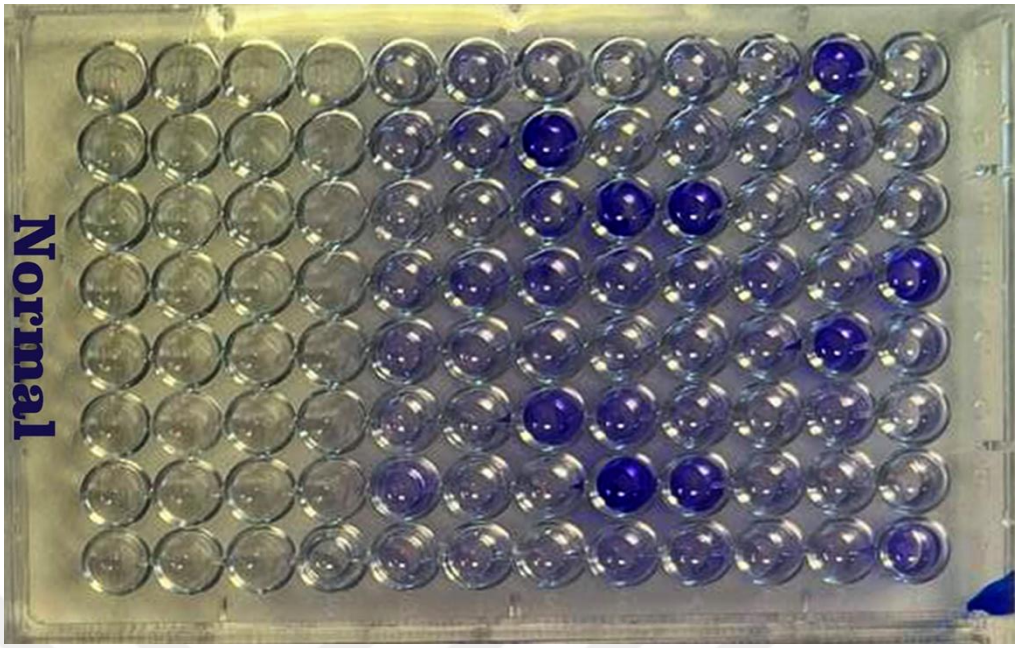

Figure 2: Biofilm formation on a microtiter in normal conditions

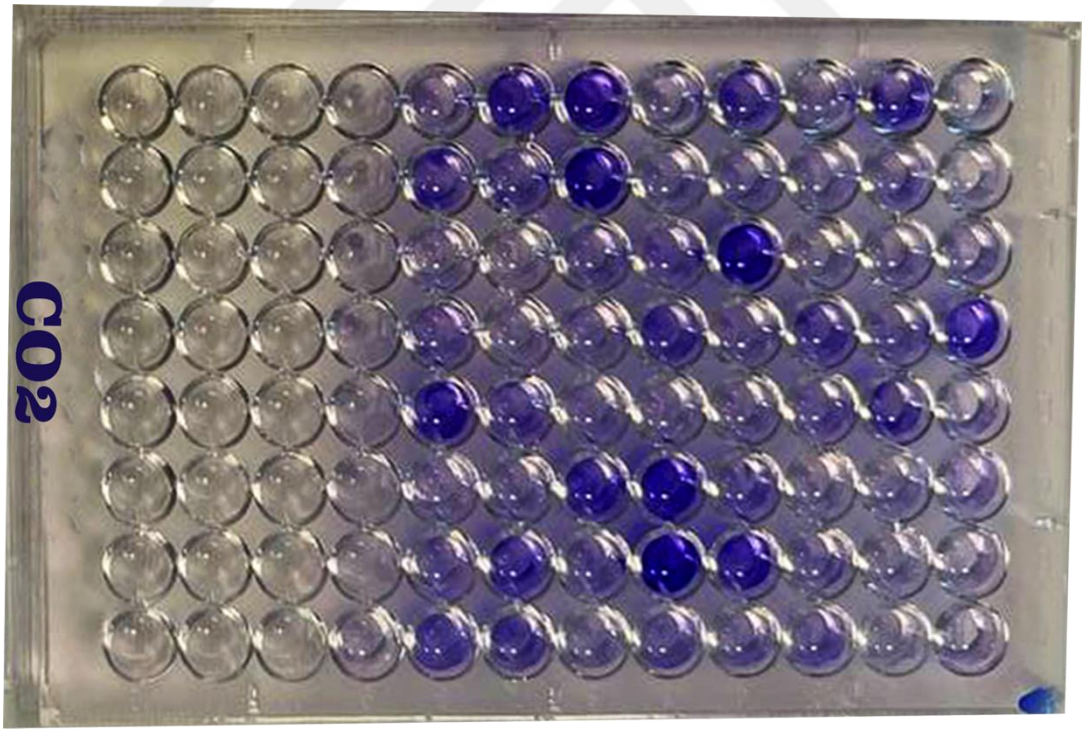

Figure 3: Biofilm formation on a microtiter in CO_2 conditions

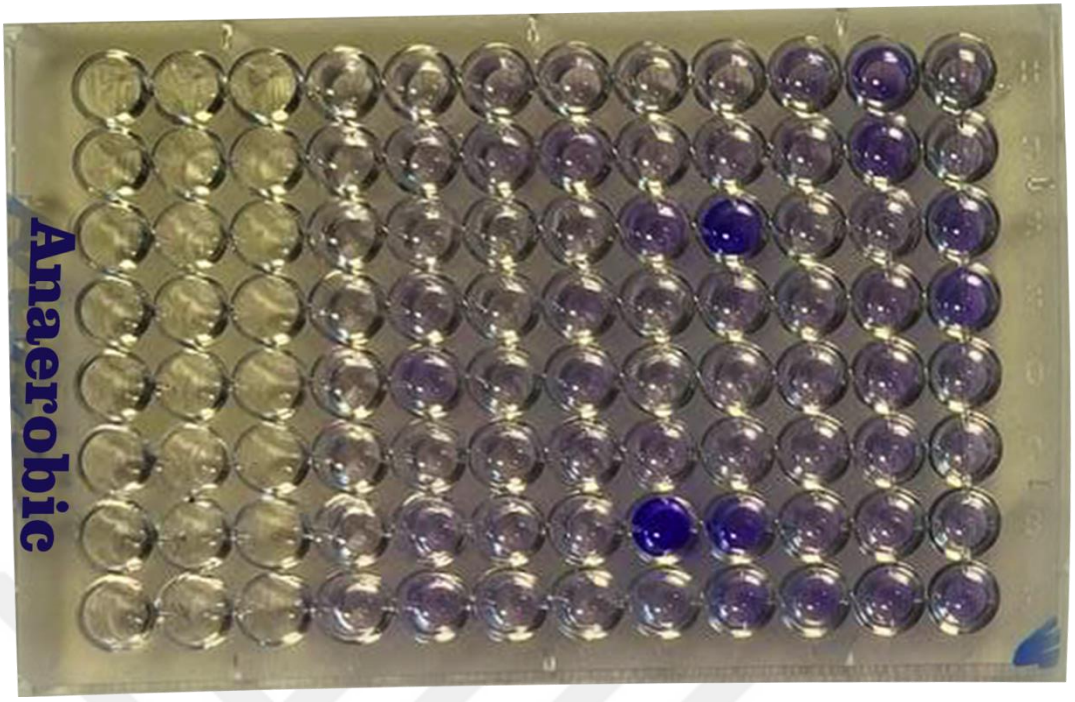

Figure 4: Biofilm formation on a microtiter in anaerobic conditions

The results showed a difference in all the different conditions. The normal condition and CO_2 were 97% (number = 61) biofilm-producing and 3% (number = 2) not biofilm producing in all staphylococcal strains, in contrast to the anaerobic condition, 76% (number = 48) produced biofilm and 24% (number = 15) did not produce biofilm of all staphylococcal strains. The ability to produce biofilm was summarized by taking different types and strains of *Staphylococcus* from clinical sources, all the results that appeared show that each type and strain of staphylococcus has the ability to produce biofilm under the same conditions as well as in different conditions (Table 2). When the ability for biofilm creation was taken into consideration, the circumstances had no effect on the bacteria that produced strong biofilms ($p>0.05$). On the contrary, there was a significant difference for non-biofilm producers, weak/moderate biofilm producers, their biofilm production is affected by the different conditions ($p\leq0.05$).

