NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

INVESTIGATION OF STAPHYLOCOCCAL TOXIC SHOCK SYNDROM TOXIN-1 IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

M.Sc. THESIS

Osaid Abdallah Abedalqader MOMANI

Nicosia

October, 2021

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Supervisor

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Nicosia

October, 2021

Approval

We certify that we have read the thesis submitted by **Osaid Abdallah Abedalqader MOMANI** titled **"Investigation of Staphylococcal Toxic Shock Syndrome Toxin-1 in Methicillin-Resistant *Staphylococcus aureus*"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Medical and Clinical Microbiology.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Osaid Abdallah Abedalqader MOMANI

…../…../…..

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Osaid Abdallah Abedalqader MOMANI

Abstract

Investigation of Staphylococcal Toxic Shock Syndrome Toxin-1 in Methicillin-Resistant *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive opportunistic bacterium that causes infections in humans and animals. It can asymptomatically colonize healthy people, giving it the ability to transmit between individuals. *S. aureus* is able to cause a broad range of mild to severe infections. Resistance to the antimicrobials being used has risen and has become a global problem as therapeutic alternatives are rapidly running out. Resistance to commonly used antibiotics such as methicillin, oxacillin, penicillin and vancomycin has resulted in the emergence of resistant pathogens such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). Virulence factors are the primary pathogenicity factors of *S. aureus* inducing innate and adaptive immunological responses in the host. *S. aureus* produces a diverse range of pathogenic virulence factors, including enterotoxins, exfoliative toxins, superantigens, phenol-soluble modulins, and cytolysins. The aim of this thesis project was to investigate the presence of *tsst-1* gene in MRSA clinical isolates at Near East University Hospital. Seventy-six MRSA isolates were identified using coagulase tube test, cefoxitin disc diffusion test, and *tsst-1* gene amplification by polymerase chain reaction (PCR). The presence of *tsst-1* gene was detected only in two (2.63 %) of 76 MRSA isolates screened. One of the TSST-1 positive isolates was from a tracheal aspirate from a female patient. The other TSST-1 positive isolate was from a blood sample from a male patient. This is the first study to investigate presence of toxic shock syndrome toxin 1 carriage in MRSA in Northern Cyprus, and it is another step towards a better understanding of the molecular properties of *S. aureus* strains present in hospitals in Cyprus.

Keywords: *Staphylococcus aureus*; MRSA; molecular typing; virulence factor, toxic shock syndrome toxin-1

Özet

Metisilin Direncli *Staphylococcus aureus* Klinik İzolatlarında Stafilokokal Toksik Şok Sendromu Toksin-1'in Araştırılması

Osaid Abdallah Abedalqader Momani

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Danışman: Doç. Dr. Buket Baddal

Ekim, 2021, 66 sayfa

Staphylococcus aureus, insanlarda ve hayvanlarda enfeksiyonlara neden olan Gram pozitif fırsatçı bir bakteridir. Sağlıklı bireylerde asemptomatik olarak kolonize olabilmesi nedenı ile bireyler arasında bulaş kapasitesi bulunmaktadır. *S. aureus,* hafif seyirli veya şiddetli enfeksiyonlara neden olabilir. Kullanılan antimikrobiyallere karşı direnç artışı küresel bir sorun haline gelmiştir. Metisilin, oksasilin, penisilin ve vankomisin gibi yaygın olarak kullanılan antibiyotiklere karşı gelişen direnç mekanizmaları, metisilin-dirençli *S. aureus* (MRSA) ve vankomisin-dirençli *S. aureus* (VRSA) gibi dirençli patojenlerin ortaya çıkmasına neden olmuştur. Salgılanan virülans faktörleri, konakta doğal ve edinsel immünolojik tepkileri indükleyen *S. aureus*'un birincil patojenite faktörleridir. *S. aureus* enterotoksinler, eksfolyatif toksinler, süperantijenler, fenol çözünür modülinler ve sitolizinler dahil olmak üzere çeşitli patojenik virülans faktörleri üretmektedir. Bu tez projesinin amacı, Yakın Doğu Üniversitesi Hastanesi'ndeki MRSA klinik izolatlarında toksik şok sendromu toksin-1 (*tsst-1*) gen varlığının araştırılmasıdır. Koagülaz tüp testi, sefoksitin disk difüzyon testi ve polimeraz zincir reaksiyonu (PZR) ile *tsst-1* gen amplifikasyonu kullanılarak 76 MRSA izolatı tanımlanmıştır. PZR ile yapılan taramada *tsst-1* gen varlığı 76 izolatının sadece ikisinde (%2.63) saptanmıştır. TSST-1 pozitif izolatlardan biri, bir kadın hastadan alınan trakeal aspirat örneğinden elde edilmiştir. Diğer TSST-1 pozitif izolat ise, erkek bir hastadan alınan kan örneğinden izole edilmiştir. Bu çalışma, Kuzey Kıbrıs'ta MRSA izolatlarında toksik şok sendromu toksin-1 varlığını araştıran ilk çalışma olmakla birlikte, Kıbrıs'taki hastanelerde bulunan *S. aureus* suşlarının moleküler özelliklerinin incelenmesine yönelik önemli bir adım teşkil etmektedir.

Anahtar Kelimeler: Staphylococcus aureus; MRSA; moleküler tiplendirme; virülans faktörü, toksik şok sendromu toksin-1

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List of Abbreviations

MSSA: methicillin-susceptible *Staphylococcus aureus*

MRSA: methicillin-resistant *Staphylococcus aureus*

CA-MRSA: community-associated methicillin-resistant *Staphylococcus aureus*

HA-MRSA: healthcare-acquired methicillin-resistant *Staphylococcus aureus*

HCWs: healthcare workers

LA-MRSA: livestock-associated methicillin-resistant *Staphylococcus aureus*

VRSA: vancomycin-resistant *Staphylococcus aureus*

PRSA: penicillin-resistance *Staphylococcus aureus*

ECDC: European Center for Disease Prevention and Control

EUCAST: European Committee on Antimicrobial Susceptibility Testing

CP: capsular polysaccharide

LTA: lipoteichoic acid

WTA: wall teichoic acid

PFT: pore forming toxin

ADAM-10: a disintegrin and metalloprotease 10

PVL: Panton-Valentine leukocidin

SAg: super antigen

SAE: *Staphylococcus aureus* enterotoxins

ET: exfoliative toxin

ETA: exfoliative toxin A

TSS: toxic shock syndrome

TSST: toxic shock syndrome toxin

SSSS: staphylococcal scalded skin syndrome

SSSI: skin and skin structure infection

SEls: Staphylococcal enterotoxin-like proteins

MSCRAMM: microbial surface components recognizing adhesive matrix molecules

FnBP: fibronectin-binding proteins

spA: protein A

OP: opsonophagocytic

ClfA: clumping factor A

hla: staphylococcal alpha-toxin

exfA: exfoliative toxin A

PBP: penicillin binding protein

SCC: staphylococcal cassette chromosome

IR: inverted repeat

DR: direct repeat

MGEs: mobile genetic elements

TCS: two component system

CCR: chromosome recombinase

agr: accessory gene regulator

ICU: intensive care unit

NLR: node-like receptor

PSM: phenol-soluble modulin

MDR: multi-drug resistant

HoR: homogeneous resistance

HeR: heterogeneous resistance

SaPIs: pathogenicity islands

IVIG: intravenous immunoglobulins

CLSI: Clinical and Laboratory Standards Institute

CDC: Centers for Disease Control and Prevention

TRNC: Turkish Republic of Northern Cyprus

NHANES: National Health and Nutrition Examination Survey

WHO: World Health Organization

CHAPTER I

INTRODUCTIONS AND AIMS

The human body is the host of diverse bacterial communities with potentially harmful and healthy qualities. For this cause, the strains associated with different regions of the body under different health circumstances have been thoroughly characterized in recent decades. The harmless commensal bacterium *Staphylococcus aureus* is now recognized to be a key opportunistic pathogen responsible for many diseases throughout the world (Chessa et al., 2015; Abernethy et al., 2017).

S. aureus is a commensal bacterium often found asymptomatically in healthy individuals, including the nose, intestines, on the skin, skin glands, and mucous. It can also be a key opportunistic pathogen associated with various human diseases. Studies indicate that up to 20% of *S. aureus* is persistent in general population and approximately 30% are intermittent carriers, while approximately 50% are non-carriers (Lakhundi & Zhang, 2018). In humans, anterior nares are the most common site of colonization, which increases the chance of infection when the host defenses are compromised (Pinchuk et al., 2010).

Several signal transduction pathways as well as virulence genes encoded in the genome of *S. aureus*, gives the bacterium the ability to cause disease in a broad range of metabolic niches. Studying bacterial virulence factors and their role in human disease has made significant progress in recent years (Campbell et al., 2021). *S. aureus* has been proven to be a successful pathogen during colonization as well as in disease. It harbors a wide range of virulence factors including hemolytic enzymes, exfoliative toxins, and superantigen proteins comprising toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxin-like proteins (SEls), and staphylococcal enterotoxins (SEs), which are usually located on mobile genetic elements (MGEs). MGEs aid pathogenicity by carrying various virulence and resistance genes through horizontal gene transfer. Due to the plethora of virulence characteristics, *S. aureus* has the ability

to cause a variety of infections ranging; from skin and soft tissue infections to life-threatening conditions such as pneumonia and endocarditis (Tuffs et al., 2019; Rai & Khairnar, 2021; Benkerroum, 2018).

Medically, *S. aureus* poses a significant challenge due to its unusual ability to develop resistance to multiple antibiotic classes which complicates antibiotic treatment. 94% of *S. aureus* strains are resistant to penicillin and its derivatives because of the release of the penicillinase enzyme, beta-lactamase, which inhibits penicillin by hydrolyzing the beta-lactam ring (Algammal et al., 2020; El Feghaly et al., 2012). One of the most common types of *S. aureus* is methicillin-resistant *S. aureus*, or MRSA, which can be diagnosed clinically by using polymerase chain reaction (PCR) to detect the *mecA* gene and cefoxitin resistance (Gajdács, 2019). Methicillin resistance results from the mutation of the *mecA* gene, in which the transcription is triggered by the synthesis of a unique penicillin-binding protein known as PBP2a. PBP2a has a low-affinity for beta-lactam antibiotics in comparison to other PBPs. (Moosavian et al., 2018; Taylor and Unakal 2021). Healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-MRSA (LA-MRSA) are the three types of MRSA infection documented in literature (Kumar, 2020). In the late 1980s, vancomycin became the preferred antibiotic for treating MRSA infections in hospitals. Vancomycin resistant *S. aureus* (VRSA) isolates were first reported in the United States in 2002 (McGuinness et al., 2017).

As a result of the proliferation of new antibacterial drugs, bacteria that are resistant to these agents have increased the number. This has become a global concern as therapeutic alternatives are rapidly running out. Antimicrobial resistance is a problem in both the healthcare and community settings, requiring a broad approach with joined efforts to overcome the associated problems. Infections with MRSA occur in 8-33% of patients who are colonized with the bacterium. Additionally, the number of HA-MRSA infections caused by CA-MRSA is increasing. According to the Centers for Disease Control and Prevention (CDC), over 2 million cases of diseases and 23 thousand deaths per year are attributed to antibiotic resistance in the United States (U. Okwu et al., 2019).

Recently, MRSA has become the main focus of infection prevention and control practices in hospital settings. MRSA infections also represent a major cost and monetary pressure to the healthcare system worldwide. Therefore, there is a need for further understanding of its transmission, prevalence, risk factors, associated diseases and pathogenicity as well as treatment of MRSA (Chukwunonso et al., 2018).

There is limited data on the virulence characteristics of MRSA clinical isolates in Northern Cyprus. The current study aims to investigate the presence and the prevalence of TSST-1 in MRSA clinical isolates collected at Near East University Hospital, and to evaluate the potential relationship between toxin presence level and disease outcome.

CHAPTER II

LITERATURE REVIEW

Staphylococcus is a genus named after the Greek word staphyle (grape) and Kokkos (berry) (Gnanamani et al., 2017). Sir Alexander Ogston, a Scottish surgeon, defined *staphylococci* in the pus of a surgical abscess in a knee joint in 1880. Friedrich Julius Rosenbach, a German physician, differentiated bacteria by the colour of their colonies in 1884: *S. aureus* (from the Latin aurum, gold). The genus *Staphylococcus* is a member of the phylum *Actinobacteria's* family *Micrococcaceae* (Liu et al. 2005).

Some *staphylococcal* species can cause infections in the human body and termed 'pathogenic' by clinical microbiologists. There are more than 37 species and 8 subspecies in the genus *Staphylococcus,* many of which preferentially colonize the human body (Aryal, 2020).

