NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

PREVALENCE OF EXFOLIATIVE TOXIN GENES IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES AT NEAR EAST UNIVERSITY

M.Sc. THESIS

BAKARE BUSAYO OMOWUMI

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Approval

We certify that we have read the thesis submitted by Bakare Busayo Omowumi titled "**Prevalence of Exfoliative Toxin Genes in Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates at Near East University**" 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.

Bakare Busayo Omowumi

4/07/22

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Bakare Busayo Omowumi

Abstract

Prevalence of Exfoliative Toxin Genes in Methicillin-Resistant Staphylococcus aureus Clinical Isolates at Near East University

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M.Sc., Department of Medical Microbiology and Clinical Microbiology

Supervisor: Assoc. Prof. Buket Baddal

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Staphylococcus aureus, a Gram positive bacterium, is a famous human pathogen associated with hospital- as well as community-acquired infections worlwide. Infections caused by this bacterium may range in severity, varying from mild skin infections to life-threatening conditions. The current challenges faced by the healthcare systems are mostly due to two factors; first, the emergence and rapid spread of resistance to currently available antimicrobials as well as the development of resistance to new therapeutic alternatives; second, the wide range of virulence determinants harbored by the bacterium and exerting action promoting colonization and infection. These two mentioned factors render treatment and management of infections a difficult task. Therefore, in depth investigations of bacterial molecular mechanisms that control them is a critical area of research. This project was prompted by the scarcity of data on the virulence profiles of methicillin-resistant *S. aureus* (MRSA) in Northern Cyprus. By prospectively screening seventy-six MRSA clinical isolates at Near East University Hospital Microbiology Laboratory, we aimed at characterizing the presence and assessing the prevalence of a class of virulence determinants: the exfoliative toxins which are responsible for the severe skin condition, staphylococcal scalded skin syndrome. Molecular screening of toxin genes yielded no positive strains for exfoliative toxin D gene, and one positive sample (%1.32) for the exfoliative toxin B homologous gene. Our results demonstrated a low prevalence of exfoliative toxins in MRSA isolates circulating at NEU Hospital. The results presented in this study represent a point in time snapshot of the assessed parameters, and therefore will serve as reference for further and more comprehensive studies.

Keywords: *Staphylococcus aureus,* MRSA, virulence factors, exfoliative toxins

Özet

Yakın Doğu Üniversitesi'nde Metisilin Dirençli *Staphylococcus aureus* Klinik İzolatlarında Eksfolyatif Toksin Prevalansının Araştırılması

Bakare Busayo Omowumi

Yüksek Lisans, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı

Danışman: Doç. Dr. Buket Baddal

Haziran, 2021, 56 sayfa

Gram pozitif bir bakteri olan *Staphylococcus aureus*, dünya çapında hastane ve toplum kaynaklı enfeksiyonlarla ilişkili bir insan patojenidir. *S. aureus*'un neden olduğu enfeksiyonlar, hafif cilt enfeksiyonundan yaşamı tehdit eden hastalıklara kadar değişkenlik göstermektedir. Sağlık sistemlerinin karşılaştığı zorluklar çoğunlukla iki faktörden kaynaklanmaktadır; ilk olarak, mevcut antimikrobiyallere karşı direncin ortaya çıkması ve hızla yayılması, ayrıca yeni terapötik alternatiflere karşı direncin gelişmesi; ikincisi ise, bakteriye ait kolonizasyon ve enfeksiyonu tetikleyici çeşitli virülans faktörleridir. Bahsedilen bu iki faktör, enfeksiyonların tedavisini ve yönetimini zorlaştırmaktadır. Bu nedenle, bu faktörleri kontrol eden bakteriyel moleküler mekanizmaların araştırılması önem taşımaktadır. Kuzey Kıbrıs'ta metisilin dirençli S. *aureus* (MRSA) virülans profillerine ilişkin veri çok azdır. Bu çalışma, Yakın Doğu Üniversitesi Hastanesi Mikrobiyoloji Laboratuvarı'nda MRSA klinik izolatlarını prospektif olarak tarayarak, şiddetli cilt enfeksiyonlarından sorumlu eksfolyatif toksinlerin varlığını karakterize etmeyi ve prevalansını belirlemeyi amaçlamaktadır. Yetmiş-altı MRSA izolatında yapılan moleküler tarama sonucunda, eksfolyatif toksin D geni için pozitif suş bulunmazken, eksfolyatif toksin B homolog geni için bir örnek (%1.32) pozitif olarak saptanmıştır. Sonuçlarımız, YDÜ Hastanesi'nde bulunan MRSA izolatlarındaki eksfolyatif toksin prevalansının düşük olduğunu göstermiştir. Bu çalışmada sunulan sonuçlar, ileriki kapsamlı çalışmalar için bir referans olarak sunulmaktadır.