Under anaerobic condition, the ability of biofilm production of the isolates was decreased ($p\leq0.05$). There was no significant difference between normal and CO_2 conditions in terms of capacity to produce biofilm ($p>0.05$).

Table 2: Comparison of biofilm formation under different conditions

Biofilm production %	Different Conditions			
	Normal	CO_2	Anaerobic	
None (0)	2 (3%)	2 (3%)	15 (24%)	
Weak (+)	22 (35%)	19 (30%)	27 (43%)	$p \leq 0.05$
Moderate (++)	20 (32%)	24 (38%)	14 (22%)	
Strong (+++)	19(30%)	18 (29%)	7 (11%)	$p > 0.05$
Total	63 (100 %)	63 (100%)	63 (100%)	

4.2.2. Biofilm formation among species level under different conditions

These types of staphylococci for (N= 63) were studied from clinical samples and were classified as follows: "*S. aureus* (N= 17), *S. haemolyticus* (N= 3), *S. epidermidis* (N= 22), *S. hominis* (N= 16), *S. capitis* (N= 5)". Table 3 When the types of the bacteria and their ability to produce biofilm under different conditions were evaluated, no significant difference were detected ($p > 0.05$). It worth mentioning that, under anaerobic conditions, bacterial isolates were affected not significantly but very close to the statistical significance.

On the other hand, when *S. aureus* and coagulase negative bacteria were examined, they were significant difference in terms of biofilm production between them under normal and anaerobic conditions ($p \leq 0.05$).

Under different conditions, the capacity of biofilm formation among *S. aureus* was not affected. But there was a significant difference among coagulase negative staphylococci ($p \leq 0.05$).

Isolation of *staphylococcus species* that were affected by different conditions (normal, CO_2, anaerobic), and compared with each other in terms of biofilm production based on [none (0), weak (+), moderate (++), strong (+++)]. (Figure 5,6)

In normal and anaerobic condition, the highest concentration of S34 strain was significantly able to produce biofilm (strong +++) among the strains obtained. (Figure 5) In CO_2 condition, the highest concentration of S47 strain was significantly able to produce biofilm (strong +++) among the strains obtained. (Figure 5)

In Figure 4, it was noted that there is a clear difference between the types of staphylococci to produce biofilm in the same conditions and in other conditions. In

Staphylococcus epidermidis in anaerobic condition, no biofilm production was observed, in contrast to normal and CO_2 conditions, as well as in normal conditions and CO_2, it has the ability to produce moderate (++) and strong (+++) biofilm. (Figure 6) In *Staphylococcus hominis*, we observe in the anaerobic condition the inability to produce a strong biofilm, in contrast to the normal and CO_2 conditions. (Figure 6)

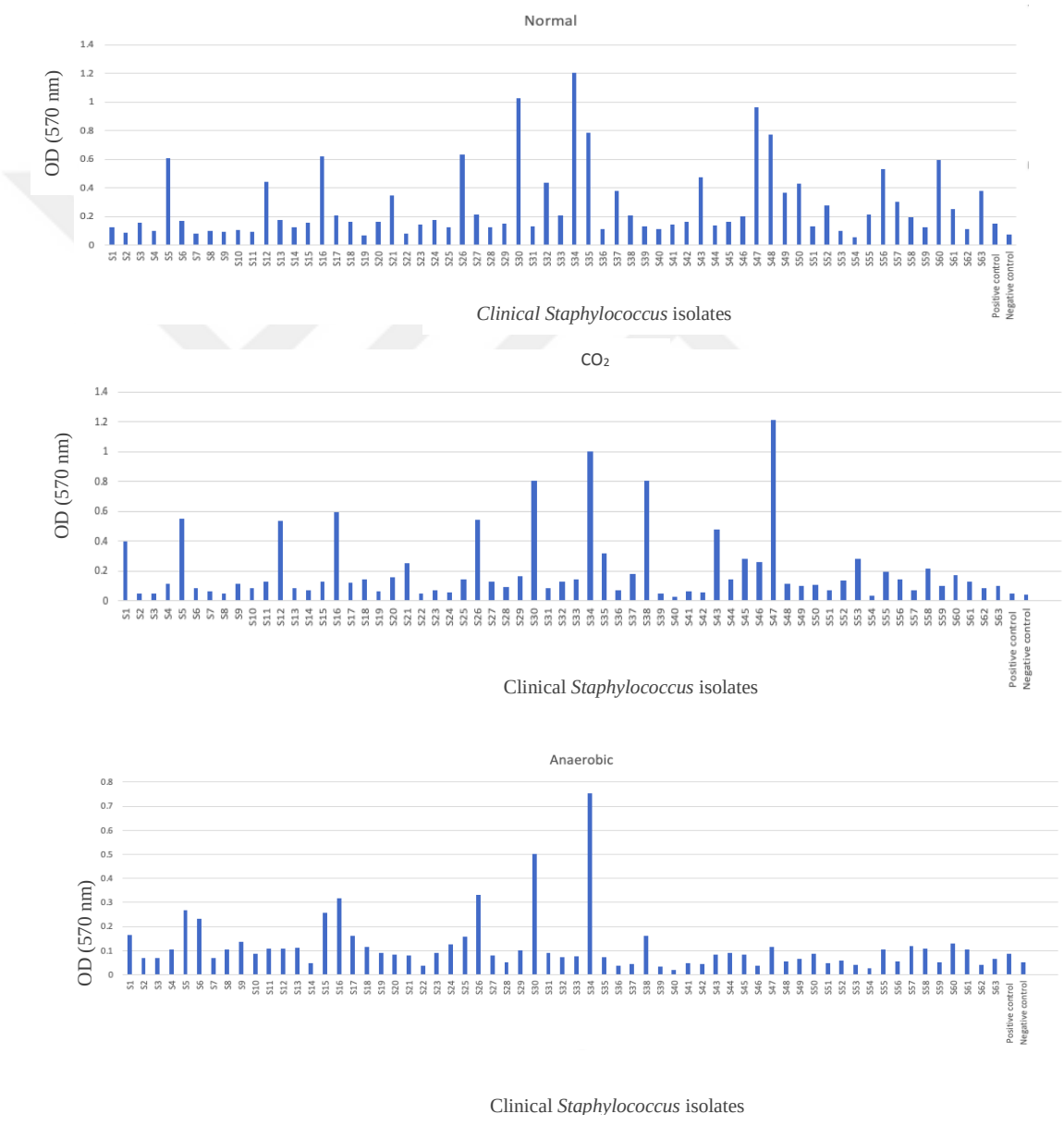

Figure 5: Biofilm formation clinical Staphylococcus isolates determined at 570 nm OD in different conditions