2. *Staphylococcus aureus*

2.1. Bacterial characteristics

S. aureus are Gram-positive and spherical in shape with a low genomic G + C content **(Figure 1)**. They are frequently found in clusters resembling a cluster of grapes when observed under a light microscope after Gram staining with a diameter varying between 0.5 and 1.0 μM. These species can grow aerobically or anaerobically (facultative anaerobe) and can grow up to 10% of salt. These organisms are non-spore-forming, produce catalase and have the ability to ferment mannitol (Gajdács, 2019). *S. aureus* cells are coagulase-positive which distinguishes it from other *staphylococcus* species. The colonies are often golden or yellow with smooth, round, raised and glistening colonies **(Figure 2).** It grows most rapidly at 37°C. Due to the synthesis of four forms of hemolysins, the organism is often hemolytic (alpha, beta, gamma, and delta) on blood agar (Ta and Cg 2017).

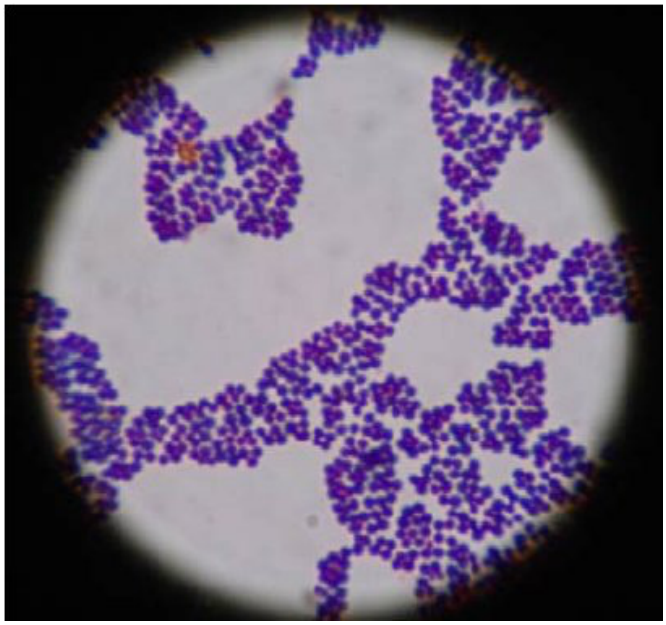

Figure 1*: S. aureus* observed under the light microscope after Gram's staining (Paudyal et al., 2014)

Figure 2*: S. aureus colonies* on the blood agar

2.2. Bacterial Structural Components

2.2.1 Cell wall components

S. aureus produces a capsular polysaccharide (CP), which acts as a pathogenic factor and aid in the bacterial resistance to phagocytosis. CP serotypes are numerous and the most notable of which are CP5 and CP8. Most clinical *S. aureus* isolates form either CP5 or CP8, allowing the bacterium to attach to a surface or host cell (Nanra et al., 2012). After host cell invasion, the bacterium is able to grow within it, where it can easily avoid detection by host immune system components (PMNs) (Batte et al., 2016*)*. The serotype of CP8 appears less virulent than the serotype of CP5, showing CP5's capability to cause bacteremia more than the CP8 serotype. *S. aureus* related strains that produce the CP5 capsule have significantly enhanced mortality rates as well as higher occurrence and severity of arthritis inducing sepsis (Watts et al., 2005).

S. aureus cell wall consists of a thick peptidoglycan (PG) layer which is 20–30 nm and extracellular polymers that are structurally supported by teichoic acids (Ultee et al., 2020). The peptidoglycan is also called a murein layer, which consists of glycan chains composed of the alternating amino sugars N-acetylglucosamine and N-acetylmuramic acid. Trunk pentapeptides (L-Ala-D-iso-Gln-L-Lys-D-Ala-D-Ala) are attached to the carboxyl group of each N-acetylmuramic acid, and interpeptide bridges link the lysine component of a trunk peptide to the penultimate D-alanine of an adjacent trunk peptide where the glycan strands are being cross-linked with short peptide motifs (Szweda et al., 2012).

Teichoic acids (TAs) are a second part of the cell wall of *S. aureus*, however, can be found in a variety of gram positives and can be defined as lipo-TAs (LTA). TA anchored in the membrane cytoplasm and cell wall TAs (WTAs) that are within the bacterial cell wall are covalently connected to peptidoglycan. WTAs help staphylococcal adhesion and colonization and play an important role in cell division, and biofilm formation (Mistretta et al., 2019).

2.2.2 Surface proteins

S. aureus expresses multiple surface proteins that play a critical role in the adherence of the bacterium to host cells, supporting bacterial invasion and evasion of host's immune response. Therefore, cell wall connected proteins are critical factors for *S. aureus* survival under commensal and invasive conditions, and could combat staphylococcal disease if targeted by vaccination (Foster et al., 2014).

Microbial surface components recognizing adhesive matrix molecules "MSCRAMMs" are several exposed proteins which are involved in the colonization, invasion, and proliferation of *S. aureus* in host cells. These MSCRAMMs link *S. aureus* and the host cells directly or indirectly. Direct method involves the proteins anchoring in the cell wall binding directly to the ligands which form an infection in tissue areas, however, subsequent development of toxins aid in infection dissemination, such as endovascular infections, bone, joint infections, and prosthetic device infections (Ashraf et al., 2017). MSCRAMMs include protein A and B (FnBPA and FnBPB), the adhesive of collagen (Cna), staphylococcal protein A (SpA), and clumping factor A and B (ClfA and ClfB) (Jin et al., 2021).

2.2.2.1 Fibronectin-binding proteins

The fibronectin-binding proteins (FnBPs), FnBPA and FnBPB, have structural resemblances to the surface proteins of other Gram-positive bacteria, which are also fibronectin-binding proteins found in *S. aureus* (Massey et al., 2001). For host cell adhesion, FN mainly forms a bridge between cell-side α5β1-integrin and bacterial binding protein FN, followed by an A-dome similar to Clot Factor A, and a fibrinogen-binding protein that contains an amino-terminal secretion signal sequence. This domain is FN active and can tie fibrinogen and elastin to the other domain (MSCRAMMs). Only 40% of the sequences of the A domain of FnBPA and FnBPB are identical (Josse et al., 2017).

2.2.2.2 Protein A

The protein A (spa) is a 45-kD surface-bound and secreted protein expressed by a large number of clinical isolates. It has been shown to bind to Fc portion of immunoglobulins (Goldmann & Medina, 2018). It can also prevent destruction by neutrophil granulocytes through opsonophagocytosis (OP). The spa can inhibit phagocytosis via the prevention of antibody binding to bacterial cell surface components and consequent complement-dependent uptake of bacteria by phagocytic cells (Nanra et al., 2012).

2.2.2.3 Clumping factor A

Clumping factor A (ClfA) is a *staphylococcal* fibrinogen (Fg) binding protein which is responsible for clumping of the blood plasma (Ghasemian et al., 2015). It is an important virulence factor of *S. aureus* involved in various infections including arthritis and endocarditis.

2.3 Extracellular Enzymes

Catalase, proteases, hyaluronidase, lipases, nucleases and staphylokinase are enzymes produced by *S. aureus* **(Figure 3)**. In addition to harvesting and transforming host tissues into bacterial nutrition, *S. aureus* enzymes can also help to invade and evade the immune system (Jin et al., 2021).

2.3.1 Coagulase

Coagulase is the main criteria utilized for *S. aureus* identification in the clinical microbiology laboratory. Several publications have shown that site-specific coagulase inactivation does not decrease microbial endocarditis pathogenicity, subcutaneous or mammalian infections (Arvidson, 2014).

2.3.2 Staphylokinase

Staphylokinase (Sak) is a cofactor that masks plasmin for the activation of plasminogen to disintegrate fibrin clots and hence promotes the spread of bacteria. Sak is generated from staphylococci's lysogenic strains; prophage encoding Sak usually has other genes that encode virulence such factors as enterotoxin A and inhibitory proteins of chemotaxis (Tam and Torres 2019).

2.3.3 Nucleases

The first detection of staphylococcal nuclease (DNase) in *S. aureus* culture filtrates was in 1956. Staphylococcal nuclease is a Ca^{2+}-dependent enzyme that is heat stable (Algammal et al., 2020). The purpose of staphylococcal nuclease is to break down the DNA and RNA substrates, as well as to affect the cleavage of the 5-phosphorylester bond (Tam and Torres, 2019).

2.3.4 Hyaluronidase

Hyaluronic acid (HA) is A linear polysaccharide consisting of recurring units of N-acetylglucosamine and glucuronic acid, connected by alternate β1,3 and β−1,4 glycoside linkages. The enzymes which degrade HA are called hyaluronate lyase or hyaluronidase respectively (Tam and Torres, 2019).

2.3.5 Lipases

S. aureus contains two lipases: lipase 1 and *S. aureus* lipase 2 (SAL1 & SAL2). SAL1 and SAL2 genes are often labeled as *gehA* and *fehB* for glycerol ester hydrolase, respectively. Lipase pro-peptides were proven to be essential for lipase transport to the

extracellular environment as well as protein stabilization to avoid degradation (Tam & Torres, 2019).

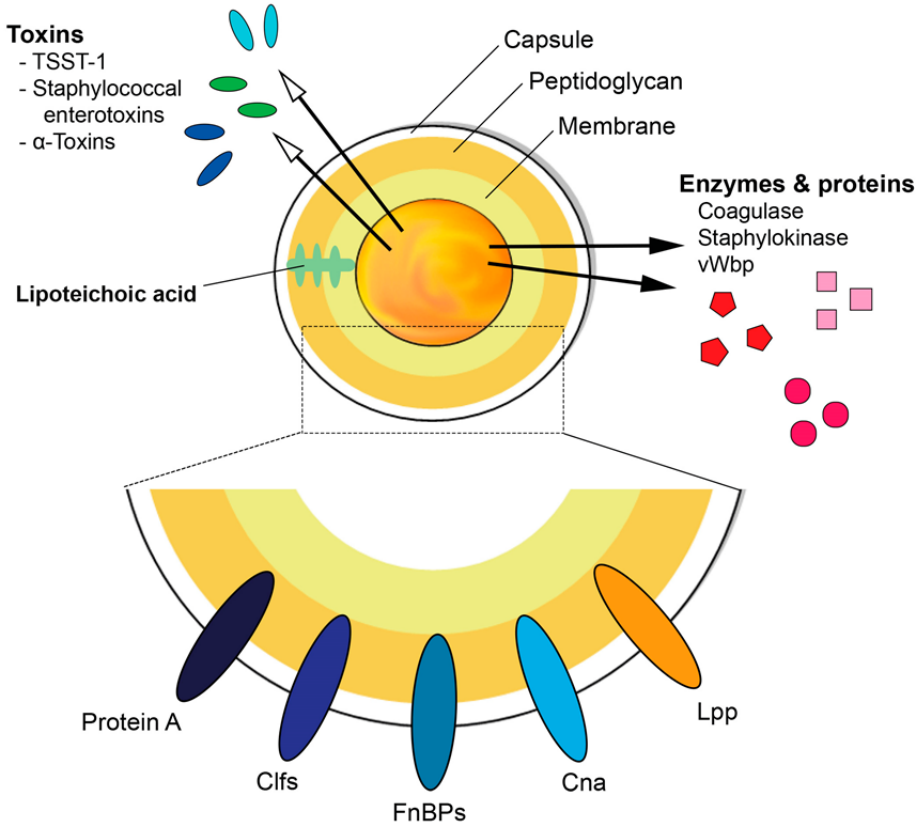

Figure 3: *S. aureus* fundamental structure and capacity to express different determinants of pathogenicity (Jin et al., 2021).

2.4 *Staphylococcal* diseases

Staphylococcal infections may vary from mild conditions of the skin to endocarditis and can lead to life threatening heart infections such as endocarditis. As a result, the indications of staphylococcal infections and their symptoms may vary greatly according to the location and severity of disease **(Table 1).**

Table 1: An overview of *S. aureus* infections (Zurita et al., 2010**)**

Source of infection	**Disease**
Skin and soft tissue	Impetigo, boils, carbuncles, abscesses, cellulitis, fasciitis, pyomyositis, surgical and traumatic wound infections
Foreign body-associated	Intravascular catheter, urinary catheter
Intravascular	Bacteremia, sepsis, septic thrombophlebitis, infective carditis
Bone and joints	Septic osteomyelitis, septic arthritis
Respiratory	Pneumonia, empyema, sinusitis, otitis media
Other invasive infections	Meningitis, surgical space infection
Toxin-mediated disease	*Staphylococcal* toxic shock, food poisoning, *staphylococcal* scalded skin syndrome, bullous

2.5 Epidemiology

2.5.1. Carriage

To understand *S. aureus* biology and how it causes disease, it is necessary to comprehend the bacterial mechanism of transmission, colonization, and preferred habitat. *S. aureus* is a major opportunistic pathogen associated with numerous conditions in humans, a commensal that is frequently present asymptomatically on the skin, skin glands, and mucous membranes of healthy persons. *S. aureus* colonizes a variety of human body niches, but the major colonization site is the anterior nares. Bacterial carriage rate can be divided into three categories: 20-30% of people are persistent carriers of *S. aureus*, 30-35% are intermittent carriers, and 40-50% are

noncarriers (Emaneini et al., 2017). Furthermore, in addition to the nares, oropharyngeal, axillary, perineal, rectal, perirectal, and even intestinal samples have also been shown to have MRSA colonization **(Figure 4)** (Turner et al., 2019).