Anahtar kelimeler: *Staphylococcus aureus,* MRSA, virülans faktörleri, eksfolyatif toksinler

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LIST OF ABBREVIATIONS

ADAM: A Disintegrin and Metalloprotease

AGR*:* Accessory Gene Regulator

AIP: Auto-Inducing Protein

APC: Antigen Presenting Cell

CA-MRSA: Community-Acquired MRSA

CP: Capsular Polysaccharide

DNA: Deoxyribonucleic Acid

DSG: Desmoglein

ET: Exfoliative Toxin

HA-MRSA: Hospital-Acquired MRSA

HCW: Healthcare Worker

HLA: Hemolysin Alpha

LTA: Lipoteichoic Acid

MH: Mueller-Hinton

MHC: Major Histocompatibility Complex

MRSA: Methicillin resistant *Staphylococcus aureus*

MSCRAMM: Microbial Surface Components Recognizing Adhesive Matrix Molecule

PBP: Penicillin Binding Protein

PFT: Pore Forming Toxin

PSM: Phenol Soluble Modulin

PVL: Panton-Valentin Leukocidin

SAg: Super-Antigen

SCC: Staphylococcal Chromosome Cassette

SE: Staphylococcal Enterotoxin

SSSS: Staphylococcal Scalded Skin Syndrome

SSTI: Skin and Soft Tissues Infection

TBE: Tris-Borate EDTA

TCR: T-Cell receptor

TSS: Toxic-Shock Syndrome

TSST: Toxic Shock Syndrome Toxin

WHO: World Health Organization

WTA: Cell Wall Teichoic acid

CHAPTER I

INTRODUCTION AND AIMS

1.1. Background of the research

Staphylococcus aureus is a renowned causative pathogen of wide variety of diseases such as respiratory, skin and soft tissue infections (Esposito et al., 2016; Pivard et al., 2021) as well as life-threatening conditions such as sepsis, necrotizing fasciitis, and other related toxin-mediated syndromes (Dayan et al., 2016; Tong et al., 2015).

S. aureus was first isolated from pus drainage in surgical abscesses by Sir Alexander Ogston in 1880. The name of this bacterium — recurrently involved in post-surgical suppurative infections — was coined by its tendency to arrange in grape-like clusters. The ability of the bacterium to induce skin infections was later confirmed by *in vivo* experiments (Rasheed & Hussein, 2021). The bacterium is a commensal and an opportunistic pathogen which may inhabit different parts of the human body, but predominantly prefer skin and nostrils (Van Wamel, 2017). Epidemiological and clinical studies of *S. aureus* have been topic of interest for nearly half of a century and results presented so far have stated that approximately 30% of individuals are permanently colonized with it (Laux et al., 2019; Tong et al., 2015). Although unproblematic in healthy individuals, permanent nasal carriage have been reported as at high-risk for hospitalized individuals to develop staphylococcal infections (Akhtar Danesh et al., 2020; Mallet et al., 2018).

The ability of *S. aureus* to colonize and subsequently infect diverse tissues and cause the wide range of diseases aforementioned suggests an important fact: it possesses several distinct molecular mechanisms to thrive in host environment (Horino & Hori, 2020; Kim, 2019; Kong et al., 2016). Among these virulence determinants are toxins; several studies have explored the effects of toxins on disease severity (O'Callaghan, 2018; Oliveira et al., 2018). Moreover, recent hype around the bacterium is to a large extent due to the rapid emergence of antimicrobial resistance. Whenever a new antibiotic was introduced into clinical use, *S. aureus* always

developed resistance in an almost instantaneous timescale fashion (T. J. Foster, 2017a; Gajdács, 2019). Regarding this matter, World Health Organization (WHO) has classified methicillin-resistant *Staphylococcus aureus* (MRSA) as one of the priority pathogen to address. MRSA strains have emerged soon after the introduction of methicillin into clinical practice (Lee et al., 2018), and have also rapidly developed a unique and distinctive feature: multidrug resistance (Kot et al., 2020).

From the history of its discovery until today, *S. aureus* (and its contemporary MRSA) has been one of the most important pathogens associated with hospital-acquired infections and was therefore termed as hospital-acquired MRSA (HA-MRSA). A new lineage, recently characterized, and mostly associated to infections in individuals with no previous hospitalization record as well as weaker multidrug resistance phenotype was termed as community-acquired MRSA (CA-MRSA). Although the definition was clear, distinguishing between the two lineage has become difficult, with an increasing rate of report presenting CA-MRSA strains as causative agents of hospital associated infections (Henderson & Nimmo, 2018; Kateete et al., 2019).

Clinically, infections caused by MRSA are often associated with increased duration and cost of hospitalization, due to difficulties in management and treatment. To illustrate such fact, a study assessing the risk factors for subsequent surgical site infection in neonates surgical patients have identified MRSA colonization as an important risk factor (Inoue et al., 2018). Another study associated MRSA infection with an increased economic impact (Zhen et al., 2020).

Since what have been discussed above can have serious consequences on global and regional public health, and given the fact that previous studies providing evidences and insight are scarce, this study aims to address the concern in a two-step fashion; Firstly, we aim at investigating the virulence determinants and associated genes, focusing on exfoliative toxins, in *S. aureus* strains isolated from clinical specimens at Near East University Hospital. We ultimately aim to provide a point in time reference, based on epidemiological pattern, to serve further studies and help in monitoring the evolution of the investigated parameters in a timeline.