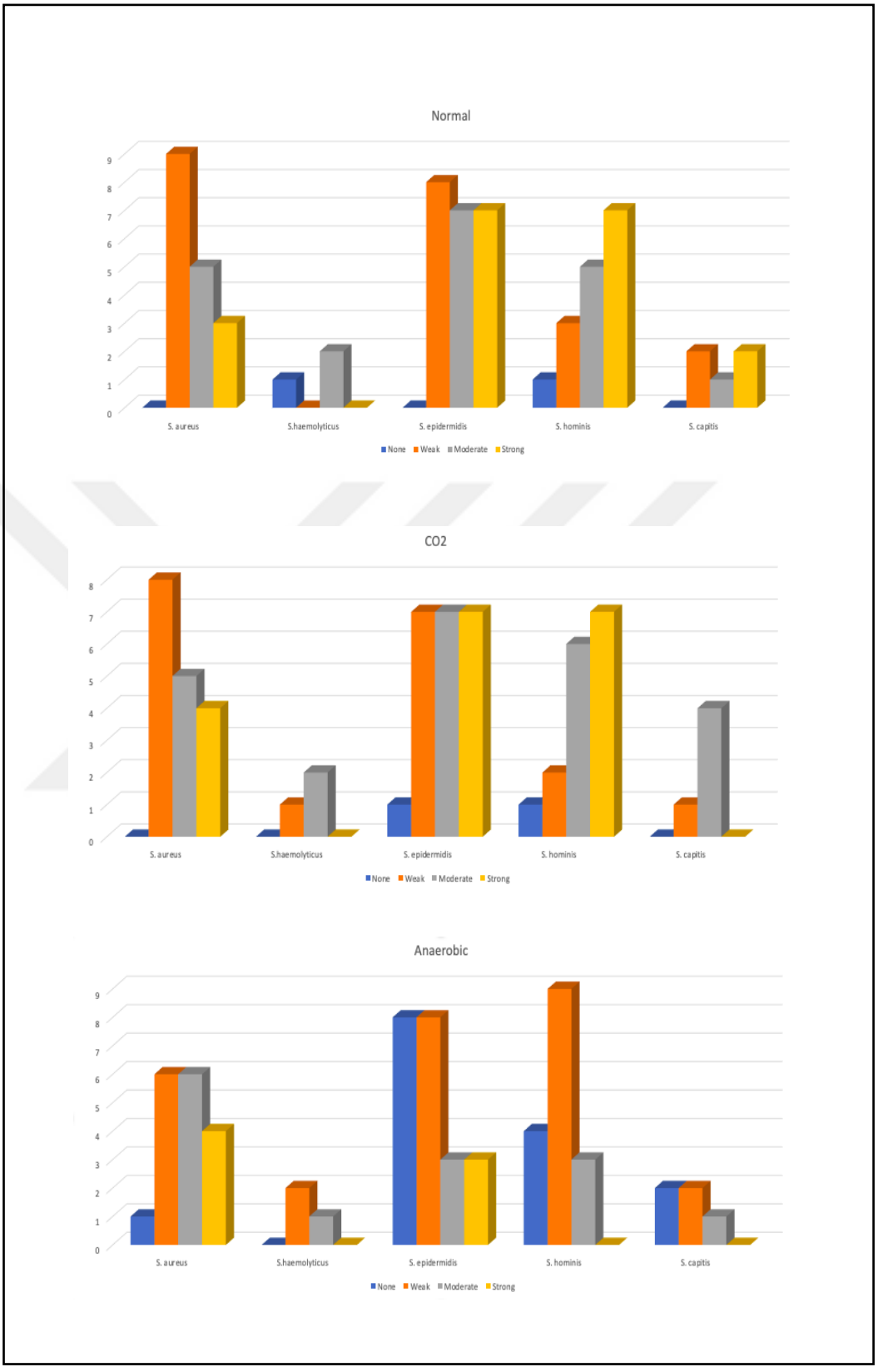

Figure 6: Biofilm formation at the level of staphylococcal species in different conditions

Table 3: Comparison of biofilm formation among species level under different conditions

Clinical Isolates			*S. aureus*	*S. haemolyticus*	*S. epidermidis*	*S. hominis*	*S. capitis*	Total	
Number			17(27%)	3 (5%)	22 (35%)	16(25%)	5 (8%)	63(100 %)	
Different conditions	**Normal**	None	0 (0%)	1 (33%)	0 (0%)	1 (6%)	0 (0%)	2	
		Weak	9 (53%)	0 (0%)	8 (36%)	3 (19%)	2 (40%)	22	63
		Moderate	5 (29%)	2 (67%)	7 (32%)	5 (31%)	1 (20%)	20	
		Strong	3 (18%)	0 (0%)	7 (32%)	7 (44%)	2 (40%)	19	
	CO_2	None	0 (0%)	0 (0%)	1 (4%)	1 (6%)	0 (0%)	2	
		Weak	8 (47%)	1 (33%)	7 (32%)	2 (12%)	1 (20%)	19	63
		Moderate	5 (29%)	2 (67%)	7 (32%)	6 (38%)	4 (80%)	24	
		Strong	4 (24%)	0 (0%)	7 (32%)	7 (44%)	0 (0%)	18	
	Anaerobic	None	1 (6%)	0 (0%)	8 (36%)	4 (25%)	2 (40%)	15	
		Weak	6 (35%)	2 (67%)	8 (36%)	9 (56%)	2 (40%)	27	63
		Moderate	6 (35%)	1 (33%)	3 (14%)	3 (19%)	1 (20%)	14	
		Strong	4 (24%)	0 (0%)	3 (14%)	0 (0%)	0 (0%)	7	

p>0.05

4.2.3. Biofilm formation and Methicillin resistance (MR)

It was noted that *Staphylococcus aureus* 76% is Methicillin- sensitive. Rest of the species staphylococci is (*Staphylococcus epidermidis* 77% is Methicillin-Resistant, *S. hominis* 81% is Methicillin-Resistant, etc). The mean there is a difference between *S. aureus* and the rest of the species. Table 4

In the results it was found that the biofilm in *Staphylococcus aureus* and *Staphylococcus hemolyticus* is highly production on different conditions (normal, CO_2, anaerobic). There is no effect under normal and CO_2 conditions on biofilm in all species, in contrast to the anaerobic condition, it affects *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Staphylococcus capitis* to a small extent. Table 4.

In general, it was found that anaerobic condition has a clear effect on the biofilm production through the results, and that the normal and CO_2 conditions have no clear effect. Table 4

Table 4: Methicillin resistance vs Biofilm formation

Clinical isolate	Number	Methicillin Resistance*		Biofilm Positives		
				Normal	CO_2	Anaerobic
S. aureus	17	MR	4 (24%)	4 (100%)	4 (100%)	4 (100%)
		MS	13 (76%)	13(100%)	13 (100%)	12 (92%)
S. haemolyticus	3	MR	2 (67%)	1 (50%)	2(100%)	2 (100%)
		MS	1 (33%)	1 (100%)	1(100%)	1 (100%)
S. epidermidis	22	MR	17 (77%)	17(100%)	16 (94%)	11 (65%)
		MS	5 (23%)	5(100%)	5(100%)	3(60%)
S. hominis	16	MR	13 (81%)	13 (100%)	13(100%)	11(85%)
		MS	3 (19%)	2 (67%)	2 (67%)	1(33%)
S. capitis	5	MR	4 (80%)	4 (100%)	4 (100%)	3 (75%)
		MS	1 (20%)	1 (100%)	1(100%)	0 (0%)
Total	63		63	61(97%)	61(97%)	48(76%)