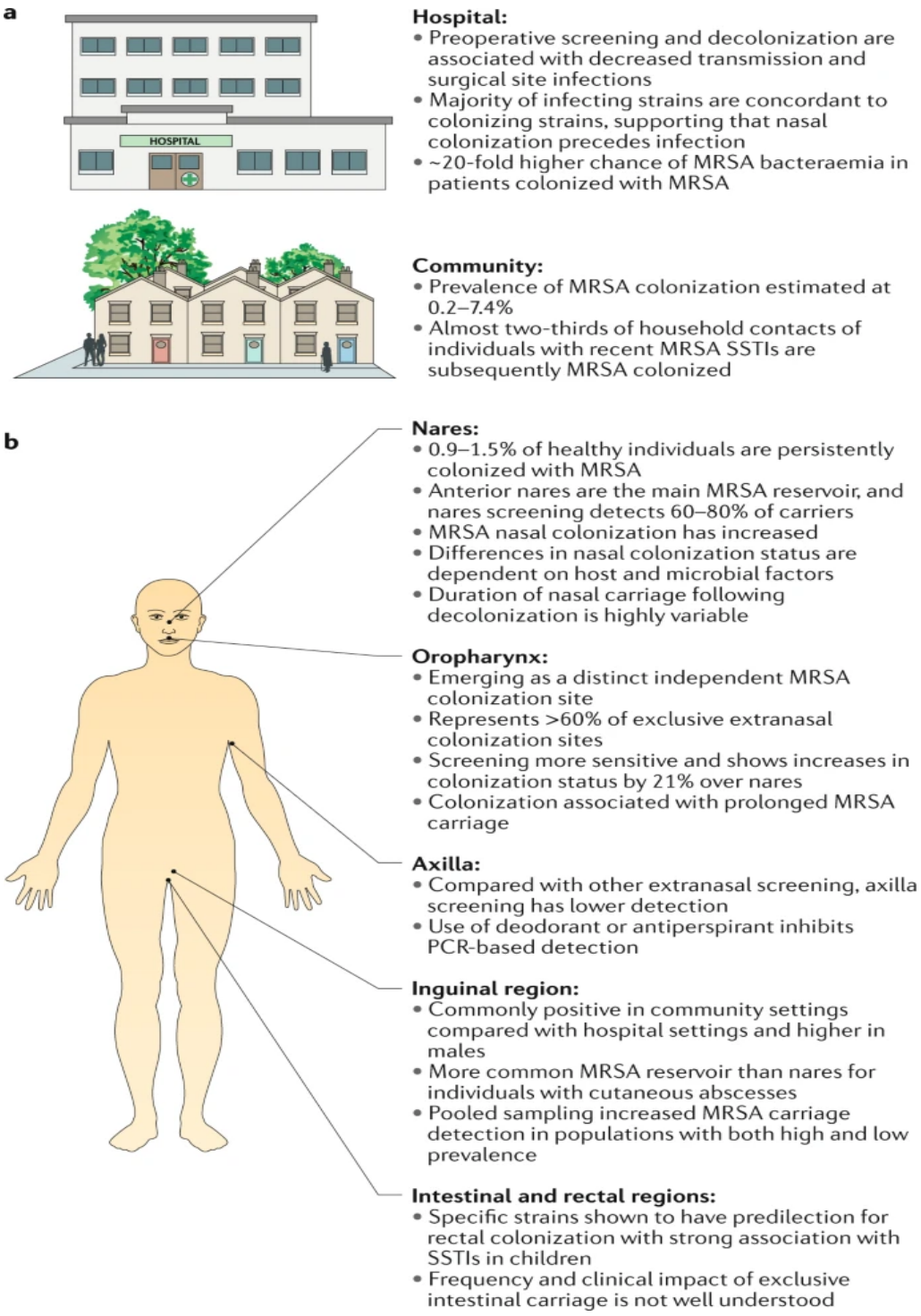

Figure 4: Methicillin-resistant *S. aureus* colonization (Turner et al., 2019).

2.5.2. Transmission

S. aureus is mostly transmitted by nasal carriage among healthcare workers (HCWs). Moreover, *S. aureus* can be transmitted by the respiratory system, which comes into direct contact with that of healthy individuals, newborn, nasopharynx or open wounds. Bacteria from healthy carriers can be transmitted to the immunocompromised population. As a result, the transfer must take a more indirect path that includes (1) exit from the respiratory tract, (2) transit to the recipient, and (3) entry into tissues (Emaneini et al., 2017; Hare & Thomas, 1956).

Hospitals take significant measures to avoid direct patient-to-patient transmission as well as transmission via personnel and the environment. Transmission may happen even with effective infection control practices, colonized healthcare personnel have been identified as potential sources of transmission in outbreaks, although the utility of healthcare worker screening and *S. aureus* eradication as regular control strategies is debatable (Price et al., 2017).

2.5.3. Risk factor

Children, the elderly, athletes, military personnel, people who inject drugs, people with indigenous backgrounds or who live in urban, underserved areas, individuals with underlying conditions, people who have frequent healthcare contact, and people in institutionalized populations, including prisoners, are more exposed to the risk of *S. aureus* (Long et al., 2014). In American women age ≥ 60 years, diabetes and poor household income were risk factors for colonization of MRSA, however, in United States exposure to healthcare was identified as the sole important risk factor (Hassoun et al., 2017).

Infections of *S. aureus* are common in children. The greatest infection risk is observed in this category, with annual incidences around 452 instances per 100 000

newborns of the general population, and death of 4.5% compared to the highest number among infants between 1 and 17 years old in the USA (Schaumburg et al., 2014).

The highest risk of infection was among newborns in hospitals under the age of 1 year. The risk factors of early post-natal baby colonization have been discovered by maternal *S. aureus* carriage and breastfeeding. Between 53% and 80% of newborn mother-dyads were simultaneously colonized with the same *S. aureus* isolate (Schaumburg et al., 2014).

However, the MRSA isolates show that increased indiscriminate and uncontrolled use of antimicrobials in the community is a substantial determinant of risk for community-related infection with extremely limited treatment options. This situation requires efforts to avoid the indiscriminate use of antimicrobials and to ensure good personal hand hygiene to avoid the expansion of these multi-drug resistant (MDR) strains into other communities of immunocompromised population (Onanuga et al., 2021).

2.6 Antibiotic resistance in *S. aureus*

S. aureus infections can be extremely hard to treat due to the acquired resistance to antimicrobial agents. A variety of resistance mechanisms, including horizontal gene transmission transfer via plasmids or other mobile genetic elements, mutations and selection of drug resistance can be observed in *S. aureus*. This has resulted in several strains that are resistant to one or more antibiotics, such as penicillin-resistant *S. aureus* (PRSA), methicillin- resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus* (VRSA) and multidrug-resistant *S. aureus* (MDRSA) (Haddad Kashani et al., 2018).

2.6.1 Penicillin-resistant *S. aureus*

Scottish microbiologist Fleming discovered penicillin in 1928, which was used in clinical practice by the early 1940s. Penicillin-resistant *S. aureus* (PRSA) has been documented in the early 1942s after few years of the initial treatment of human infections with penicillin. There were more than 80% PRSA infections in both hospitals and the community between 1950 and the late 1960s (Szweda et al., 2012). At this time, significant numbers of clinical isolates were classified as 80/81 phage-type and subsequently as multi-locus sequence type (MLST or ST) 30 and clonal complex 30 (CC30). Penicillin resistance is conferred by two pathways in *S. aureus* (McGuinness et al., 2017). Beta-lactamase synthesis promotes the gene *blaZ* and inhibits penicillin by hydrolyzing the beta-lactam ring. *blaZ* is an 846-bp gene that is regulated by two consecutive regulatory genes, the anti-repressor blaR1 and the repressor blaI. It has been observed that after beta-lactam is exposed, blaR1, a transmembrane sensor–transducer, undergoes autocatalytic cleavage that induces slicing of the repressor gene, *blaI*, and thereby enabling transcription of blaZ. Serotype analysis distinguishes four categories of blaZ; three of them (A, C, and D) are frequently found on plasmids, whereas B is typically located on the chromosome (El Feghaly et al., 2012). The second type of penicillin resistance is caused by a mutated penicillin-binding protein, PBP2a, which is encoded by *mecA* (Makgotlho et al., 2009).

2.6.2 Methicillin-resistant *S. aureus*

Methicillin was developed in the late 1950s as a treatment for PRSA infections. Although methicillin is effective in treating PRSA infections, the first methicillin-resistant *S. aureus* (MRSA) strains have been reported within two years of clinical use. In 1961, the first case of methicillin-resistant MRSA was discovered in a British hospital (Henderson & Nimmo, 2018). Over the decades, the prevalence of MRSA has risen worldwide. There is a large proportion of *S. aureus* infections in hospitals caused by MRSA, which is associated with high mortality rate (McGuinness et al., 2017).

MRSA is currently the most prevalent resistant pathogen discovered in many parts of the world, such as Europe, the US, North Africa, the Middle East, or East Asia (Guo et al., 2020). In a 2013 study by the Center for Disease Control and Prevention (CDC), MRSA was stated to be a major concern in the US for antibiotic resistance. Although the MRSA isolation rates have declined over the years, according to recent studies, additional observation and prevention measures may be needed to further reduce the spread of this pathogen. Between 2005 and 2008, the international rates for MRSA were 44.2 %. In 2014, parts of Southeast Asia, the Western Pacific, and other parts of the world observed that more than 80% of *S. aureus* infections were caused by MRSA. By 2016, this percentage had dropped to 39.0% and there was a noticeable change in MRSA rates (Schulte & Munson, 2019).

MRSA can cause a variety of illnesses, including skin and soft tissue infections, pneumonia, osteoarticular infections, toxic shock syndrome, and bacteremia. In the United States, MRSA is the second most common type of infection in children under the age of five. Clinical manifestations and infection risk factors differ between hospital acquired-MRSA (HA-MRSA), community acquired-MRSA (CA-MRSA), and livestock acquired-MRSA (LA-MRSA) strains (Chukwuma's et al., 2018).

2.6.2.1 Hospital-acquired MRSA

HA-MRSA stands for hospital-acquired MRSA isolates, which are increasing over time around the world. In the late 1970s, the majority of cases had been HA-MRSA (Khokhlova et al., 2015). There are high rates of HA-MRSA infections in the United States, Asia, and Malta (>50%). HA-MRSA infections are more common in Asian countries such as South Korea (77.6%), Vietnam (74.1%), Taiwan (65%) and Hong Kong (56.8%), The prevalence rate in Africa, China, and Europe ranges from 25 to 50 percent while in some parts of Europe, it is less than 50 percent (Kumar, 2020; Grema, 2015). Dermatitis, septicemias, and heart and lung diseases are the most common HA-MRSA complications reported (Lee et al., 2018; Bennett & Thomsen, 2020).

2.6.2.2 Community-acquired MRSA

It was unlikely for MRSA to cause infections among members of the community or hospitals until the beginning of the 20th century. The first epidemic of CA-MRSA infections was documented in 1991 which resulted in necrotic pneumonia, lung abscess, and sepsis; however, aggressive MRSA infections was reportable in the late 1990s. MRSA isolates accounted for more than half of *S. aureus* infections in US intensive care units by the mid-1990s (Dukic et al., 2013). First documented cases of MRSA infection in a community occurred in 1997 in otherwise healthy children in the US. Despite the fact that none of these children had any risk factors for developing MRSA, they all died from severe infections, suggesting that these CA-MRSA strains were particularly virulent (Szweda et al., 2012). CA-MRSA was recognized as a distinct clinical organization in 1999 after an investigation of four infant deaths in the Midwestern United States. In Detroit and Western Australia, CA-MRSA cases had previously been linked to intravenous drug users. The CDC established a new case definition for MRSA infections among healthy people: any infection diagnosed in patients without health care-associated MRSA risk factors such as hospitalization, hemodialysis, surgery and presence of indwelling catheters and other medical devices. (Kong et al., 2016). The most common clinical manifestation associated with CA-MRSA is skin and soft tissue infection (Otto, 2013).