CHAPTER II

LITERATUTE REVIEW

2.1. Microbiology of staphylococci

2.1.1. Taxonomy

The genus *Staphylococcus* along with several other genera to the family of *Staphylococcaceae.* It is reported in the literature that the genus comprises as much as 45 different species and 24 subspecies (Gherardi et al., 2018). Other than *S. aureus*, clinically relevant species which are increasingly reported as aetiologic agents in human infections include *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus* (Becker et al., 2020; Heilmann et al., 2019). They are human commensals and can be isolated from various sites such as skin, nares and mucous membranes.

2.1.2. Laboratory identification

Members of the genus staphylococci are Gram-positive cocci. They vary in size from 0.5-1.5µm and may occur in irregularly shaped "grape-like" clusters. They are non-motile, non-spore forming and non-capsulated (although some species harbor capsule-encoding genes) (

Figure 1) (Argemi et al., 2019). Regarding oxygen requirements, they can behave as either aerobic or facultative anaerobe; they are sodium chloride (NaCl) resistant, indicating that they can readily grow on culture medium containing up to 10% NaCl. Biochemically, they are mostly oxidase-negative, catalase-positive (this latter characteristic distinguish them from streptococci genus, which are catalase-negative).

Laboratory identification is achieved by growth on most of the non-selective and rich media. The optimal growth temperature is 37°C, however, most of staphylococci can thrive at temperatures ranging from 15°c to 45°C. The differentiating feature between *S. aureus* and other staphylococci is the production of

coagulase (routinely assessed via coagulase tube test), and hemolysis halo on solid media culture (Bonar et al., 2018; T. J. Foster & Geoghegan, 2015).

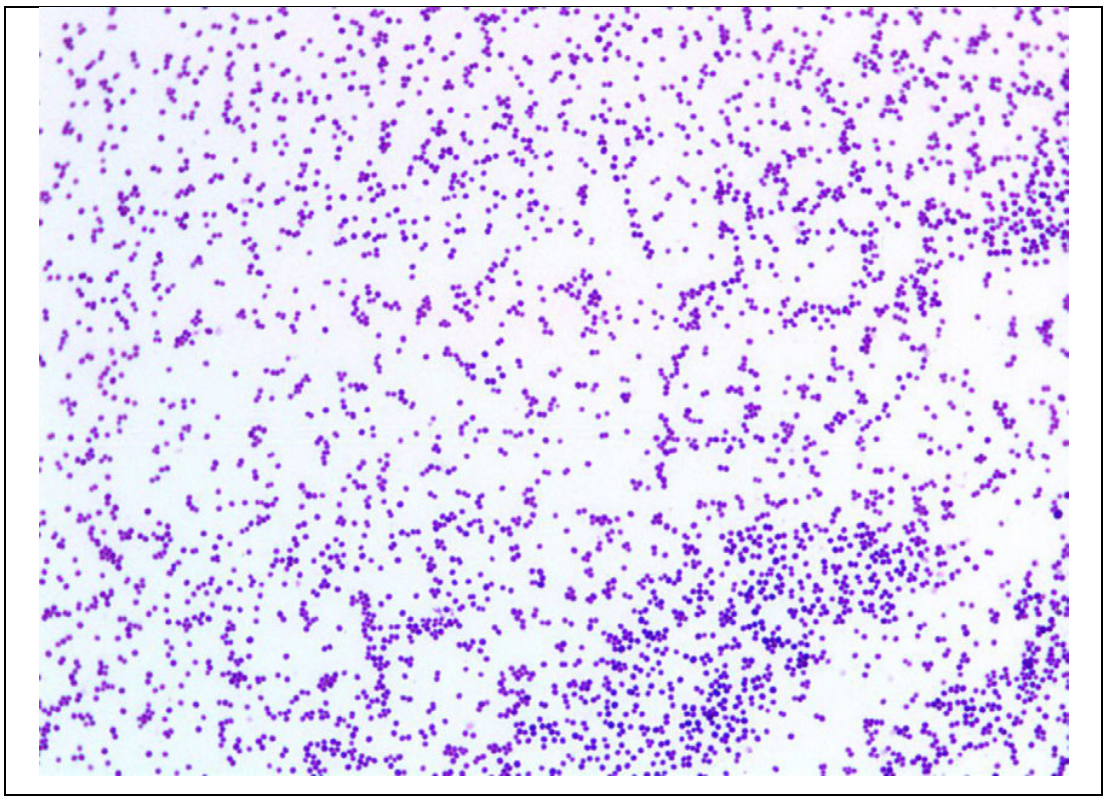


Figure 1: *S. aureus* morphology after Gram staining

2.2. *Staphylococcus aureus*

2.2.1. The genome

A series of studies performed have emphasized the distinctive features of *S. aureus* genome (Lakhundi & Zhang, 2018). The pan-genomic investigations highlight the following three major entities:

i. The core genome: as the concept of "core" appears, this part of the genome represents approximately 75% of the whole genetic material and contains genes necessary for the maintenance of survival mechanisms as well as many virulence genes. It is shared across all strains and therefore shows very low genetic variability (T. J. Foster & Geoghegan, 2015).

ii. The accessory genome: this is the part of the genome which encompasses non-housekeeping genes. They play an important role in virulence and resistance mechanisms and include plasmids, bacteriophages, transposons and insertion sequences, cassette chromosome components.