*MR: Methicillin Resistant, MS: Methicillin sensitive

5. DISCUSSION AND CONCLUSION

Staphylococci are a type of bacteria that are found throughout the world. are found in humans and animals' normal microflora. Depending on whether or not they produce coagulase, staphylococci are divided into two primary groups: coagulase-negative staphylococci (CoNS) and coagulase-positive staphylococci (CoPS). *Staphylococcus species* were first identified by Friedrich Rosenbach, who discovered that yellow/orange colonies corresponded to CoPS species and white colonies corresponded to CoNS species. *Staphylococcus aureus*, a member of the CoPS group, and *Staphylococcus epidermidis*, a member of the CoNS group, are the most isolated members of their respective groups, which is why the vast majority of CoNS investigations are focused on this particular strain of bacteria. However, CoNS encompasses a diverse group of bacteria, including *Staphylococcus haemolyticus*, *Staphylococcus hominis*, and *Staphylococcus capitis*, totaling approximately 50 species that are currently classified into approximately 40 major species and approximately 20 subspecies. Because of their widespread distribution as skin colonizers, CoNS have typically been treated as culture pollutants rather than as the primary agent of severe diseases. In spite of their seemingly benign host interaction, it is now known that these species are capable of causing serious infections, particularly in immunocompromised patients. As a result, they are now classified as opportunistic pathogens and are becoming increasingly important in the healthcare environment. (França et al., 2021)

One of the leading causes of hospital and community-acquired infections is *S. aureus*, an opportunistic bacterium with high pathogenic potential. *S. aureus* clonal strains are capable of producing biofilms of various shapes and sizes, which have been linked to persistent infection. For all that we know, there is still a lot we don't know about this pathogen's molecular mechanisms that control biofilm development, even though this has been an area of study for quite some time. Antibiotic-resistant infections affect more than 2.8 million people each year in the United States, and as the post-antibiotic age approaches, we must take extreme efforts to discover alternative treatment and prevention tactics. A very virulent opportunistic pathogen, *S. aureus* has been linked in over 320,000 illnesses, making it the most common pathogen in the world. It is

common for *S. aureus* to infect patients in hospitals, infecting a third of the population on a constant basis. It is also a key cause of dormant organ infections produced by biofilms in hospitals. In most cases, bacterial cells clinging to the surface of an implant, such as a catheter, a heart valve, or an artificial joint, cause a device-associated infection to develop. Bacteria produce DNA, carbohydrates, and proteins once they have been bonded together, resulting in the formation of an extracellular polymeric matrix. Population heterogeneity, persistent cell production, stress response regulation, and microclimate change can all be found in biofilms. As a result, a combination of these characteristics impacts medication stability and penetration, making *S. aureus* and other biofilm-forming bacteria resistant to the vast majority of the antimicrobials in our arsenal of weapons. Moreover, because biofilms are associated with chronic and persistent infection, aggressive treatment measures such as implant removal as well as the removal of infected tissue and bone are required (Tomlinson, et al., 2021)

Microorganisms, such as *Staphylococcus aureus*, have the astonishing capacity to encase themselves in a protective environment known as a biofilm, which allows them to live and cause stubborn infections for extended periods. Specifically, the primary goal of this review was to explore the impact of various environmental circumstances on the production of biofilms in *S. aureus* in general, as well as the clinical implications of these findings. Techniques such as microtiter screening in conjunction with crystal violet staining, Confocal laser scanning microscopy, and mass spectrometry, as described in this review, provide detailed high-throughput information on biofilm morphology in various states and aid in the identification of individual EPS components. The mechanism of action of anti-biofilm drugs used to prevent or disrupt *S. aureus* biofilm development remains a mystery, despite tremendous progress in understanding the structure, architecture, and dynamics of *S. aureus* biofilm formation. Also lacking is knowledge on the effect of anti-biofilm agents, as well as their method of action, on the physiology of cells trapped in different levels of biofilms, which is a major concern. To close these knowledge gaps, additional study is required. Intriguingly, is it possible that these anti-biofilm agents have specific targets (EPS/polysaccharide proteins), such as eDNA or urate cells, or whether they inhibit and proliferate biofilms through a number of different mechanisms? It will be possible

to forecast whether or not hospital infections such as *S. aureus* will acquire resistance to these novel drugs once the precise mechanism of action has been uncovered. (Idrees et al., 2021)

Use of innovative combination antibiotic and antimicrobial agents with diverse modes of action can avoid the development of antibiotic and antimicrobial resistance in microorganisms' Functional excipients, such as amino acids and anti-biofilm compounds, can contribute in the creation of novel medications in various therapeutic areas if their multimodal effects are understood. Because these medicines can target specific biofilm-related diseases produced by this notorious pathogen, they can contribute in the fight against antimicrobial resistance by providing focused treatment for these infections. Idrees and colleagues (Idrees, et al, 2021)

A biofilm is formed when bacteria attach themselves to surfaces in a range of environments. This allows germs to survive longer periods of time and continue to represent a major threat to the patient's recovery. The bacteria *Staphylococcus aureus* creates permanent biofilms that are difficult to eliminate due to the bacteria's enhanced resistance to drugs and environmental stressors. As with pathogenicity, the ability to master biofilms differs between clonal *S. aureus* strains on a phenotypic level. In part, this is due to the fact that the majority of what we now know about active regulation in biofilms is based on mature populations, and hence little is known about the transitory regulation that drives association, proliferation, maturation, and dissemination in biofilms. (Tomlinson, et al. 2021)

This coating of host proteins such as fibrinogen and fibronectin helps to stabilize and protect the implant after it has been implanted into the human body. To form a biofilm, *S. aureus* bacteria must be in close contact, reproduce, grow into adulthood and disperse. Intercellular adhesion factors and host colonization factors, such as MSCRAM, are the first steps in this process. Factors are grouped together. FBF-binding proteins Clf and fibronectin Both Fnb and Sdr interact with the host protein and aid in the colonization of implant surfaces. The bacteria in the biofilm secrete DNA, polysaccharides, and proteins to carry out biofilm replication. The peptididoglycan hydrolase protein is responsible for releasing these components from cells, while the enzyme icaADBC helps to form the matrix by producing the amino

acid IPA. Implant surfaces and host proteins become inaccessible to proliferating cells within this matrix, requiring them to adhere to EPS. Although some MSCRAM, such as Sdr and Fnb, have the ability to self-associate in order to perform this job, IgG-binding protein A is the predominant component of the extracellular matrix that aids in the assembly of the bacterium. Different microcolonies within a biofilm display different growth characteristics and protein expression patterns as the biofilm matures, indicating that the biofilm is becoming more complex. The procedure is then repeated in a different location. (Tomlinson et al.,2021)

The virulence factors of CoNS were found to be lower than those of CoPS due to their 'absence' of thrombosis. The outcomes and frequent updates on species and subspecies have revealed a diverse set of species that range from non-pathogenic to facultatively pathogenic, with variable degrees of virulence potential, as well as species that are non-pathogenic but have facultatively pathogenic potential. Certain isolates have lately been discovered as pathogenic bacteria with a high virulence effect. This coating of host proteins such as fibrinogen and fibronectin helps to stabilize and protect the implant after it has been implanted into the human body. To form a biofilm, *S. aureus* bacteria must be in close contact, reproduce, grow into adulthood and disperse. Intercellular adhesion factors and host colonization factors, such as MSCRAM, are the first steps in this process. Factors are grouped together. FBF-binding proteins Clf and fibronectin Both Fnb and Sdr interact with the host protein and aid in the colonization of implant surfaces. The bacteria in the biofilm secrete DNA, polysaccharides, and proteins to carry out biofilm replication. The peptididoglycan hydrolase protein is responsible for releasing these components from cells, while the enzyme icaADBC helps to form the matrix by producing the amino acid IPA. Implant surfaces and host proteins become inaccessible to proliferating cells within this matrix, requiring them to adhere to EPS. Although some MSCRAM, such as Sdr and Fnb, have the ability to self-associate in order to perform this job, IgG-binding protein A is the predominant component of the extracellular matrix that aids in the assembly of the bacterium. Different microcolonies within a biofilm display different growth characteristics and protein expression patterns as the biofilm matures, indicating that the biofilm is becoming more complex. The procedure is then repeated in a different location. (Tomlinson et al.,2021)

Antimicrobial susceptibility testing and methicillin resistance were determined in this study for previously identified clinical isolates of *Staphylococci species* "*S. aureus, S. haemolyticus, S. epidermidis, S. hominis,* and *S. capitis*". According to this study, 63% of staphylococci clinical isolates are methicillin-resistant, while 37% are methicillin-sensitive. However, there was a difference in the species tested for methicillin resistance; *Staphylococcus aureus* was 76 percent sensitive and 24 percent resistant, whereas the rest of the species were 20-25 percent sensitive and 75-80 percent resistant.