In 2008, the CDC confirmed that around 12% of MRSA infections were linked with the community (Choi et al., 2014). According to the CDC, CA-MRSA refers to cases where MRSA is isolated less than 48 hours after hospital admission without a history of hospitalization or surgery in the previous 12 months, permanent indwelling catheters or percutaneous medical devices, residence in a long-term-care facility, dialysis, or previous culture of MRSA (Henderson & Nimmo, 2018).

2.6.2.3 Livestock-acquired MRSA

LA-MRSA is related to locally-assigned infections, such as skin and soft tissue infections and otitis media along with critical and invasive infections including bacteremia. Members of households with animals are more likely to be colonized by LA-MRSA, which can be transmitted to other members of the household through direct contact with the livestock including cows, horses, chickens, and mainly pigs. Cases of LA-MRSA also occurred in people who had no relationship with livestock and in these situations, the infection was spread by contamination or by food transmission (Lee et al., 2018).

2.6.3 Vancomycin resistance

In 1958, vancomycin was used for treating *S. aureus* which was resistant to penicillin, but soon methicillin was displaced. With the growth of MRSA, vancomycin has become popular and has been the basis for MRSA since then. It is a glycopeptide with antibacterial and inhibition of gram-positive diseases by the production of cell walls. Although vancomycin was used for over 50 years, the best way to use this drug is still discussed (Holmes et al., 2015). *S. aureus* isolates are divided into three categories by Clinical and Laboratory Standards Institute (CLSI) with reduced susceptibility to vancomycin. Vancomycin-susceptible *S. aureus* (VSSA) has a MIC ≤ 2 g/ml, vancomycin-intermediate *S. aureus* (VISA) has a MIC of 4-8 g/ml, and VRSA has a MIC ≥ 16 g/ml. The presence of *vanA* or other vancomycin resistance factors must be verified by molecular techniques to determine whether an isolate belongs to VRSA (Cong et al., 2020).

2.7 Mechanisms of antibiotic resistance in *S. aureus*

Resistance to β-lactams in *S. aureus* is usually described in a diverse manner within a particular population. The vast majority of cells in a diverse population are susceptible or borderline sensitive to β-lactams. A subpopulation of around 0.1% may

tolerate antibiotics, and when exposed to the antibiotic again, a homogeneously resistant population form. The processes behind this transition from heterogeneous resistance (HeR) to homogeneous resistance (HoR) are linked to accessory alterations outside of *mecA*. High-level β-lactam resistance is associated with substantial energy demands, which impose a selection penalty on the cell (Campbell et al., 2021). The resistance of β-lactam was identified in *S. aureus* via two major mechanisms, β-lactamases, and more crucially by utilizing penicillin-binding protein (PBP2a) with a low affinity.

2.7.1 β-lactamases

The first known resistance mechanism of *S. aureus* to β-lactams was due to the formation of a β-lactamase (penicillinase), a stimulated extracellular enzyme produced in response to lactam exposure that hydrolyzes the β-lactam ring, producing inert derivatives. The functional gene *blaZ* encodes β-lactamases, which are regulated by two regulatory genes, *blaI* and *blaR1*. In the existence of penicillin, the sensor protein BlaR1 initiates a signaling cascade that results in the cleavage of the transcriptional repressor BlaI, allowing for the high-level synthesis of β-lactamase. Semisynthetic-lactamase-resistant penicillin such as methicillin and oxacillin were introduced in early 1960 to tackle the resistance problem (Chin et al., 2021).

2.7.2 Penicillin-binding proteins

Penicillin-binding proteins (PBPs) are membrane-bound proteins found in methicillin-susceptible *S. aureus* (MSSA). A disruption of peptidoglycan occurs as a result of beta-lactam antibiotics binding to PBPs. PBP2a or PBP2′ are other names of PBPs which encoded by the *mecA* gene that is found in the *staphylococcal cassette chromosome mec (SCCmec)* element of MRSA. β-lactam antibiotics such as methicillin and oxacillin have a low affinity for the alternative protein. Since the

peptidoglycan is unaffected, the MRSA can continue to survive (Rai & Khairnar, 2021).

Often, MRSA strains are labeled as multidrug-resistant (MDR) due to the integration of many other resistance determinants into the sequence of *SCCmec*, which allows it to act as a hotspot of integration for genetic mobile elements such as plasmids and transposons (Szweda et al., 2012; Lv et al., 2021).

2.8 *Staphylococcal cassette chromosome*

Staphylococcal cassette chromosome (SCC) components are a distinct class of mobile genetic elements (MGEs) found in staphylococci, including *SCCmec*, which contains the *mec* genes that encode resistance to methicillin and almost all β-lactam antibiotics such as methicillin and oxacillin (Shore & Coleman, 2013). SCCmec consist of 3 basic structural components: 1- *mec* gene complex, which include (*mecA, mecB, mecC,* and *mecD*) with regulatory elements control (mecR1 and mecI which are encoding a signal transducer and repressor protein respectively); 2-cassette chromosome recombinase (*ccr*) gene complex encoding the site-specific recombinases (ccrAB and ccrC); 3- joining region (J regions) **(Figure 5**) (Lakhundi & Zhang, 2018; Baig et al., 2018).

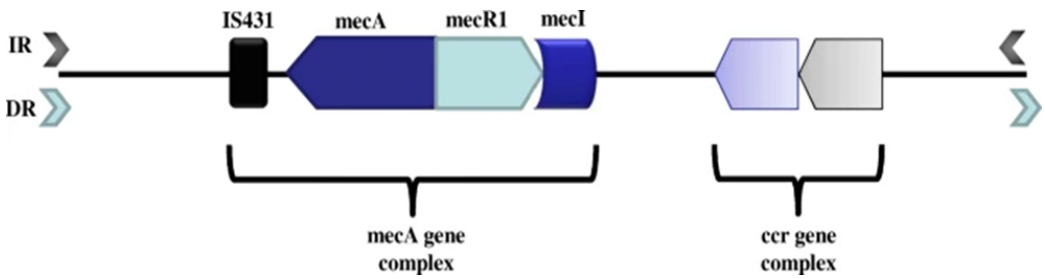

Figure 5: Schematic illustration of the structure of *SCCmec* (Abbreviations: IR, inverted repeat; DR, direct repeat) (Gill et al., 2019)

The genotypic characteristics of the isolates were used to classify them as CA-MRSA or HA-MRSA. In other words, SCCmec types I, II, and III are typically restricted to HA-MRSA and not found in healthy populations, whereas SCCmec types IV and V are primarily associated with CA-MRSA (Kateete et al., 2019).

Both HA- and CA-MRSA possess the staphylococcal cassette chromosome mec (SCCmec) and exhibit a variety of genetic courses, typically classified by multilocal sequence types (ST -types), protein A gene|(spa) types, and SCCmec types (Khokhlova et al., 2015).

3.Virulence factors of *S. aureus* pathogenesis

Innate immunity plays an important role in the host's protection against infections triggered by *S. aureus*. Many virulence factors of *S. aureus* are remarkable, and can cause a variety of infections of the skin, wounds, and deep tissues, as well as life-threatening illnesses such as pneumonia, endocarditis, septic arthritis, and septicemia. *S. aureus* also secretes multiple toxins, and can result in food poisoning, scalds, and toxic shock syndrome (Stark, 2013).

3.1 Extracellular toxins

Unlike other bacterial pathogens that depend on only one or a few toxins resulting in diseases, *S. aureus* develops a staggering number of virulence factors. During infection, a variety of toxins, immune evasion factors, a variety of protein and non-protein factors can allow host colonization. The toxins of *S. aureus* can be divided into three main groups - the pore-forming toxins (PFTs), the exfoliative toxins (ETs) and the superantigens (Cheung et al., 2021).

3.1.1 Pore-forming toxins

Pore-forming toxins (PFT) is responsible to impede complement activation, neutralize antimicrobial resistance peptides, suppress neutrophil chemotaxis or lyse neutrophils. PFTs cause numerous cellular host responses, including macrophage recruitment, and play a key role in host cell membrane damage, that includes hemolysins (alpha, beta, delta and gamma) and leukocidin Panton Valentine Leukocidin (PVL) (Jordan et al., 2020).

3.1.1.1 α-Hemolysin

α-hemolysin or α-toxin (Hla) is a polypeptide of 33 kDa that is released by 95% of *S. aureus* clinical strains. Hla is a β-barrel tissue barrier that facilitates bacterial penetration. Moreover, it produces water-soluble α-toxin monomers in the target cell membrane producing heptameric β-barrel holes that lead to cell lysis (Oliveira et al., 2018). The α-toxin heptamer is like a mushroom and has 3 key areas: an extracellular cap domain, a β-barrel-polar stem domain, and a rim domain which provides recipient specificities (Tam & Torres, 2019). Even at sub-lytic concentrations, Hla was shown to impair effector cells of the innate immune system, enhance a hyperinflammatory response characteristic of bacterial pneumonia, in addition, it impacts cells, monocytes, macrophages, epithelial and endothelial cells (Wang et al., 2021). His-35 mutation, which disrupts interprotomer stabilization and prevents pore formation while inactivating the toxin, is an example of how important the amino latch is in forming β-barrel pores. α-toxin is secreted in monomer form. Hla adheres to ADAM-10 and forms a heptameric prepore on the surface of the target cell. The preparation of pre-stem domains can then expand to create a β-barrel pore, penetrating the target cell (**Figure 6)** (Tam & Torres, 2019).

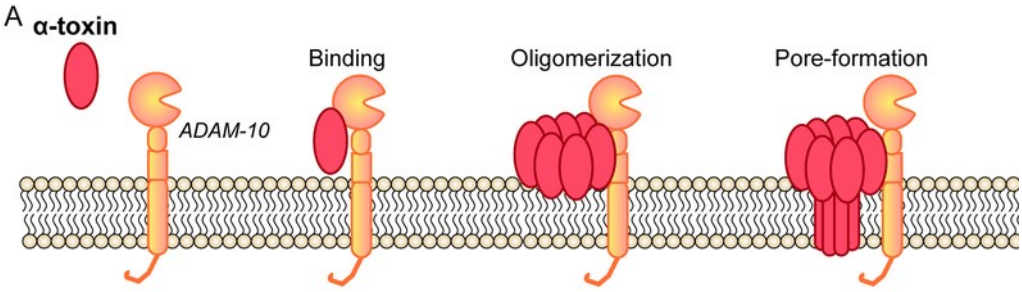

Figure 6: α-toxin secretion (Tam & Torres, 2019)

3.1.1.2 Panton-Valentine leucocidin

Panton-valentine leukocidin (PVL) is a leukocyte-destroying exotoxin produced by 2-5% of *S. aureus* strains (Castellazzi et al., 2021). PVL-positive MRSA infections usually affect individuals living in the community (CA-MRSA) and are most common in healthy young adults and children. The prevalence of MSSA and HA -MRSA is often in the low 2% (Ahmad et al., 2020). In 1932, PVL was identified as a virulence factor in the family of synergohymenotropic toxins. It is responsible for a number of necrotizing illnesses, including pneumonia and skin and soft tissue infections (Ayepola et al., 2018). The toxin is encoded by a prophage and consists of two protein subunits, LukF-PV with a molecular mass of 38 kDa and LukS-PV with a molecular mass of 32 kDa (toxin components), acting as sub-units in the cell membrane of the host cell that results in cells leakage and mortality. The genes *lukS-PV* and *lukF-PV* are found in the chromosome, respectively (Zhang et al., 2018) (Mazzoleni et al., 2021).

3.1.3 Superantigens

S. aureus superantigens (SAgs) are a unique family of non-glycosylated low-molecular-weight exoproteins that are distinctly resistant to heat, acids (stomach acid), proteolysis (trypsin and pepsin) and desiccation. In addition, the ability to produce excessive and non-conventional T-cell activation and cytokine release, that will cause

interference with immune system function systemically (Zhao et al., 2019; Spaulding et al., 2013).

Superantigen toxins have a molecular weight of 19-30 kDa with proteins, which are made up of 168–261 amino acids. Related to the resistance to methicillin, the toxin genes are found in mobile genetic elements such as υSa genomes islands, pathogenic islands as well as phage genomes, plasmids, and *SCC*. (Hu et al., 2021). There are three types of SAgs: staphylococcal enterotoxins (SEs), exfoliative toxins (ETs) and toxic shock syndrome toxin-1(TSST-1) (Andrey et al., 2010).