- Plasmids

Plasmids harbored by *S. aureus* are categorized in three different classes — Class I, II and III—on the basis of their size and functional determinants (Firth et al., 2019; T. J. Foster & Geoghegan, 2015). They play an important role in the spread of antibiotic resistance genes, and constitute together with bacteriophages, the major vehicles of horizontal gene transfer (Lerminiaux & Cameron, 2019).

- Bacteriophages

In a broader context, bacteriophage refers to a class of viruses that infect bacteria. At a bacterial genome level, and with specific interest in gene transfer, bacteriophages are relevant when it comes to consider the transduction process. Transduction is an important process in the evolution of *S. aureus* strains, for both virulence and genetic diversity. In the first case, several virulence factors have been identified to be carried by phages (T. J. Foster & Geoghegan, 2015). Phages may also serve as vehicle to transport set of genes involved in mechanisms other than virulence, rendering them responsible for the majority of horizontal gene transfer that occurs among *S. aureus* strains. Of note, generalized transduction phages carrying exclusively bacterial DNA may deliver it into a new bacterium; there, the imported genome fragment may remain and replicate independently as a plasmid, or may be integrated into the chromosome. In either cases, as a consequence of the transfer, the receiver bacterium may later express new phenotypes (due to the expression of novel genotypes). For example, in a set of experiments, authors have confirmed the latter discussed hypothesis; *S. aureus* generalized transduction phage Φ11 enabled survival of the transductants to antibiotic exposure (Fillol-Salom et al., 2019).

Moreover, phages have garnered more attention and studied are still ongoing. Apart from genetic transfer function, they hold other relevant clinical implication. The ΦSA169, have been demonstrated to increase MRSA fitness and survival during endovascular infections (Li et al., 2020). Other phages are responsible for *S. aureus* host adaption (Moller et al., 2021; Rohmer & Wolz, 2021).

- Staphylococcal chromosome cassette

20 Kb to 70 Kb fragments are found in most of MRSA strains; they are mobile genetic elements referred to as staphylococcal chromosome cassette (SCC) (Firth et al., 2019). Resistance to methicillin is generically mediated via *mec* segment of the SCC, therefore termed as SCC*mec*. The *mecA* gene, and its recently discovered homologous, namely *mecB*, *mecC*¸ *mecD*, all encode for a PBP2a, a low affinity penicillin binding protein (PBP) (Becker et al., 2018; Lozano et al., 2020; Schwendener et al., 2021).

Structurally, SCC*mec* is made of three distinct domains namely (see **Figure 2**): the ***ccr*** domain, a set of functional genes mediating integrity, recombination and insertion features. They are crucial in converting methicillin-sensitive strains into MRSA, as they drive SCC*mec* integration into *S. aureus* chromosome (Firth et al., 2019; Lakhundi & Zhang, 2018); the *mec* domain, composed of the methicillin resistance effector as aforementioned, along with regulatory genes (Carretto et al., 2018); and finally, the ***J*** domain, considered as a landing site for additional genes insertion. As a hot-spot for gene addition, this latter domain holds a high genetic diversity across strains and is used in SCC*mec* subtyping (see http://www.sccmec.org, (Firth et al., 2019)). The unique organization of *mec* and *ccr* domains helped identify at present, 14 different allotypes (Urushibara et al., 2020).

Of note, SCC*mec* is not a feature unique to *S. aureus* (Chanchaithong et al., 2019; G. Foster & Paterson, 2020), and the range of feature exhibited by SCC genetic elements is not limited to encoding for methicillin resistance. For example, non-*mec* SCC structures encoding for other resistance determinants have been reported (H. J. Chen et al., 2016).

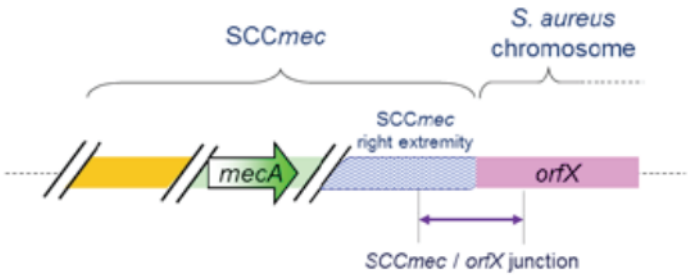


Figure 2: Diagram of SCC*mec* and its insertion site (BioMérieux 2011)

2.2.2. Virulence determinants

S. aureus secrete a wide range of virulence determinants (**Figure 3**). A good way to illustrate how various *S. aureus* virulence determinant repertoire is, is to simulate an infection scenario. Abscesses are the most common manifestation of staphylococcal skin and skin structures infections and therefore are a good example to showcase possible involvement of virulence determinants at early stage of the infection (Tong et al., 2015).