The effect of different conditions (normal, CO_2, anaerobic) on the biofilm analysis of these clinical isolates was then examined, results indicating that natural and CO_2 conditions had no effect on biofilm formation, 97 percent positive and 3% negative, respectively, while anaerobic condition had 76 percent positive and 24 percent negative.

When the capacity for biofilm production was taken into consideration, the conditions had no effect on the bacteria that produced strong biofilms ($p>0.05$). On the contrary, there was a significant difference for non-biofilm producers, weak/moderate biofilm producers, their biofilm production is affected by the different conditions ($p\leq0.05$). Under anaerobic condition, the ability of biofilm production of the isolates was decreased ($p\leq0.05$). There was no statistically significant difference in the ability to form biofilm between normal and CO_2 conditions ($p>0.05$).

When the types of the bacteria and their ability to produce biofilm under different conditions were evaluated, no significant difference were detected ($p>0.05$). It worth mentioning that, under anaerobic conditions, bacterial isolates were affected not significantly but very close to the statistical significance. On the other hand, when *S. aureus* and coagulase negative bacteria were examined, they were significant difference in terms of biofilm production between them under normal and anaerobic conditions ($p\leq0.05$). Under different conditions, the capacity of biofilm formation among *S. aureus* was not affected. A statistically significant difference was found among CoNS ($p \leq0.05$).

Researchers Idrees and colleagues discovered that the biofilm had a great tolerance to a variety of different environmental conditions. According to the findings of the study by Tomlison et al., the biofilm exhibits great resilience to a wide range of environmental conditions as well as increased resistance to antibiotics. In the study of France et al., the biofilm of CoNS is a major virulence factor. According to our study, biofilm formation is not affected under normal and CO_2 conditions. But; under anaerobic condition, biofilm formation decreased significantly.

This study discusses Staphylococcal bacteremia in patients, which is caused by implanted devices (prosthetic limbs, joints, and catheters in blood vessels), immunocompromised patients, contaminated medical devices, and inappropriate antibiotic use by patients. The findings indicate that eradicating this infection will be difficult due to its resistance to antibiotics and its resistance to a variety of environmental conditions due to its ability to produce biofilm.

REFERENCES

Abolghait SK, Fathi AG, Youssef FM, Algammal AM. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. Int. J. Food Microbiol. 328, 108669.

Adams M. (2009). *Staphylococcus aureus* and other pathogenic Gram-positive cocci. In Foodborne Pathogens; de Blackburn, C.W, McClure, P.J, Eds; Woodhead Publishing Series in Food Science, Technology and Nutrition; Elsevier: Cambridge, UK, pp. 802–819. ISBN 9781845693626.

Agostinho A, James G, Wazni O, Citron M, Wilkoff BD. (2009). Inhibition of *Staphylococcus aureus* Biofilms by a Novel Antibacterial Envelope for Use with Implantable Cardiac Devices. Clin. Transl. Sci. 2, 193–198.

Algburi A, Comito N, Kashtanov D, Dicks LMT, Chikindas ML. (2017). Control of biofilm formation: Antibiotics and beyond. Appl. Environ. Microbiol. 83, e02508-16.

Alonzo F, Torres VJ. (2014). The Bicomponent Pore-Forming Leucocidins of *Staphylococcus aureus*. Microbiol Mol Biol Rev 78, 199–230.

Al-Sultany ZK, Al-Charrakh AH. (2020). Antibiotic resistance patterns of coagulase negative *Staphylococcus* (CoNS) strains isolated from blood stream infections in Babylon province, Iraq. Annals of Tropical Medicine and Public Health, 23, 231-633.

Amyes SG. (2005). Treatment of staphylococcal infection. BMJ, 330, 976–977.

Archer NK, Mazaitis MJ, William Costerton J, Leid JG, Powers ME, Shirtliff ME. (2011) *Staphylococcus aureus* biofilms: Properties, regulation and roles in human disease.Virulence , 2, 445–459.

Arciola CR, Campoccia D, Montanaro L. (2018). Implant infections: Adhesion, biofilm formation and immune evasion. Nat. Rev. Microbiol. 16, 397–409.

Argemi X, Hansmann Y, Prola K, Prévost G. (2019). Coagulase-negative staphylococci pathogenomics. International journal of molecular sciences, 20(5), 1215.

Aswani V, Najar F, Pantrangi M, Mau B, Schwan WR, Shukla SK. (2019). Virulence factor landscape of a *Staphylococcus aureus* sequence type 45 strain, MCRF184. BMC Genomics 20, 123.

Azmi K, Qrei W, Abdeen Z. (2019). Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of *Staphylococcus aureus* isolated from Palestinian patients. BMC Genom. 20, 571–578.

Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. (2008). Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J. Bacteriol. 190, 300-310.

Bazargani MM, Rohloff J. (2016). Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control, 61, 156–164.

Bhattacharya M, Wozniak DJ, Stoodley P, Hall-Stoodley L. (2015) Prevention and treatment of *Staphylococcus aureus* biofilms. Expert Rev. Anti. Infect. Ther. 2015, 13, 1499–1516.

Cao Z, Casabona MG, Kneuper H, Chalmers JD, Palmer T. (2016). The type VII secretion system of *Staphylococcus aureus* secretes a nuclease toxin that targets competitor bacteria. Nat. Microbiol. 2, 16183.

Casey AL, Lambert PA, Elliott TSJ. (2007). Staphylococci. International journal of antimicrobial agents, 29, S23-S32.

Chen Q, Xie S, Lou X, Cheng S, Liu X, Zheng W, Zheng Z, Wang H. (2020). Biofilm formation and prevalence of adhesion genes among *Staphylococcus aureus* isolates from different food sources. Microbiologyopen, 9, e00946.

Chmielewski RAN, Frank JF. (2003). Biofilm formation and control in food processing facilities. Compr. Rev. Food Sci. Food Saf. 2, 22–32.

Chung HY, Kim YT, Kwon JG, Im HH, Ko D, Lee JH, Choi SH. (2021). Molecular interaction between methicillin-resistant *Staphylococcus aureus* (MRSA) and chicken breast reveals enhancement of pathogenesis and toxicity for food-borne outbreak. Food Microbiology, 93, 103602.

Corrigan RM, Rigby D, Handley P, Foster TJ. (2007). The role of *Staphylococcus aureus* surface protein SasG in adherence and biofilm formation. Microbiology 153, 2435–2446.

Crémet L, Corvec S, Batard E, Auger M, Lopez I, Pagniez F, Dauvergne S, and Caroff N. (2013). Comparison of three methods to study biofilm formation by clinical strains of Escherichia coli. Diagnostic Microbiology and Infectious Disease, 75(3), 252–255.

DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. (2010). Community-associated meticillinresistant *Staphylococcus aureus*. Lancet 375, 1557–1568.

Donlan RM (2002). Biofilms: Microbial Life on Surfaces. Emerg. Infect. Dis. 8, 881 890.

Donlan RM. (2000). Role of biofilms in antimicrobial resistance. ASAIO J. 46, S47–S52.

Dupre JM, Jonson WL, Ulanov AV, Li Z, Wilkinson BJ, Gustafson JE. (2019). Transcriptional profiling and metabolomic analysis of *Staphylococcus aureus* grown on autoclaved chicken breast. Food. Microbiol. 82, 46-52.