3.1.3.1 Staphylococcal enterotoxins

SEA to SEE and SEG are the seven distinct SEs. Additionally, SEB and SEC variants were identified. *Staphylococci* were originally named for their ability to stimulate vomiting and diarrhea. Both of these are common side effects of SE ingestion as they contain 9-19 amino acids from the disulfide loop, which is found in the protein. However, the disease is self-limiting (Tam & Torres, 2019; Zhao et al., 2017; Grumann et al., 2014).

3.1.3.2 Exfoliative toxins

Exfoliative toxin (ET) is a superantigen, and also known as epidermolytic toxins, are highly selective serine proteases produced by *S. aureus*. The proteases identify and hydrolyze cadherins in the skin's surface layers. ETs are exotoxins that cause keratinocyte junction rupture and cell-cell adhesion in the host's epidermis, causing skin peeling and blister production (Bukowski et al., 2010; Imanishi et al., 2019). There are also three different *S. aureus* ET serotypes (ETA, ETB and ETD) found and related to human staphylococcal skin infections such as staphylococcal scalded skin syndrome (SSSS) or bull impetigo (Imanishi et al., 2019). This blistering skin

condition is classified into two clinical forms: localized and generalized. The generalized type, known as Ritter's disease, is common in newborns and children. Nevertheless, the localized type, known as bullous impetigo, can affect people of all ages (Staiman et al., 2018).

3.2 Regulation of toxin production in *S. aureus*

S. aureus virulence and toxin genes are regulated, which determines the course of the disease. “Accessory gene regulator (Agr)" is considered a major *S. aureus* virulence regulator in terms of global virulence (Derakhshan et al., 2021). According to a recent study on the HA-MRSA strain USA100, the *agr-II* gene regulates virulence-associated genes and the expression of toxins that cause skin disease. Furthermore, the Agr-II system of USA 100 MRSA was correlated with the optimal bacterium that could survive sublethal antibiotic doses (Rai & Khairnar, 2021).

The Agr loci are recognizably polymorphic in a significant sequence. Four variations have been recognized (Agr types I to IV). These *S. aureus* strains are marked by mutations in the histidine kinase AgrC sensor domain and by polymorphisms in sequences that influence the three agr-specific determinants of the autoinduction peptides (AgrB, AgrD, and the sensor domain of AgrC). Since agr is an integrated system, various variants must be developed in conjunction, in order to maintain agr functioning, so that bacteria may escape host defenses, spread to host cells and tissues (Painter et al., 2014).

Agr system components are coordinated by the cross-activated P2 and P3 devices, which produce RNAII and RNAIII. The transcription of the agr system's core machinery (RNAII), which is an operon of 4 genes AgrBDCA is controlled by P2, whereas transcription of RNAIII is made by P3, which is a regulatory RNA of the Agr system **(Figure 6)** (Butrico and Cassat 2020). AIP is synthesized by the transmembrane endopeptidase AgrB and SpsB, which cyclizes AgrD, the peptide precursor of the autoinducer peptide, AIP, into an octapeptide, which is then exported

by AgrB. Histidine protein kinase sensors and response regulators, AgrC and AgrA, are homologs in bacteria. Regarding AgrC's extracellular receptor, AIP is capable of binding to it at a threshold concentration of AIP. The autophosphorylation of AgrC leads to AgrA becoming active, which in turn triggers P2 and P3-driven transcription, followed by RNAIII translation, which results in an up-regulation of toxins and enzymes like hemolysins, proteases, lipases, enterotoxins, and TSST-1. Additionally, AgrA binds to the transcriptional promoter and increases the production and activity the transcription of RNAII, which in turn feeds into the agrBDCA operon. There's also a possibility that AgrA can turn on phenol-soluble modules (PSM) transcription without the need for RNAIII. RNAIII regulates transcriptional and translational expression of virulence factors. A hemolysin-encoded gene (hld) is located within RNAIII (Kong et al., 2016) (Andrey et al., 2010).

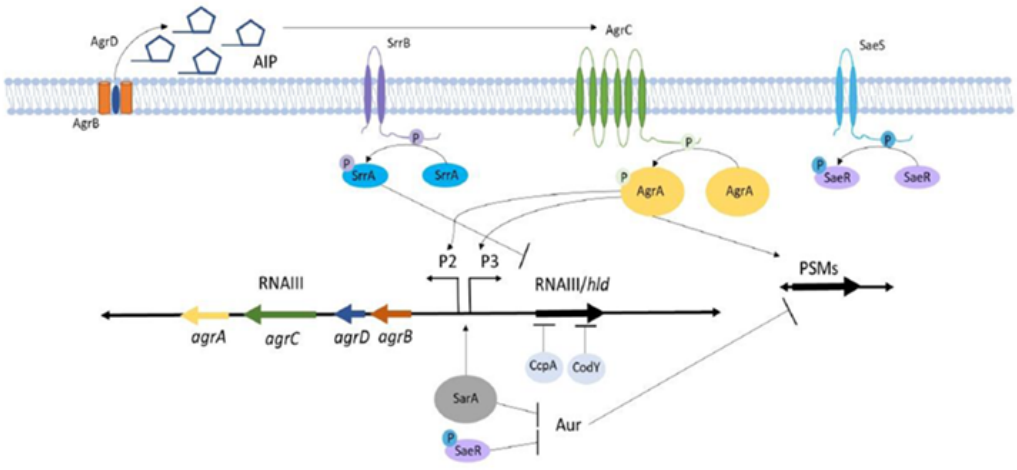

Figure 7: Accessory gene regulation (Agr) signals through self-control and interaction with other systems of two components (Butrico and Cassat 2020).

4. Toxic shock syndrome toxin-1

4.1 Overview

TSST-1 is a member of the staphylococcal group I SAgs family and exotoxin formed by 5-25% of the *S. aureus* strains of samples from various origins (Brudzinski et al., 2021). TSST-1 was renamed in 1984 to highlight the absence of emetic action and its connection with toxic shock syndrome (TSS). TSS is a toxin-mediated illness that occurs 1–2 weeks after the start of hypotension, fever, rash and desquamation. Furthermore, TSS is most often caused by invasion of Group A *streptococcal* and staphylococcal infections, which induce immunological response and large cytokine production. TSS covers at least three organ systems: gastrointestinal, musculoskeletal, or central nervous system as described by the CDC (Suga et al., 2016; Tam & Torres, 2019).

Historically, pyrogenic toxin superantigen (PTSAg) TSST-1 led in the tragedy of 1928 to the loss of 50 % of children. This cause was not identified at that time, but the epidemic led to staphylococcal *alpha-toxin* (alpha-hemolysin and alpha-cytotoxin) being identified as the most common reason. There was a minimal alpha-toxin production by *S. aureus* strain but high quantities of TSST-1 were observed. All children afflicted showed defining clinical characteristics of TSS, including fever, hypotension, and multiorgan modifications typical of staphylococcal TSS. Regrettably, it was not until 1981 that TSST-1 was discovered. In 1987, a series of TSS instances of influenza infection, called the post-influenza TSS in the Minneapolis Paul region, was brought to the attention of the Minnesota Department of Health and colleagues. The 8 children mentioned in the research died following influenza infection to *S. aureus* superinfection caused by TSST-1. However, the surviving child had *S. aureus* infection caused by staphylococcal *enterotoxin* B (SEB) (Schlievert and Davis 2020).

TSS has a high death rate and is considered to progress through three stages. It has a fast start of action and symptoms such as high temperature, hypotension, multi-organ

failure, and an erythematous rash. In the first stage, the symptoms are vague and non-specific, making the diagnosis difficult. In the diagnostic laboratory, there is a definite entry point for infection, such as cutting or wound aid and during this period, TSS can be treated with the use of antibiotics. The second stage has notable symptoms and indicators, including vomiting, fever, diarrhea, hepatitis, nausea, cardiovascular stability, and hypotension. At this stage, a cytokine storm will be produced, so broad-spectrum antibiotics are needed. The third stage is distinguished by extensive bacteremia, sepsis, systemic shock, and multiple organ failure. There is also desquamation, apparent ecchymosis, bullae, and edema. At this stage, treatment consists of providing palliative care for the failing organs. Dialysis, mechanical ventilation, and extensive surgical debridement are required, in addition to the current therapy of broad-spectrum antibiotics and intravenous immunoglobulins (IVIG) (Amreen et al., 2021).

Staphylococcal TSS was originally identified in 1978 in conjunction with a *S. aureus* disease in children and was followed by an outbreak in the 1980s associated with tampon use. However, improvements in tampon production and utilization have resulted in a considerable reduction in the number of menstrual-related staphylococcal infection (mTSS) with 95%prevalence, while non-menstrual staphylococcal infection (TSS)with 50% prevalence. TSS caused by non-menstrual staphylococci has been linked to postoperative, postpartum, postabortion, intrauterine device insertion, burns, soft tissue injuries, and localized infections (Gottlieb et al., 2018). TSS-like neonatal exanthematous lesions, sudden infant death syndrome, and Kawasaki syndrome are all increased when TSST-1 is secreted into the blood (Sultan & Nabiel, 2019). In the United States, the rate of menstruation and non-menstrual TSS is estimated to be between 0.8 and 3.4 per 100,000 women (Ross & Shoff, 2020).

The *tst*-encoded 21.9 kDa superantigen TSST-1 toxin is not widespread. *S. aureus* chromosome contains mobile genetic elements (MGE) called staphylococcal pathogenicity islands (SaPIs). Known as lineages, SaPIs are linked to specific *S. aureus* genetic families, known as lineages. *Tst* is carried by SaPI1, SaPI2, and SaP68111 in human *S. aureus* strains (Sharma et al., 2018). In healthy carriers, there

is a 13–25 % prevalence of TST-encoding strains, indicating a large disease potential. However, the case rate is relatively low (1-4/100,000) (Andrey et al., 2015; Zarei Koosha et al., 2016). TSST-1 toxin production is influenced by a variety of factors, including glucose (by the ccpA catabolite repressor), O2 (via the srrAB two-component system), pH, CO_2, NaCl, magnesium concentration, α and β of hemoglobin (Andrey et al., 2010). The toxin is chemical-stable, heat- and dry-resistant. Induced transcriptional TSST-1 expression was identified as sub-inhibited by nafcillin levels although the expression TSST-1 was reduced by clindamycin, linezolid, and tigecycline (Andrey et al., 2015).

4.2 Structure of TSST-1

There have been many structural and mutational analyses that have revealed important information about the 3D structures and the interactions between superantigens in the host cells. Researchers have explored the crystal structuring of superantigens have dictated that superantigens contain a conservated general structure consisting of 2 key protein domains: The N-terminal domain is an oligosaccharide/ oligonucleotide link layer (O/B) like β-barrel (domain B) (Spaulding et al., 2013). The bigger C-terminals (domain A) are made up of a twisted β-sheet and β-grasp. These two domains are separated by α-helix **(Figure 8)** (Proft & Fraser, 2016).

Superantigens can be classified into five major groups based on small variations in the common core structure. TSST-1 identifies superantigens in group I. The primary amino acid sequences of Group I superantigens are different from those of the other superantigens. These superantigens lack the emetic cystine loop of SEs and the extra 15 amino acid loop of group V SE-l superantigens, and only have the core structure (Spaulding et al., 2013). There is only one MHC II binding site in the O/B folds of superantigens group I, which reacts with the β-chains on MHC II molecules. TSST-1 associate with the antigenic peptide in the peptide-binding groove of MHC II of the molecule. In the orthodox view of the molecule, the V-TCR binding site of TSST-1

can be seen in a groove formed by the O/B fold and the -grasp domains **(Figure 9)** (Zeng et al., 2019; Schlievert et al., 2019).