2.2.2.1. Adherence factors

To successfully maintain host colonization and opportunistically achieve invasion of host tissues, *S. aureus* employs a large variety of surface embedded proteins. One of the most prominent member of this virulence determinants family is the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM). They exhibit various features as host structures adhesion and colonization, promoting abscess formation, bacterial survival during bacteremia episode and biofilm formation. A summary of those features is discussed by T. J. Foster and colleagues (T. J. Foster, 2019).

Another mediator of host interaction is teichoic acids; whether tethered to peptidoglycan (wall teichoic acid, WTA) or attached to the outer leaflet of plasma membrane (lipoteichoic acid, LTA), they play an important role in the protection against cell damage, mediate interactions with receptors, biomaterials, and phage (Keinhörster et al., 2019; van Dalen et al., 2020).

In encapsulated bacteria, capsular polysaccharides (CP) are important surface structures, and have been identified as potent virulence factors. Among the 11 different serotypes identified in *S. aureus*, the serotypes 5 (CP5) and 8 (CP8) are the most predominant produced by clinical isolates (B. Liu et al., 2017). CPs have been found to enhance virulence in animal infection model studies for several pathologies; however, although it could be intuitive that either acapsular mutant strains may show decreased virulence, several findings have presented those latter *S. aureus* strains to thrive at causing particular infections, suggesting that the absence of CP's in some cases is not detrimental but rather advantageous to establish specific infection (Keinhörster et al., 2019). Furthermore, capsule in addition to other functions such as immune evasion, impairs opsonophagocytic killing by neutrophils (B. Liu et al., 2017; Turner et al., 2019). So far, capsular polysaccharides are an effective target for vaccine development (Clegg et al., 2021; Miller et al., 2020).

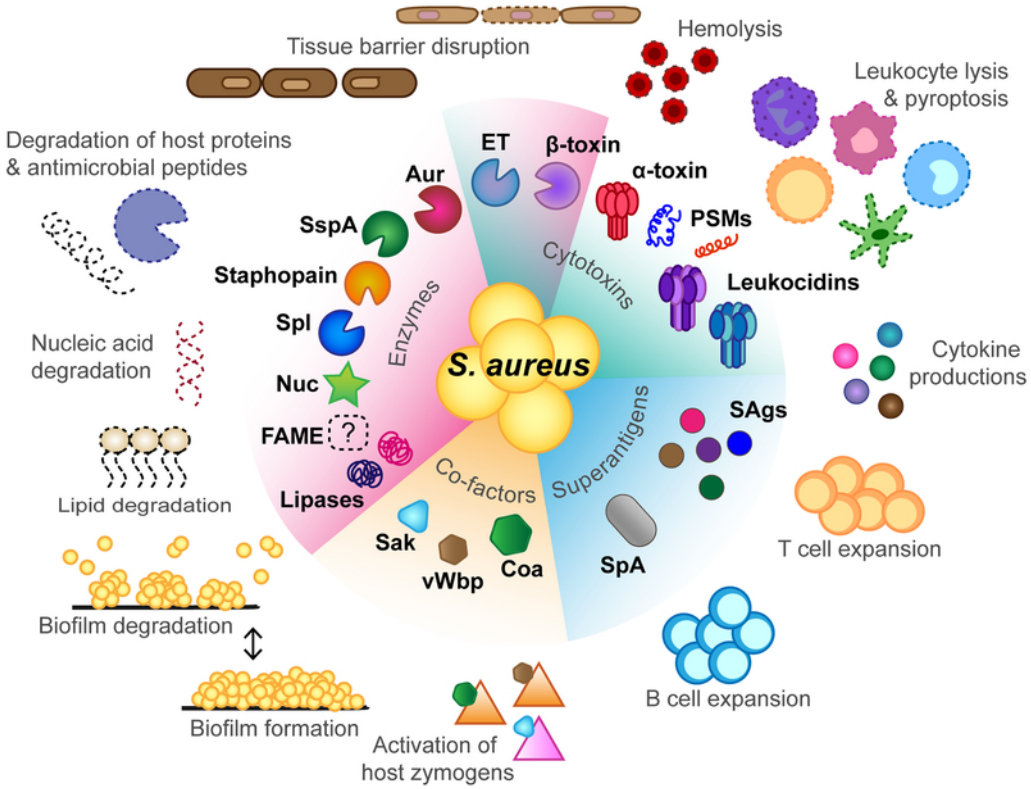


Figure 3: Overview of *S. aureus* virulence determinants (Torres, 2019)

2.2.2.2. Toxins

i. Alpha-toxin (Hla or hemolysin-α)

Pore forming toxins (PFT) is a family of cytotoxic proteins regarded as one of the most potent virulence determinants evolved by pathogenic bacteria (Hu et al., 2021). The *hla* gene, harbored by a vast majority of *S. aureus* strains (approximately 95%) encodes a 33 KDa protein known as alpha-toxin (Hla) (Oliveira et al., 2018; Seilie & Bubeck Wardenburg, 2017). Hla, regarded as the prototypic β-barrel PFT, is the toxin responsible of complete red blood cells lysis (referred to as β-hemolysis) on blood agar.