Durand G, Javerliat F, Bes M, Veyrieras JB, Guigon G, Muqnier N, Schicklin S, Kaneko G, Santiago- Allexant E, Bouchiat C, Martins-Simões P, Laurent F,

Van Belkum A, Vandenesch F, Tristan A. (2018). Routine whole-genome sequencing for outbreak investigations of *Staphylococcus aureus* in a national reference center. Front. Microbiol. 20, 511.

Dutta A, Bhattacharyya S, Kundu A, Dutta D, Das AK. (2016). Macroscopic amyloid fiber formation by staphylococcal biofilm associated SuhB protein. Biophys. Chem. 217, 32–41.

Euzeby JP. (2019) List of Prokaryotic names with standing in Nomenclature- Genus Staphylococcus. A, III

Fadeeva E, Schlie-Wolter S, Chichkov BN, Paasche G, Lenarz T. (2016). Structuring of biomaterial surfaces with ultrashort pulsed laser radiation. In Laser Surface Modification of Biomaterials: Techniques and Applications; Elsevier Inc.: Amsterdam, The Netherlands, pp. 145–172, ISBN 9780081009420.

Fogarty LR, Haack SK, Johnson HE, Brennan AK, Isaacs NM, Spencer C. (2015). *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) at ambient freshwater beaches. J. Water Health. 13, 680-692.

Fontecha-Umaña F, Ríos-Castillo AG, Ripolles-Avila C, Rodríguez-Jerez JJ. (2020). Antimicrobial activity and prevention of bacterial biofilm formation of silver and zinc oxide nanoparticle-containing polyester surfaces at various concentrations for use. Foods, 9, 442.

Foster TJ, Geoghegan JA, Ganesh VK, Hook M. (2014). Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. Nat. Rev. Microbiol. 12, 49-62.

Foster TJ. (2005). Immune evasion by Staphylococci. Nat. Rev. Microbiol. 3, 948-958.

Fox A, Pichon B, Wilkinson H, Doumith M, Hill RL, McLauchlin J, Kearns AM. (2017). Detection and molecular characterization of Livestock-Associated MRSA in raw meat on retail sale in North West England. Lett. Appl. Microbiol. 64, 239-245.

França A, Gaio V, Lopes N, Melo LD. (2021). Virulence factors in coagulase-negative Staphylococci. Pathogens, 10(2), 170.

Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. (2018) Biofilms in the Food Industry: Health Aspects and Control Methods. Front. Microbiol. 9, 898.

Guangyin Z, Youcai Z. (2017). Harvest of Bioenergy from Sewage Sludge by Anaerobic Digestion. In Pollution Control and Resource Recovery; Elsevier: Amsterdam, The Netherlands, pp. 181–273.

Hamadi F, Latrache H, Mabrrouki M, Elghmari A, Outzourhit A, Ellouali M, Chtaini A. (2005). Effect of pH on distribution and adhesion of *Staphylococcus aureus* to glass. J. Adhes. Sci. Technol. 19, 73–85.

Hamida RS, Ali MA, Goda DA, Khalil MI, Al-Zaban MI. (2020). Novel Biogenic Silver Nanoparticle-Induced Reactive Oxygen Species Inhibit the Biofilm Formation and Virulence Activities of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strain. Front. Bioeng. Biotechnol. 8, 433.

Health Protection Agency. (2007). Identification of *Staphylococcus species*, *Micrococcus species* and *Rothia species*. National Standard Method BSOP ID 7, issue 2.

Hennekinne JA, De Buyser ML, Dragacci S. (2012). *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol. Rev. 36, 815-836.

Herman-Bausier P, Labate C, Towell AM, Derclaye S, Geoghegan JA, Dufrêne YF. (2018). *Staphylococcus aureus* clumping factor A is a force-sensitive molecular switch that activates bacterial adhesion. Proc. Natl. Acad. Sci. USA, 115, 5564.

Hickey EE, Wong HS, Khazandi M, Ogunniyi AD, Petrovski KR, Garg S, Page SW, O'Handley R, Trott DJ. (2018). Repurposing Ionophores as novel

antimicrobial agents for the treatment of bovine mastitis caused by Gram-positive pathogens. J. Vet. Pharmacol. Ther. 41, 746–754.

Hochbaum AI, Kolodkin-Gal I, Foulston L, Kolter R, Aizenberg J, Losick R. (2011). Inhibitory Effects of d-Amino Acids on *Staphylococcus aureus* Biofilm Development. J. Bacteriol. 193, 5616.

Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. (2010). Antibiotic resistance of bacterial biofilms. *International journal of antimicrobial agents*, *35*(4), 322-332.

Holstege CP, Rifampin. (2014). In Encyclopedia of Toxicology: Third Edition; Elsevier: Amsterdam, The Netherlands, pp. 134–136. ISBN 9780123864543.

Holt JG. (1994). Gram-Positive cocci. In: Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST, editors. Bergey's Manual of Determinative Bacteriology. 9th ed.: Baltimore; p. 527-37. B, IV.

Hu H, Ramezanpour M, Hayes AJ, Liu S, Psaltis AJ, Wormald PJ, Vreugde S. (2019). Sub-inhibitory clindamycin and azithromycin reduce *S. aureus* exoprotein induced toxicity, inflammation, barrier disruption and invasion. J. Clin. Med. 8, 1617.

Huda S, Azmiza SJ, Tengju ZMTJ, Rosni I. (2017). A review of staphylococcal cassette chromosome mec (SCCmec) types in coagulase-negative staphylococci (CoNS) species. Malays. J. Med. Sci. 24, 7-18.

Idrees M, Mohammad AR, Karodia N, Rahman A. (2020). Multimodal Role of Amino Acids in Microbial Control and Drug Development. Antibiotics 9, 330.

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

James H, Jorgensen MAP, Karen C, Carroll, Guido Funke. (2015). Manual of clinical microbiology, 11th ed:354-422. B, III.

Kaplan JB, Mlynek KD, Hettiarachchi H, Alamneh YA, Biggemann L, Zurawski DV, Black CC, Bane CE, Kim RK, Granick MS. (2018). Extracellular polymeric substance (EPS)-degrading enzymes reduce staphylococcal surface attachment and biocide resistance on pig skin in vivo. PLoS ONE 13, e0205526.

Kavanagh KT. (2019). Control of MSSA and MRSA in the United States: protocols, policies, risk adjustment and excuses. Antimicrob. Resist. Infect. Control 8, 103.

Kennedy AD, DeLeo FR. (2009). Epidemiology and virulence of community-associated MRSA. Clin. Microbiol. Newsl. 31, 153–160.

Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. (2018) Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon, 4, 1067.

Kielian T, Cheung A, Hickey WF. (2001). Diminished virulence of an alpha-toxin mutant of *Staphylococcus aureus* in experimental brain abscesses. Infect. Immun. 69, 6902-6911.

Kim K. (2012). Neuroimmunological Mechanism of Pruritus in Atopic Dermatitis Focused on the Role of Serotonin. Biomolecules and Therapeutics 20, 506–512.

Kluytmans JA. (2010). Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency. Clin. Microbiol. Infect. 16, 11- 15.

Kobayashi SD, Malachowa N, Deleo FR. (2015). Pathogenesis of *Staphylococcus aureus* abscesses. Am. J. Pathol. 185, 1518–1527.

Kostakioti M, Hadjifrangiskou M, Hultgren SJ. (2013). Bacterial biofilms: Development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb. Perspect. Med. 3, a010306.

Kot B, Sytykiewicz H, Sprawka I. (2018). Expression of the Biofilm-Associated Genes in Methicillin-Resistant *Staphylococcus aureus* in Biofilm and Planktonic Conditions. Int. J. Mol. Sci., 19, 3487.