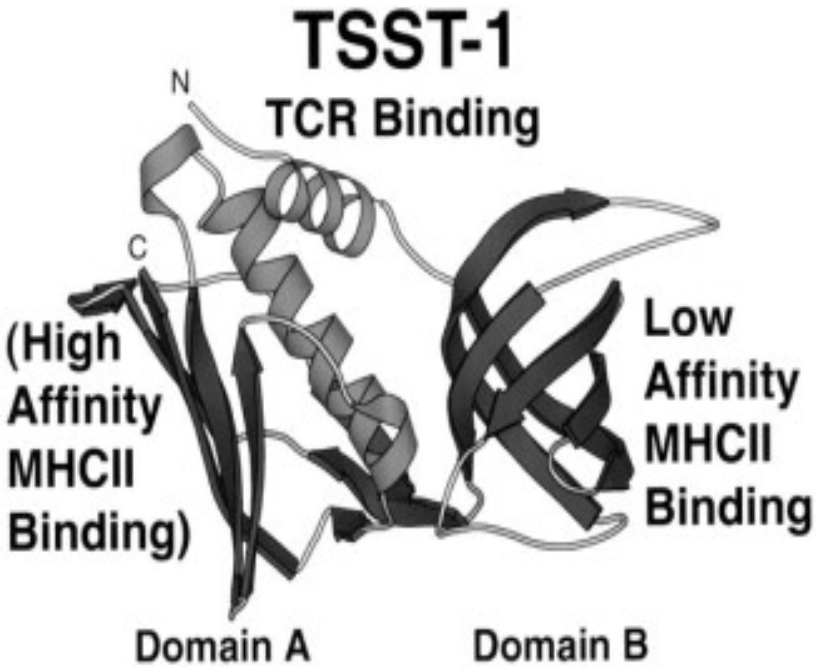

Figure 8: Structure of TSST-1: designated A and B, as well as MHC II molecules is listed as the low-affinity interaction sit (Schlievert, 2001)

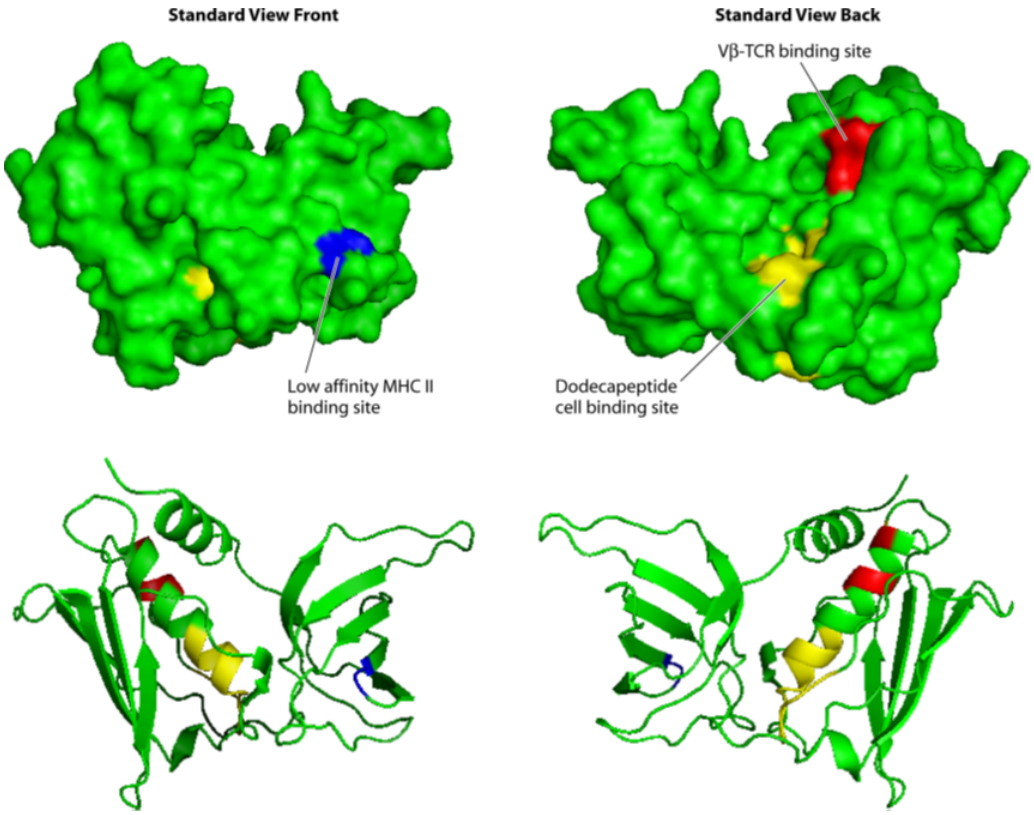

Figure 9: TSST-1, a group I superantigen, is shown in three dimensions (Spaulding et al., 2013)

4.3 Mechanism of action

The capacity of TSST-1 and other SAgs to activate T-cells and APCs is the key mechanism of action. With TSST-1, this is accomplished by non-specifically cross-linking Vβ-chains of TCRs with α-chains on APCs. The antigenic peptide in the MHCII peptide-binding region interacts with TSST-1 that is coupled to the MHCII. This results in substantial T cell activation and proliferation, as well as APC activation. A normal antigen activates 0.01% of T cells, but SAgs activate up to 50% of T cells **(Figure 10**) (Herrera, 2016; Krakauer, 2019). Therefore, when MHC II connect to T-cell receptors will cytokines and chemokines storm, interleukin-1 (IL-1) and IL-2 are examples of cytokines, as are interferon- γ (IFN-γ), tumor necrosis factors TNF-α and

TNF-β. TNF-α and IL-1 are activated early in the process and are direct agents of fever, hypotension, and shock. IFN-γ generated by activated T-cells simultaneously works with TNF-α and IL-1 to improve innate immunity by creating an inflammatory milieu for T-cell activation and differentiation (Amreen et al., 2021).

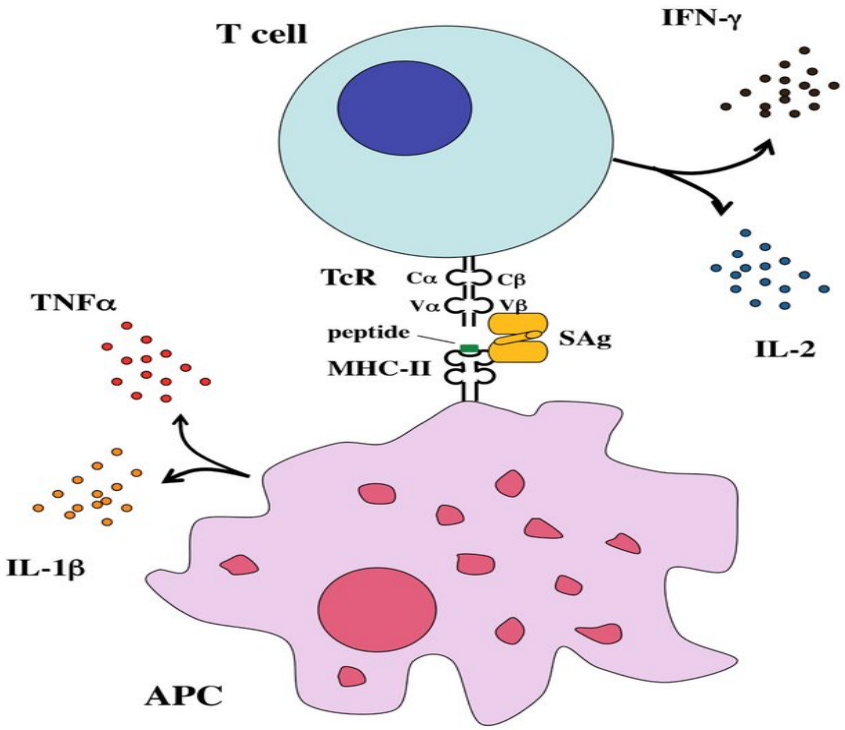

Figure 10: Superantigen interaction with TCR-MHC-II (Proft & Fraser, 2016).

CHAPTER III

MATERIALS AND METHODS

3.1 Bacterial isolates and identification

94 samples of *S. aureus* were collected between 2012 and 2021 from a variety of sites including wound, blood, tracheal aspirate, urine, nasal, and sputum at Near East University Hospital Microbiology Laboratory in Northern Cyprus.

74 samples were isolated and identified as MRSA with Beckton-Dickinson® Phoenix 100 susceptibility system. *S. aureus* isolates were cultured on 5% blood agar from the glycerol stocks and stored at - 80°C. All isolates were initially screened with the coagulase test tube. Departmental and patient demographic data was retrieved from the hospital Nucleus system.

3.2 Coagulase tube test

Individual colonies per isolate and 1 mL of human plasma were mixed in a tube. The samples were then incubated for 4 hours at 37°C. After the incubation, if coagulation was observed, samples were reported to be positive for coagulase and thus indicated the presence of *S. aureus*. The samples were reported negative for coagulase due to the lack of clots after the incubation period.

3.3 Antibiotic susceptibility testing

Susceptibility testing was performed by the disc-diffusion method. Mueller-Hinton agar plates were prepared in accordance with the manufacturer instructions. A bacterial

suspension was prepared by inoculating each isolate in saline to reach at 0.5 McFarland turbidity. Agar plates were then inoculated with the bacterial suspension using a sterile cotton swab. The cefoxitin-containing antimicrobial disc (30 µg) was then placed onto each agar plate and plates were aerobically incubated at 35 °C for 24 hours. Interpretation of susceptibility according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines was made following incubation (EUCAST, 2020). *S. aureus* isolates were reported as methicillin sensitive if the diameter of the zone was >22mm; as methicillin resistant when the diameter of the zone was <22mm **(Figure 11)**.

Figure 11: Cefoxitin susceptibility testing on Mueller-Hinton agar.

3.4 Bacterial DNA extraction

DNA extraction was performed by the boiling method. In brief, a suspension of each isolate was prepared in sterile eppendorf tubes containing 500 µl of sterile deionized water. The mixture was heated for 15 minutes in a dry bath at 95°C. The tubes were centrifuged for 5 minutes at 13000 rpm, and the supernatants containing

bacterial DNA were collected in fresh tubes. Supernatants were stored at 4°C for direct use or -20°C for long-term keeping. 4 µl of the supernatant was used as a template for PCR amplification (Socohou et al., 2021).

3.5 PCR amplification of *tsst-1* gene

For all isolates identified as MRSA, PCR amplification of the *tsst-1*gene was performed. For the first step, 25 µl of the reaction mixture was prepared which consisted of: 12.5µl of 2x PCR master mix (Thermo Scientific); 1 µl of forward primers, 1 µl of reverse primer (at a final concentration of 10pmol/µl) **(Table 2)**, 6.5 µl of nuclease free PCR grade water (Thermo Scientific) and 4 µl of DNA template.

Table 2: *S. aureus* gene-specific oligonucleotide primers used in this study

Gene	Prime sequence (5′ to 3′)	Amplified size (bp)
tst-F	ACCCCTGTTCCCTTATCATC	326-bp
tst-R	TTTTCAGTATTTGTAACGCC	

DNA amplification was performed in a BIO-RAD thermal cycler **(Figure 12**) with the following thermal cycling profile: An initial denaturation at 94°C for 5 min, 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min), and a final extension at 72°C for 7 min (Mehrotra et al., 2000).

Figure 12: BIO-RAD thermal cycler

3.6 Gel electrophoresis

The concentration of agarose in a gel depends on the size of the DNA fragments to be separated. For this study, 2% agarose gel was used. In the first step, TBE (45 mM Tris-borate, 1 mM EDTA) was added as a buffer to the agarose, then the agarose/buffer mixture was heated in the microwave, followed by the addition ethidium bromide (EtBr) at a concentration of 0.5 µg/ml and the agarose was allowed to cool down on the work surface. The comb was removed and the gel was placed in the gel tank containing TBE buffer the loading dye was added to the DNA samples to be separated. The gel loading dye (Hibrigen) was typically prepared at 6X concentration **(Figure 13**). A 50 bp DNA ladder (Hibrigen) was used as DNA molecular weight marker. The DNA bands were separated at 120V for 45 minutes. Finally, the gel was exposed to UV light using a transilluminator (DNA MiniBIS Pro Gel Imaging System.).

Figure 13: Sample loading and gel electrophoresis

CHAPTER IV

RESULTS

4.1 Patient and sample distribution

In this investigation, 76 samples collected from patients admitted to Near East University Hospital were identified as MRSA. Of these isolates, forty-four (57.9%) were isolated from male patients, and thirty-two (42.1%) were isolated from female patients as shown in **Figure 14**. The mean age of patients included in this study was 60.16 with age groups ranging from 1 to 99.