Secreted as a water soluble monomer, Hla oligomerizes upon contact with host cell membrane into a heptameric mushroom-like structure, which has the ability to successfully establish the transmembrane pore (von Hoven et al., 2019). The bifunctional extracellular domain (adhesion and protease domains), ADAM-10, serves as receptor for Hla; its sheddase activity initiation via Hla/ADAM-10 complex formation results in the cleavage of several host surface proteins, including E-cadherin's and epithelial tissue barrier dysfunction (Oliveira et al., 2018; Tam & Torres, 2019). Moreover, several other cellular cascades such as: i) dysregulation of ion-dependent mechanisms due to increased permeability to ions, ii) membrane rupture and eventually cell death, are triggered upon formation of the latter complex on cell surface (von Hoven et al., 2019). Due to its functions and ubiquitous expression across cell lineage, ADAM-10 holds an important clinical significance and is responsible for the wide cellular specificity of Hla (J. S. M. Souza et al., 2020)).

ii. Beta-toxin

Staphylococcal beta-toxin is a non-PFT with enzymatic activity toward membrane phospholipids. The toxin is known to promote intracellular proliferation of the pathogen via escape from phagocytic vacuoles (Oliveira et al., 2018). Due to its specific substrate, the activity of is tightly connected to the amount of sphingomyelin content in the target cell; thus, beta-toxin exhibits a very restricted specie-dependent activity (Tam & Torres, 2019).

iii. Phenol Soluble Modulins (PSMs)

PSMs are a class of amphipathic proteins. They have been shown to exhibit functions — although some have only been demonstrated via *in vivo* experiments— as many as immune modulation, biofilm development, proinflammatory, cytolytic and antimicrobial activities (Dastgheyb et al., 2015).

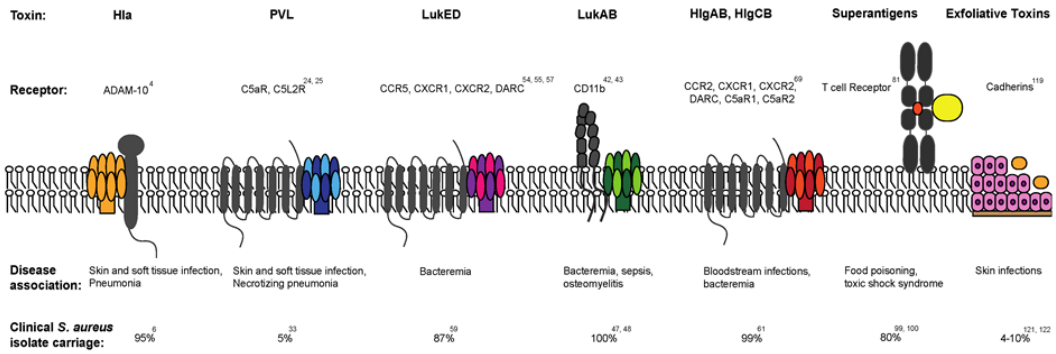


Figure 4: Staphylococcal toxins, with their respective receptors and associated clinical manifestations (Bennett & Thomsen, 2020)

iv. Leukotoxins

This group of toxins specifically attack leucocytes (

Figure *4*), and is therefore termed as leucocidins. They consist of two fractions "F" and "S" and exhibit an AB toxin-like mechanism of action. In this way, similarly to the "B" component in AB toxins, the "S" component is the binding fraction — thus conferring cell specificity to the toxin —; then "F" component is subsequently recruited and engage into oligomerization and pore formation (Spaan et al., 2017).

The Panton-Valentin leucocidin (PVL), is by far the most studied member of this group and is overly found in *S. aureus* strains causing necrotizing pneumonia and CA skin infections (Hanawa et al., 2020; Jiang et al., 2017; X. Wang et al., 2016). The γ-hemolysin, although less studied is another member of this family which interestingly shows dual activity towards leucocytes and red blood cells (Seilie &

Bubeck Wardenburg, 2017). The role of other members of this group as LukED and LukAB in pathogenesis remains unclear.

v. Super antigens (SAgs)

The staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST) are members of the pyrogenic exotoxins family (Tam & Torres, 2019). Their ability to initiate connection between T-cell receptors (TCR) and major histocompatibility complex (MHC) class II on antigen presenting cells (APC) results in hyper activation of T-cell dependent leucocytes , and overproduction of proinflammatory cytokines: the so-called “cytokines storm” (Krakauer, 2019).