Krasowska A, Sigler K. (2014). How microorganisms use hydrophobicity and what does this mean for human needs? Front. Cell. Infect. Microbiol., 4.

Lacey KA, Leech JM, Lalor SJ, McCormack N, Geoghegan JA, McLoughlin RM. (2017). The *Staphylococcus aureus* cell wallanchored protein clumping factor A is an important T cell antigen. Infect. Immun. 85.

Landini P, Antoniani D, Burgess JG, Nijland R. (2010). Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Appl. Microbiol. Biotechnol. 86, 813–823.

Le KY, Otto M. (2015). Quorum-sensing regulation in staphylococci-an overview. Front. Microbiol. 6, 1174.

Lister JL, Horswill AR. (2014). *Staphylococcus aureus* biofilms: Recent developments in biofilm dispersal. Front. Cell. Infect. Microbiol. 4, 178.

Löffler B, Tuchscherr L. (2021). *Staphylococcus aureus* Toxins: Promoter or Handicap during Infection.

Lowy FD. (1998). *Staphylococcus aureus* infections. N. Engl. J. Med. 339, 520-532.

Maikranz E, Spengler C, Thewes N, Thewes A, Nolle F, Jung P, Bischoff M, Santen L, Jacobs K. (2020). Different binding mechanisms of: *Staphylococcus aureus* to hydrophobic and hydrophilic surfaces. Nanoscale, 12, 19267–19275.

Manzur A, Gavalda L, de Gopegui ER, Mariscal D, Dominguez MA, Perez JL, Segura F, Pujol M. (2008). Spanish Network for Research in, I.D. Prevalence of methicillin-resistant *Staphylococcus aureus* and factors associated with colonization among residents in community long-term-care facilities in Spain. Clin. Microbiol. Infect. 14, 867–872.

Marques SC, Rezende JDGOS, Alves LADF, Silva BC, Alves E, de Abreu LR, Piccoli RH. (2007). Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. Braz. J. Microbiol. 38, 538–543.

Miao J, Lin S, Soteyome T, Peters BM, Li Y, Chen H, Su J, Li L, Li B, Xu Z, et al. (2019). Biofilm Formation of *Staphylococcus aureus* under Food Heat Processing Conditions: First Report on CML Production within Biofilm. Sci. Rep. 9.

Min SH, Kang MH, Sur JH, Park HM. (2014). *Staphylococcus pseudintermedius* infection associated with nodular skin lesions and systemic inflammatory response syndrome in a dog. Can. Vet. J. 55, 480–483.

Monteiro-Neto V, de Souza CD, Gonzaga LF, da Silveira BC, Sousa NCF, Pontes JP, Santos DM, Martins WC, Pessoa JFV, Carvalho Júnior AR, et al. (2020). Cuminaldehyde potentiates the antimicrobial actions of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. PLoS ONE, 15.

Moormeier DE, Bayles KW. (2017). *Staphylococcus aureus* biofilm: A complex developmental organism. Mol. Microbiol. 104, 365–376.

Nair D, Memmi G, Hernandez D, Bard J, Beaume M, Gill S, Francois P, Cheung AL. (2011). Wholegenome sequencing of *Staphylococcus aureus* strain RN4220, a key laboratory strain used in virulence research, identifies mutations that affect not only virulence factors but also the fitness of the strain. J. Bacteriol. 193, 2332-2335.

Nair S, Desai S, Poonacha N, Vipra A, Sharma U. (2016). Antibiofilm activity and synergistic inhibition of *Staphylococcus aureus* biofilms by bactericidal protein P128 in combination with antibiotics. Antimicrob. Agents Chemother. 60, 7280–7289.

Nanra JS, Buitrago SM, Crawford S, Ng J, Fink PS, Hawkins J, Scully IL, McNeil LK, Aste- Amézaga, JM, Cooper D, Jansen KU, Anderson AS. (2013). Capsular

polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Hum. Vaccin Immunother. 9, 480- 487.

Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, Dahbi G, Blanco M, del Carmen Ponte M, and Soriano F. (2008). Correlation between virulence factors and in vitro biofilm formation by *Escherichia coli* strains. Microbial Pathogenesis, 45(2), 86–91.

Nazir R, Zaffar MR, Amin I. (2019). Bacterial biofilms: The remarkable heterogeneous biological communities and nitrogen fixing microorganisms in lakes. In Freshwater Microbiology: Perspectives of Bacterial Dynamics in Lake Ecosystems; Elsevier: Amsterdam, The Netherlands, pp. 307–340, ISBN 9780128174951.

Nazzaro F, Fratianni F, d'Acierno A, Coppola R, Jesus Ayala-Zavala F, Gomez da Cruz A, De Feo V. (2020). Essential Oils and Microbial Communication. In Essential Oils–Oils of Nature; IntechOpen: Lobdon, UK.

Neu TR, Lawrence JR. (2010). Extracellular Polymeric Substances in Microbial Biofilms. In Microbial Glycobiology; Elsevier Inc.: Amsterdam, The Netherlands, pp. 733–758. ISBN 9780123745460.

Nguyen HTT, Nguyen TH, Otto M. (2020). The staphylococcal exopolysaccharide PIA Biosynthesis and role in biofilm formation, colonization, and infection. Comput. Struct. Biotechnol. J. 18, 3324–3334.

Nourbakhsh F, Namvar AE. (2016). Detection of genes involved in biofilm formation in *Staphylococcus aureus* isolates. GMS Hyg. Infect. Control, 11, Doc07.

Novick RP, Geisinger E. (2008). Quorum Sensing in Staphylococci. Annual Review of Genetics 42, 541–564.

O'Neill E, Pozzi C, Houston P, Humphreys H, Robinson DA, Loughman A, Foster TJ, O'Gara JPA. (2008). Novel *Staphylococcus aureus* Biofilm Phenotype Mediated by the Fibronectin-Binding Proteins, FnBPA and FnBPB. J. Bacteriol. 190, 3835.

O'Toole GA. (2011). Microtiter dish biofilm formation assay. Journal of visualized experiments: JoVE, (47).

Otto M. (2010). Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. Annu. Rev. Microbiol. 64, 143–162.

Otto M. (2018). Staphylococcal Biofilms. Microbiol. Spectr, 6.

Ou C, Shang D, Yang J, Chen B, Chang J, Jin F, Shi C. (2020). Prevalence of multidrug-resistant *Staphylococcus aureus* isolates with strong biofilm formation ability among animal-based food in Shanghai. Food Control, 112, 107106.

Paharik AE, Horswill AR. (2016). The Staphylococcal Biofilm: Adhesins, Regulation, and Host Response. Microbiol. Spectr. 4.

Parrino B, Schillaci D, Carnevale I, Giovannetti E, Diana P, Cirrincione G, Cascioferro S. (2019). Synthetic small molecules as anti-biofilm agents in the struggle against antibiotic resistance. Eur. J. Med. Chem. 161, 154–178.

Peschel A, Otto M. (2013) Phenol-soluble modulins and staphylococcal infection. Nat. Rev. Microbiol.11, 667–673.

Petrova OE, Sauer K. (2012). Sticky situations: Key components that control bacterial surface attachment. J. Bacteriol. 194, 2413–2425.

Priest NK, Rudkin JK, Feil EJ, van den Elsen JM, Cheung A, Peacock SJ, Laabei M, Lucks DA, Recker M. (2012). From genotype to phenotype: can systems biology be used to predict *Staphylococcus aureus* virulence? Nat. Rev. Microbiol. 10, 791-797.

Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO. (2015). Agents that inhibit bacterial biofilm formation. Future Med. Chem. 7, 647–671.

Rahdar HA, Malekabad ES, Dadashi AR, Takei E, Keikha M, Kazemian H, and Karami- Zarandi M. (2019). Correlation between biofilm formation and

carbapenem resistance among clinical isolates of *Klebsiella pneumoniae*. Ethiopian journal of health sciences, 29(6), 745–750.