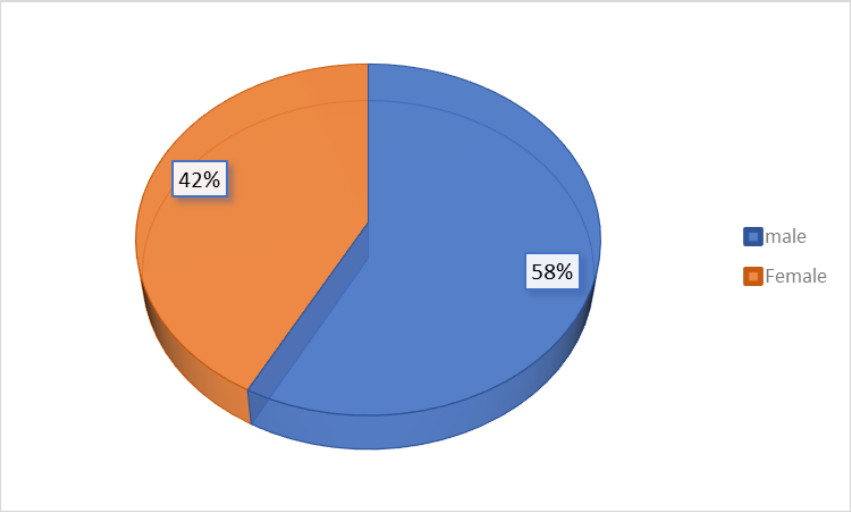

Figure 14: Distribution of patient gender

Most MRSA infections occurred in hospitalized patients (n=57, 75%), with the remaining (n=19; 25%) occurring in outpatients. Figure 16 shows the distribution of MRSA cases in inpatient and outpatient groups based on gender **Figure 15**.

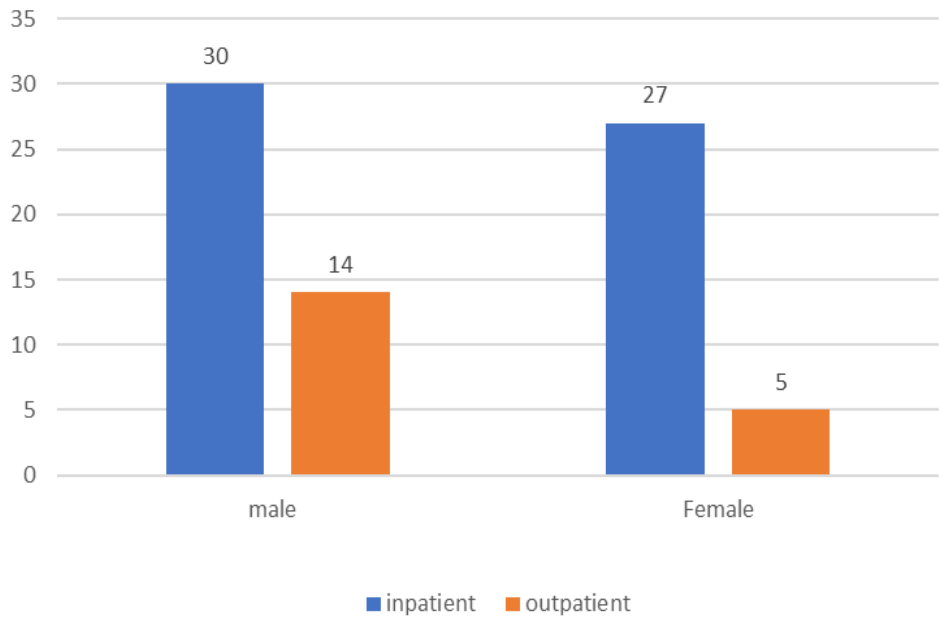

Figure 15: Distribution of inpatient and outpatient cases based on gender

According to the age categories of the patients, patients over the age of 50 had the highest incidence (n= 58, 76.3%) of MRSA infections, followed by the 18-35 and 36-50 age groups. The distribution of patient age groups is shown in **Table 3.**

Table 3. Distribution of age category

Age Group	Frequency	Percent (%)
1-17	2	2.6
18-35	10	13.2
36-50	6	7.9
≥51	58	76.3
Total	76	100.0

According to the types of specimens that were collected from several different body sites, the majority of MRSA was isolated from the abscess-wound (n=19, 25%), followed by blood (n=17; 22.4%) and tracheal aspirate (n=13; 17.1%). The distribution of MRSA according to body sites is shown in **Figure 16**.

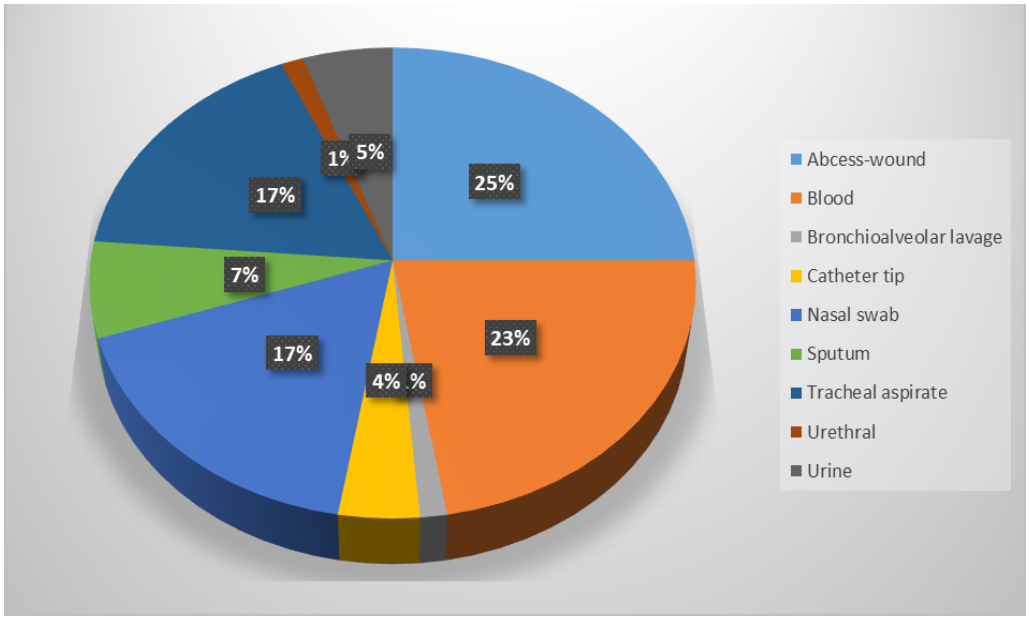

Figure 16: Distribution of the collected specimen types

When the departments where clinical samples were collected was investigated, it was found that the highest number of MRSA were collected from Cardiology department (n=14; 18.4 %) followed by the Pulmonary Infections department (n=10; 13.2%), as shown in **Table 4**.

Table 4. The distribution of clinical specimens among different hospital departments

Department	Frequency	Percent
Cardiology	14	18.4
Pulmonary Infections	10	13.2
Anesthesiology	7	9.2
Laboratory	7	9.2
Neurosurgery	6	7.9
Orthopedics and traumatology	6	7.9
Cardiovascular surgery	5	6.6
General surgery	5	6.6
Dermatology	4	5.3
Gastroenterology	2	2.6
Intensive Care Unit	2	2.6
Dialysis	1	1.3
Emergency	1	1.3
Geriatrics	1	1.3
Infectious Diseases	1	1.3
Neurology	1	1.3
Pediatrics	1	1.3
Plastic surgery	1	1.3
Urology	1	1.3
Total	76	100.0

4.2 Phenotypic characterization

In this study, out of 94 *S. aureus* isolates, 76 isolates were confirmed to be MRSA with coagulase tube test and cefoxitin disc diffusion assays **(Table 5)**. 18 isolates which were found to be coagulase negative and cefoxitin susceptible were excluded from this study.

Table 5: Collective data for all MRSA isolates used in this study

Sample No	Gender	Age	Clinical sample	Inpatient/outpatient	Service	Coagulase	Cefoxitin (mm)	MRSA (TSST-1)
1	Female	85	Tracheal aspirate	inpatient	Brain Surgery	Positive	14	positive
2	Female	59	Abcess-wound	inpatient	Orthopedics and Traumatology	Positive	17	Negative
3	male	76	Blood	inpatient	Cardiology	Positive	9	Negative
4	male	75	Blood	inpatient	Cardiology	Positive	19	Negative
5	male	20	Abcess-wound	outpatient	Orthopedics and Traumatology	Positive	15	Negative
6	male	55	Abcess-wound	outpatient	Dermatology	Positive	15	Negative
7	male	57	Blood	inpatient	Cardiology	Positive	14	Negative
8	female	99	Tracheal aspirate	inpatient	Pulmonary Infections	Positive	16	Negative
9	female	25	Urine	outpatient	Infectious Diseases	Positive	19	Negative
10	male	65	Blood	inpatient	Neurosurgery	Positive	15	Negative
11	male	65	Blood	inpatient	Neurosurgery	Positive	12	Negative
12	male	87	Tracheal aspirate	outpatient	Anesthesia	Positive	13	Negative
13	female	1	Urine	outpatient	Pediatrics	Positive	15	Negative
14	female	63	Nasal swab	inpatient	Cardiovascular surgery	Positive	15	Negative
15	male	41	Tracheal aspirate	inpatient	Pulmonary Infections	Positive	16	Negative
16	male	62	Tracheal aspirate	inpatient	Neurology	positive	10	Negative
17	male	53	Urine	inpatient	Cardiology	Positive	13	Negative
18	male	66	Catheter tip	inpatient	Cardiology	Positive	10	Negative
19	male	55	Nasal swab	inpatient	Cardiology	Positive	18	Negative
20	male	75	Blood	inpatient	Emergency	Positive	16	Negative
21	female	58	Bronchioalveolar lavage	inpatient	Pulmonary Infections	Positive	11	Negative
22	female	80	Tracheal aspirate	inpatient	Pulmonary Infections	Positive	15	Negative
23	female	80	Tracheal aspirate	inpatient	Pulmonary Infections	Positive	14	Negative
24	female	77	Nasal swab	inpatient	cardiovascular surgery	Positive	15	Negative
25	female	58	Sputum	inpatient	Pulmonary Infections	Positive	8	Negative
26	female	80	Sputum	inpatient	Pulmonary Infections	Positive	13	Negative
27	male	88	Abcess-wound	inpatient	General surgery	Positive	11	Negative
28	male	88	Blood	inpatient	General surgery	positive	11	Negative
29	male	88	Blood	inpatient	General Surgery	Positive	8	Negative
30	male	27	Catheter tip	inpatient	Neurosurgery	Positive	15	Negative
31	male	27	Nasal swab	outpatient	Laboratory	positive	22	Negative
32	male	60	Blood	inpatient	Pulmonary Infections	Positive	7	Negative
33	male	63	Blood	inpatient	Laboratory	Positive	13	Negative
34	female	51	Abcess-wound	inpatient	Laboratory	Positive	14	Negative
35	female	65	Nasal swab	inpatient	Cardiovascular surgery	positive	14	Negative
36	male	63	Nasal swab	outpatient	Pulmonary Infections	Positive	15	Negative
37	male	67	Blood	inpatient	Geriatrics	Positive	17	Negative
38	male	61	Abcess-wound	outpatient	Orthopedics and traumatology	Positive	12	Negative
39	male	61	Abcess-wound	outpatient	Orthopedics and traumatology	Positive	17	Negative
40	male	63	Abcess-wound	outpatient	Orthopedics and traumatology	Positive	18	Negative
41	male	55	Blood	outpatient	Laboratory	Positive	14	Negative
42	male	88	Blood	inpatient	General Surgery	Positive	19	Negative
43	male	25	Nasal swab	outpatient	Laboratory	Positive	20	Negative
44	female	76	Nasal swab	inpatient	Laboratory	Positive	14	Negative
45	male	39	Nasal swab	inpatient	Cardiology	Positive	16	Negative
46	male	63	Blood	outpatient	Gastroenterology	Positive	15	Negative
47	female	80	Sputum	inpatient	Cardiology	Positive	14	Negative
48	male	41	Blood	inpatient	Pulmonary Infections	Positive	18	positive
49	male	19	Tracheal aspirate	inpatient	Neurosurgery	Positive	16	Negative
50	male	74	Catheter tip	inpatient	Cardiology	Positive	17	Negative
51	female	76	Abcess-wound	inpatient	Dialysis	Positive	16	Negative
52	male	70	Abcess-wound	inpatient	Cardiology	Positive	16	Negative
53	female	69	Abcess-wound	inpatient	Gastroenterology	Positive	15	Negative
54	female	74	Nasal swab	inpatient	Cardiovascular surgery	Positive	17	Negative
55	male	73	Nasal swab	inpatient	Cardiovascular surgery	Positive	18	Negative
56	female	79	Nasal swab	inpatient	Cardiology	Positive	17	Negative
57	female	56	Blood	inpatient	Anesthesiology	Positive	15	Negative
58	female	2	Abcess-wound	inpatient	Plastic surgery	positive	20	Negative
59	female	29	Abcess-wound	inpatient	General surgery	positive	12	Negative
60	male	70	Tracheal aspirate	inpatient	Anesthesiology	positive	19	Negative
61	male	95	Abcess-wound	inpatient	Cardiology	positive	17	Negative
62	female	28	Abcess-wound	outpatient	Dermatology	positive	9	Negative
63	male	39	Nasal swab	outpatient	Cardiology	positive	13	Negative
64	female	71	Sputum	inpatient	Anesthesiology	positive	15	Negative
65	female	42	Sputum	inpatient	Anesthesiology	positive	14	Negative
66	female	54	Tracheal aspirate	inpatient	Intensive Care Unit	positive	5	Negative
67	male	80	Tracheal aspirate	inpatient	Anesthesiology	Positive	16	Negative
68	female	96	Tracheal aspirate	inpatient	Anesthesiology	Positive	16	Negative
69	female	36	Urine	outpatient	Laboratory	Positive	15	Negative
70	female	20	Abcess-wound	outpatient	Dermatology	Positive	13	Negative
71	male	23	Urethral	outpatient	Urology	Positive	13	Negative
72	female	85	Tracheal aspirate	inpatient	Brain Surgery	Positive	16	Negative
73	female	59	Abcess-wound	inpatient	Orthopedics and traumatology	Positive	20	Negative
74	male	76	Blood	inpatient	Cardiology	Positive	17	Negative
75	male	55	Abcess-wound	outpatient	Dermatology	Positive	19	Negative
76	male	66	Abcess-wound	inpatient	Intensive Care Unit	Positive	12	Negative

4.3 Amplification of *tsst-1*gene

Based on the PCR results, the *tsst-1* gene was identified as a 326 bp band using a DNA molecular marker (50 bp ladder) **(Figure 17)**. The presence of *tsst-1* gene was detected only in two (2.63 %) of 76 MRSA isolates screened.