To date, 23 distinct SEs have been identified (Medvedova et al., 2017). They are causative agents of staphylococcal food poisoning, due to their heat stability and resistance to gastric enzymes. Besides they also exhibit SAgs activity (Bae et al., 2021). Toxic shock syndrome (TSS) is caused by TSST and is clinically characterized by fever, skin desquamation and eventually organ involvement (Bennett & Thomsen, 2020).

vi. Exfoliative toxins (ETs)

Several serologically distinct ETs have been described. They cause a toxin mediated infection known as staphylococcal scalded skin syndrome (SSSS), characterized by blisters formation in skin. The condition mostly occurs in neonates, although some cases have been presented in adult patients (Bukowski et al., 2018).

vii. Enzymes and other virulence determinants

- Coagulase

Coagulase production is one of the principal identification criteria of *S. aureus* (Tam & Torres, 2019). The bacterium may produce two types of coagulase: cell-bound coagulase (Coa), the so-called ”clumping factor”, and a free form coagulase known as

von Willebrand factor binding protein (vWbp), both coagulase factors are potent in converting fibrinogen into fibrin. Although classified as exoenzymes, they bear no enzymatic activity but rather function as cofactors enhancing enzymatic activity (Bonar et al., 2018). The role of coagulase in pathogenesis is well documented (Bonar et al., 2018; Karer et al., 2020; Yu et al., 2017).

- Hyaluronidase

This enzyme hydrolyses hyaluronic acid, a major component of extracellular matrix. Due to its action, this enzyme promotes spreading of *S. aureus*, especially in wound infections (Abdul Halim et al., 2020; Park et al., 2017). Other potent enzymes worth citing are catalase, which catalyzes the conversion of hydrogen peroxide into water and oxygen; nuclease, lipases, serine proteases, *etc.* (Tam & Torres, 2019).

viii. Mechanisms of virulence regulation

S. aureus virulence determinants such as adhesins, and other major surface molecules which promote colonization and adherence are known to be produced in early growth stage; their production is down-regulated at late growth phase. Most of the aforementioned exotoxins are produced in the late exponential growth phase. Regulation of all those virulence determinants is controlled by a centralized set of regulatory genes among which, one the most studied is the accessory gene regulator locus *agr* (Guo et al., 2017).

The *agr* locus encodes the quorum sensing system. This system senses and reacts to the environmental accumulation of molecules referred to as auto-inducer peptides (AIPs). The production of AIPs is tightly proportional to the bacterial population density in the environment (Jenul & Horswill, 2019).

Basically, while bacteria multiply in the environment, there is gradually more cells producing AIPs; the component concentration in the extracellular environment increases until a threshold, when it triggers a cascade of reactions resulting in down regulation of colonization factors and high expression of tissue degradation

compounds (Jenul & Horswill, 2019; S. Mukherjee & Bassler, 2019; Zhang et al., 2018).

Other major virulence regulation systems are summarized in **Error! Reference source not found.**.

Table 1: Major virulence regulation clusters (Jenul & Horswill, 2019)

Regulator system	***Role***	***In vivo***
agr	Cell-to-cell communication (quorum sensing) with AIPs as signal; *agr* activation leads to expression of exo-toxins and exo-enzymes	Required for virulence in animal models of skin infection, pneumonia, and endocarditis
SaeRS	Induction of exo-protein production, including many virulence factors	Required for virulence in animal models of skin infection and pneumonia
SrrAB	Oxygen-responsive TCS; induction of *plc* and *ica* expression; repression of *agr*, TSST-1, and *spa*	Required for defense against neutrophils
ArlRS	Autolysis and cell surface TCS; induction of MgrA expression and repression of *agr* and autolysis	Required for virulence in animal models of skin infection and endocarditis
SarA	Cytoplasmic regulator; induction of exo-proteins and repression of *spa*	Required for virulence in animal models of biofilm infection
Rot	Cytoplasmic regulator of toxins and extracellular proteases; *agr* activation prevents Rot translation	Mutation of *rot* restores virulence in *agr*-null background in rabbit endocarditis model
MgrA	Cytoplasmic regulator; induction of efflux pumps and capsule expression; repression of surface proteins	Required for virulence in animal models of skin infection and endocarditis
SigB	Stationary phase sigma factor; inhibits *agr* activity	Important for the establishment of chronic infection in rat lung model

2.2.3. Mechanisms of antibiotic resistance

S. aureus has been developing resistance to several antibiotics and has become widely multidrug resistant (R. Mukherjee et al., 2021). PBPs represent a class of protein involved in the synthesis of bacterial cell wall. As target of β-lactam antibiotics, several mechanisms of resistance have been evolved (C Reygaert, 2018).

Penicillin is the first antibiotic to be introduced into routine clinical care in year 1941. It works via the inactivation of PBP function which results in impaired cell wall synthesis and cell death (Shalaby et al., 2020). As early as 1942, resistance to penicillin was reported and spread at such a rate that currently it is the most common resistance type observed in *S. aureus* (Lobanovska & Pilla, 2017). The resistance to penicillin (encoded by *blaz* gene) is achieved via hydrolysis of β-lactam ring of penicillin and penicillin-based antibiotics (C Reygaert, 2018; R. Mukherjee et al., 2021).

Another mechanism of resistance has emerged after introduction of methicillin; the bacterium evolved through modification of drug target. β-lactam antibiotic resistance in MRSA strains is based on the low affinity of the novel PBP: PBP2a. Whereas the interaction of PBP with β-lactams results in cell death, PBP2a is capable of taking over cell wall biosynthesis and hence ensure bacterial survival (Gajdács, 2019; Shalaby et al., 2020). PBP2a is encoded by the *mecA* gene, carried on *SCCmec.*

Regarding alternatives to treatment of MRSA infections, vancomycin is a glycopeptide antibiotics frequently used. However, resistance to vancomycin, first described in enterococci, has been transferred to *S. aureus* as result of genetic material exchange (T. J. Foster, 2017b; Rasheed & Hussein, 2021).