Rasmussen RV, Fowler VG Jr, Skov R, Bruun NE. (2011). Future challenges and treatment of *Staphylococcus aureus* bacteremia with emphasis on MRSA. Future Microbiol. 6, 43–56.

Rees CED, Green LH, Goldman E, Loessner MJ. (2015). Practical Handbook of Microbiology; Goldman, E., Green, L.H., Eds.; CRC Press: Boca Raton, FL, USA, ISBN 9780429168932.

Reffuveille F, Josse J, Vallé Q, Gangloff CM, Gangloff SC. (2017). *Staphylococcus aureus* Biofilms and their Impact on the Medical Field. In The Rise of Virulence and Antibiotic Resistance in *Staphylococcus aureus*; InTech: Rijeka, Croatia, Chapter 11, p. 187.

Riedel S, Morse SA, Mietzner TA, Miller S. (2019). Jawetz Melnick & Adelbergs Medical Microbiology 28 E. McGraw Hill Professional.

Rizzato C, Torres J, Kasamatsu E, Camorlinga-Ponce M, Bravo MM, Canzian F, Kato I. (2019). Potential role of biofilm formation in the development of digestive tract cancer with special reference to helicobacter pylori infection. Front. Microbiol. 10, 846.

Roy R, Tiwari M, Donelli G, Tiwari V. (2018). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence, 9, 522–554.

Sabat A, Melles DC, Martirosian G, Grundmann H, Van Belkum A, Hryniewicz W. (2006). Distribution of the serine-aspartate repeat protein-encoding sdr genes among nasal-carriage and invasive *Staphylococcus aureus* strains. J. Clin. Microbiol. 44, 1135–1138.

Saggu SK, Jha G, Mishra PC. (2019). Enzymatic Degradation of Biofilm by Metalloprotease from Microbacterium sp. SKS10. Front. Bioeng. Biotechnol. 7, 192.

Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. (2018). *Staphylococcus aureus* nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Front. Microbiol. 9, 2419.

Salem AH, Elkhatib WF, Noreddin AM. (2011). Pharmacodynamic assessment of vancomycin-rifampicin combination against methicillin resistant *Staphylococcus aureus* biofilm: A parametric response surface analysis. J. Pharm. Pharmacol. 63, 73–79.

Sanchez CJ, Akers KS, Romano DR, Woodbury RL, Hardy SK, Murray CK, Wenke JC. (2014). D-amino acids enhance the activity of antimicrobials against biofilms of clinical wound isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 58, 4353–4361.

Sani NA, Sapri H, Neoh H-M, Hussin S. (2014). First report on the molecular epidemiology of Malaysian *Staphylococcus epidermidis* isolated from a university teaching hospital. BMC Research Notes 7, 597.

Sauer K. (2003). The genomics and proteomics of biofilm formation. Genome Biol. 4, 219.

Schaumburg F, Pauly M, Anoh E, Mossoun A, Wiersma L, Schubert G, Flammen A, Alabi AS, Muyembe-Tamfum JJ, Grobusch MP, et al. (2015). *Staphylococcus aureus* complex from animals and humans in three remote African regions. Clin. Microbiol. Infect. 21, 345.e1–345.e8.

Schilcher K, Horswill AR. (2020). Staphylococcal biofilm development: structure, regulation, and treatment strategies. Microbiology and Molecular Biology Reviews, 84(3), e00026-19.

Sergelidis D, Angelidis AS. (2017). Methicillin-resistant *Staphylococcus aureus*: a controversial food-borne pathogen. Lett. Appl. Microbiol. 64, 409- 418.

Sharma D, Misba L, Khan AU. (2019) Antibiotics versus biofilm: An emerging battleground in microbial communities. Antimicrob. Resist. Infect. Control, 8, 1–10.

Shrestha P, Cooper BS, Coast J, Oppong R, Thuy NDT, Phodha T, Celhay O, Guerin PJ, Wertheim H, Lubell Y (2018). Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. Antimicrob. Resist. Infect. Control, 7, 98.

Sinha B, Francois P, Que YA, Hussain M, Heilmann C, Moreillon P, Lew D, Krause KH, Peters G, Herrmann M. (2000). Heterologously Expressed *Staphylococcus aureus* fibronectin-binding Proteins Are Sufficient for Invasion of Host Cells. Infect. Immun. 68, 6871.

Sonesson A, Przybyszewska K, Eriksson S, Mörgelin M, Kjellström S, Davies J, Potempa J, Schmidtchen A. (2017) Identification of bacterial biofilm and the *Staphylococcus aureus* derived protease, staphopain, on the skin surface of patients with atopic dermatitis. Sci. Rep. 7, 8682–8689.

Taglialegna A, Lasa I, Valle J. (2016). Amyloid structures as biofilm matrix scaffolds. J. Bacteriol. 198, 2579–2588.

Tomlinson BR, Malof ME, Shaw LN. (2021). A global transcriptomic analysis of *Staphylococcus aureus* biofilm formation across diverse clonal lineages. Microbial genomics, 7(7).

Van Kessel KP, Bestebroer J, van Strijp JA, (2014). Neutrophil-mediated phagocytosis of *Staphylococcus aureus*. Front. Immunol. 5, 467.

Vatan A, Saltoglu N, Yemisen M, Balkan II, Surme S, Demiray T, Mete B, and Tabak F. (2018). Association between biofilm and multi/extensive drug resistance in diabetic foot infection. International Journal of Clinical Practice, 72(3).

Verma J, Bag S, Saha B, Kumar P, Ghosh TS, Dayal M, Senapati T, Mehra S, Dey P, Desigamani A, et al. (2019). Genomic plasticity associated with antimicrobial resistance in Vibrio cholerae. Proc. Natl. Acad. Sci. USA, 116, 6226–6231.

W HO. (2019). New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis; WHO: Geneva, Switzerland, Volume 29, pp. 2019–2021.

Wang B, Muir TW. (2016). Regulation of Virulence in *Staphylococcus aureus*: Molecular Mechanisms and Remaining Puzzles. Cell Chemical Biology 23, 214–224.

Warraich AA, Mohammed AR, Perrie Y, Hussain M, Gibson H, Rahman A. (2020). Evaluation of anti-biofilm activity of acidic amino acids and synergy with ciprofloxacin on *Staphylococcus aureus* biofilms. Sci. Rep. 10.

Wells CM, Beenken KE, Smeltzer MS, Courtney HS, Jennings JA, Haggard WO. (2018). Ciprofloxacin and Rifampin Dual Antibiotic-Loaded Biopolymer Chitosan Sponge for Bacterial Inhibition. Mil. Med. 183, 433–444.

Wolz C, Goerke C, Landmann R, Zimmerli W, Fluckiger U. (2002). Transcription of Clumping Factor A in Attached and Unattached *Staphylococcus aureus* In Vitro and during Device-Related Infection. Infect. Immun. 70, 2758.

Zeaki N, Johler S, Skandamis PN, Schelin J. (2019). The Role of Regulatory Mechanisms and Environmental Parameters in Staphylococcal Food Poisoning and Resulting Challenges to Risk Assessment. Front. Microbiol. 10, 1307

Zhang K, McClure J A, Elsayed, S, Conly JM. (2009). Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A mec and type 4 ccr gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 53:531-540.

Zubair S, Fischer A, Liljander A, Meens J, Hegerman J, Gourle H, Bishop RP, Roebbelen I, Younan M, Mustafa MI, Mushtaq M, Bongcam-Rudloff E, Jores J. (2015). Complete genome sequence of *Staphylococcus aureus*, strain ILRI_Eymole1/1, isolated from a Kenyan dromedary camel. Stand.