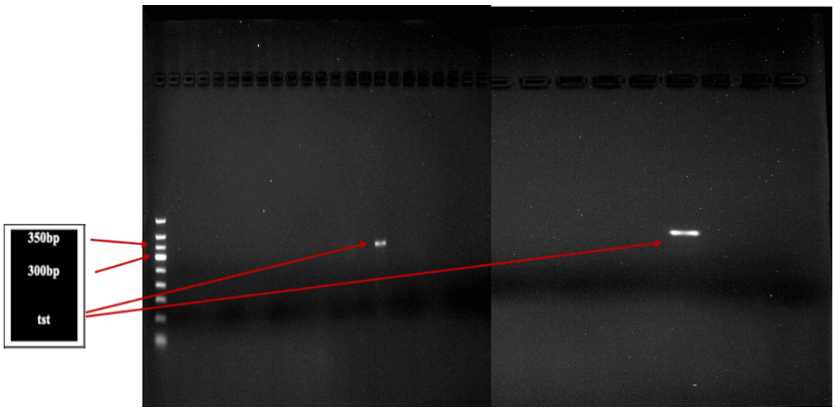

Figure 17: Results of PCR amplification of TSST-1 gene fragment by specific primers as a single band of 326 bp upon electrophoresis

One of the TSST-1 positive isolates (sample no. 1) was from a tracheal aspirate from a female patient. The patient was admitted to NEU Hospital Department of Brain Surgery on 05/08/2018 with a subdural hematoma diagnosis, and was later admitted to the Intensive Care Unit. Patient has high serum CRP levels (11.59 mg/L) and elevated neutrophil count ($11.09x10^3$/µl) on 01/10/2018 for over a week. She was diagnosed with MRSA infection on 03/11/2018. The patient was hospitalized for 2 months and 11 days in total and died on 16/10/2018.

The other TSST-1 positive isolate (sample no. 48) was from a blood sample from a male patient. The patient was initially admitted to NEU Hospital for a femoral fracture on 31.01.2016 and was discharged on 18.02.2016. Patient returned to NEU Hospital with high fever (40ºC) on 22.02.2016 and was diagnosed with MRSA infection in blood. Patient was hospitalized for over a month with positive MRSA blood culture and elevated neutrophil counts ($10.10x10^3$/µl) and was successfully discharged post treatment on 18/04/2016.

CHAPTER V

DISCUSSION

Multidrug-resistant bacterial pathogens are currently considered as a global public health concern. As of 2019, the CDC has announced that the human race is now in the "post antibiotic era," and the World Health Organization (WHO) has declared that the antibiotic resistance crisis is becoming a major healthcare problem. One of the most common commensal microorganisms and a major pathogen responsible for hospital- and community-acquired infections is *S. aureus*. Equipped with an arsenal of virulence factors for increased pathogenicity, infections with MRSA lead to longer hospital stays and higher health care costs (Lakhundi & Zhang, 2018). Therefore, new antimicrobial agents with diverse bactericidal mechanisms are urgently required to foster alternative therapeutic strategies for combating MRSA infections (Ovchinnikov et al., 2020; Hutzschenreuter et al., 2018). Unraveling of the mechanisms behind pathogenicity of MRSA also represents a critical area of research to be addressed.

In this study, virulence characteristics of MRSA isolates collected from patients at Near East University Hospital was investigated. 76 isolates were identified as MRSA strains using both phenotypic and genotypic methods. Men were the most severely affected group of patients, accounting for the majority of those who were hospitalized. The highest cases of MRSA (n= 44; 57.89%) was observed in inpatient group and the highest percentage of male patients (n=34; 44.73%) were those > 51 years of age. According to previous studies, there was no correlation between gender, clinical specimens, and methicillin resistance (Omidi et al., 2020). However, data from the large National Health and Nutrition Examination Survey (NHANES) study suggests an interplay between gender and race/ethnicity, male gender was a notable risk factor for *S. aureus* carriage in non-Hispanic white and Mexican American populations but not in the non-Hispanic black population (Sollid et al., 2014). In another study, on the contrary females in the 51-60 age group were identified to be more susceptible to

MRSA infection compared to males in Dhaka, Bangladesh (Parvez et al., 2018). Further study results revealed a link between female hormones and differences in bacterial virulence and adaptive immune response (Ali & Seiffein, 2021). In terms of the distribution of MRSA infections observed in this study, the inpatient group was more than three times affected compared to the outpatient group (n=57, n=19 respectively), with a median age of 66. A similar pattern of outcomes had been observed in a previous study (Changchien et al., 2016). Increased exposure to medical procedures such as antibiotic therapy and invasive surgeries and devices have been associated a longer hospital stay comes, which elevates the risk of developing a hospital-acquired infection, particularly MRSA infection, and adds to the overall cost of care (Hutzschenreuter et al., 2018; Efa et al., 2019).

In the current study, most of the MRSA isolates were from a wound abscess (n=19; 25%) of skin foci. This may be explained as a consequence of skin commensalism by *S. aureus*. A previous study reported that 68.3% of SSTIs were caused by MRSA in Colombia (Valderrama-Beltrán et al., 2019). Bacteremia is prevalent in individuals who have a skin and skin structure infection (SSSI) that severely necessitates hospitalization (Lipsky et al., 2010). In the United States, SSSIs continue to be among the most common HAIs, accounting for approximately 21.8% of all HAIs, resulting in increased morbidity and mortality, as well as increased costs because utilization of health care resources (Barry, 2021).

Blood samples were the second most common source of MRSA in this study, accounting for 22.4% ((n=17) of the total samples collected. In addition, it was reported 15 of the MRSA bacteremia infections were diagnosed in the inpatient group, and the mean age was 65. A similar study for the same age group has been previously published (Lee et al., 2016). Persistence of bacteremia was documented more frequently in HCA bacteremia, resulting in a higher rate of bacteriologic failure (Bishara et al., 2012). The presence of MRSA bacteremia in elderly patients is concerning. It has a high mortality rate, particularly in patients with co-morbidities such as malignancies, cardiovascular disease, and an increased risk of developing

complications (Pastagia et al., 2012). *S. aureus* bacteremia management entails identifying the infecting strain and source of infection as soon as possible, then selecting an appropriate antibiotic treatment, and implementing effective prevention strategies (Hassoun et al., 2017). MRSA infections were mostly found in the cardiology department (n=14; 18.4%) and the pulmonary infection department (n=10; 13.2%).

TSS is caused by superantigens from staphylococci and streptococci, which cause a rapid hyperinflammatory response and are characterized by a cytokine storm. Todd et al. first described this syndrome in 1978 as a rare side effect of *S. aureus* infection. MRSA infections in the postpartum period frequently result in TSS and other serious illnesses. Toxic shock syndrome is recognized to occur in the early postoperative period after various types of surgery (Komuro et al., 2017). The CDC developed a case definition for toxic shock syndrome 40 years ago. Significant criteria hold a 38.8°C fever, a diffuse, macular erythrodermic rash, skin desquamation 1–2 weeks after illness onset, and hypotension. The involvement of three or more organ systems is considered a minor criterion (Poudel et al., 2021).

In this investigation, molecular techniques were used to detect the prevalence of toxin gene carriage in MRSA. This study was focused on the staphylococcal superantigens, particularly TSST-1. Only two (2.63%) of the MRSA isolates tested in the current study have been found positive for the *tsst-1* gene. Both samples were isolated from inpatients. One of the TSST-1 positive isolates was from a tracheal aspirate from a 85 year-old female patient. The female patient had a severe MRSA infection which was hospital-acquired. Patient was hospitalized for over 2 months and later died. The virulent characteristic of the MRSA isolate may have had a negative impact on the condition of this patient. The other TSST-1 positive isolate was from a blood sample from a 41 year-old male patient. Although this patient was also hospitalized for a long period of time, upon treatment the patient was successfully discharged. According to a recent review conducted in Iran, the overall prevalence of clinical *S. aureus* isolates infected with TSST-1 was 21.3%. TSST-1 levels in clinical

S. aureus isolates ranged from 0% to 68%. However, *tsst*-carrying MRSA strains were found in high numbers in studies from Japan (75.7%) and Taiwan (75%) (Goudarzi et al., 2020). In Turkey, the *tsst-1* gene was detected in 3.3% of nasal MRSA isolates (Dincer et al., 2021). A prior study conducted in Turkey reported the prevalence of MRSA infection with *tsst-1* genes to be 14.2% (Motamedifar et al., 2015).

This study had a few limitations. Firstly, the small number of MRSA isolates studied suggests that the differences noted could be due to low genotype frequencies recorded. Secondly, the patients were drawn from a single hospital therefore may not represent the overall virulence characteristics of isolates of entire country. Thirdly, this work was also limited by lack of susceptibility testing for other antibiotics. If antibiotics are used appropriately, it can help reduce hospitalization time as well as the cost of care. Further studies that compare virulence characteristics of MSSA and MRSA isolates can be beneficial.

CHAPTER VI
CONCLUSION AND RECOMMENDATIONS

S. aureus is a major public health concern in both high- and low-income countries worldwide. The emergence of new antibiotic resistance mechanisms, as well as an increase in the occurrence of unusually severe infections further complicates the situation. Molecular biology techniques were used in this study to characterize patterns of toxin carriage in clinical MRSA isolates obtained from patients at Near East University Hospital over an eight-year period. The result of the experimental study has led to the conclusion that the MRSA strains circulating in the hospital carry the *tsst-1* gene at a low prevalence. Moreover, the study results indicated that hospitalized patients are the most affected subgroup, necessitating the development of an effective surveillance strategy.

Forthcoming research is needed to validate the conclusions presented in this study. Thus, researchers should attempt to address this question to develop a better understanding of the virulence patterns of *S. aureus* strains present in the Turkish Republic of Northern Cyprus (TRNC). The use of whole genome sequencing for genome comparison between different strains and screening of toxin genes and antibiotic resistance patterns are other areas that need to be explored.

Collectively, this is the first study to investigate presence of toxic shock syndrome toxin 1, and it is another step toward a better understanding of the molecular properties of *S. aureus* strains found in TRNC hospitals. As a result, this research could aid in the development of better strategies for infection prevention and control treatment.

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APPENDICES

Appendix A

Turnitin Similarity Report

Turnitin Originality Report

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Curriculum Vitae

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2. EDUCATION

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3. ACADEMIC EXPERIENCE

PERIOD	TITLE	DEPARTMENT	UNIVERSITY
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4. FIELD OF INTERESTS

FIELDS OF INTERESTS	KEY WORDS
Medical Microbiology	MRSA , *Staphylococcus aureus,* molecular typing; toxic shock syndrome toxin-1; virulence factors

Ethical Approval

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 27.05.2021

Toplantı No **:** 2021/91

Proje No **:**1331

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Buket Baddal'ın sorumlu araştırmacısı olduğu, YDU/2021/91-1331 proje numaralı ve **"Molecular Detection of Exfoliative Toxins and Toxic Shock Syndrome Toxin-1 in Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates from Clinical Specimens at Near East University Hospital"** başlıklı proje önerisi kurulumuzca online toplantıda değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı

Yakın Doğu Üniversitesi

Bilimsel Araştırmalar Etik Kurulu Başkanı