2.2.4. Epidemiology

S. aureus are ubiquitous. They are present as commensal and opportunistic pathogens colonizing different parts of the body. As aforementioned, approximately 30% of human individuals are persistently colonized (Rasheed & Hussein, 2021). The incidence of staphylococcal carriage in individuals is influenced by several factors; however, some groups are at higher risk than other. Among the latter groups of individuals, healthcare workers (HCWs), immunocompromised and chronically ill patients are of great concern.

Transmission of *S. aureus* (including MRSA) may occur via several sources. With carriers at the center of the transmission chain, person to person transmission is the most common route of infection that has been identified. From HCWs and colonized patients, the risk of nosocomial infections in healthcare settings is dramatically high (Salmanov et al., 2019; van Belkum, 2016). Moreover, hospital acquired strains can travel back to community and even spread worldwide (Coll et al., 2017; Lamanna et al., 2017; Ruscio et al., 2019). Studies have also discussed transmission route via airborne particles especially in patients suffering from viral respiratory infection (Fedy Morgene et al., 2018).

2.2.5. Clinical diseases

S. aureus induced infections may be broadly classified in two groups:

2.2.5.1. Suppurative infections

Regardless the bacterial pathogen at cause, suppurative infections may occur at any site of the body. Following cutaneous colonization by *S. aureus*, individuals are prone to recurrent skin and soft tissue infections (SSTIs). Bacteria may gain access to deep layer of the skin after traumatic inoculation —such as wounds and trauma caused by surgical procedures; exceptionally, in some cases, the bacterium may establish infection in patients suffering from atopic dermatitis (Blicharz et al., 2019; Tong et al., 2015). SSTIs may manifest in various forms, depending on the route of inoculation and the invasiveness of the pathogen (Ondusko & Nolt, 2018); therefore; mild infections such as folliculitis or impetigo can be seen, as well as more severe infection like necrotizing fasciitis (Tong et al., 2015).

With skin as primary infection foci, SSTIs mostly complicate in blood stream infections. In these cases, bacteria gains access to blood stream and spread to heart (thus causing infective endocarditis, a high mortality rate infection) and other parts of the body. It may result in metastatic infections such as various bone and joints

infections, central nervous system infection and ultimately multi organ involvement (Horino & Hori, 2020; Timsit et al., 2020; Tong et al., 2015).

Respiratory tract infections such as aspiration pneumonia, following invasive aspiration of respiratory secretions, is commonly seen. Deep inoculation of the bacterium in lower respiratory tract, immune status of the patient and virulence of the strains may predetermine to somehow severe infections. For example, severe form of necrotizing pneumonia have been strongly associated with PVL producing CA-MRSA strains (Masters et al., 2017; Rájová et al., 2016; Roux et al., 2017).

Other clinical diseases caused by *S. aureus* are urinary tract infections, ocular infections, and device-associated infection in patient with indwelling medical devices (Tong et al., 2015)

2.2.5.2. Toxin induced infections

Briefly, staphylococcal toxinosis is mostly caused by SAgs. Staphylococcal TSS caused by strains producing TSST-1 was firstly associated with menstruations in women; however, the disease may manifest from TSST-1 producing *S. aureus* at other body sites (Tong et al., 2015). SSSS, or Ritter disease is caused by exfoliative toxins and is characterized by sudden onset of disseminating skin blisters (Tong et al., 2015). Lastly, staphylococcal induced food poisoning is one of the most common food-borne illness. It is caused by enterotoxins present in food ingested and is rather considered as an intoxication (Ondusko & Nolt, 2018).

2.2.6. Focus on exfoliative toxins and SSSS

SSSS was first described in the 19th century by Gottfried Ritter after observation of over 290 cases of such clinical presentation. Until now, the condition have been reported to occur mostly in neonates and infants (Aydin & Alsbjørn, 2016; Liy-Wong et al., 2021; Staiman et al., 2018).

2.2.6.1. Clinical features

As aforementioned, SSSS is primarily a condition that affects neonates and infants. Clinically, its manifestations may vary from localized blisters formation to severe disseminated exfoliative affection. The localized form, known as bullous impetigo is limited to the site of infection is the moderate form of the infection and is characterized by the absence of diffuse exfoliation and systemic symptoms. Clinical sites are predominantly friction-prone areas such as perineum, and other medically exposed sites (umbilical region and post-operative wounds) (Leung et al., 2018). In the disseminated form of SSSS (**Figure 5**), there is a sudden onset of symptoms with diffuse erythema and skin desquamation. Due to the excessive fluid loss, temperature imbalance and possibility of skin lesions, the management of the condition is crucial. This is particularly important in infants who have immature immune system and low renal clearance to combat the toxin. They are also prone to complications such as secondary bacterial infection and multi-organ failure (Aydin & Alsbjørn, 2016; Liy-Wong et al., 2021; Martinez & Jordan, 2019).

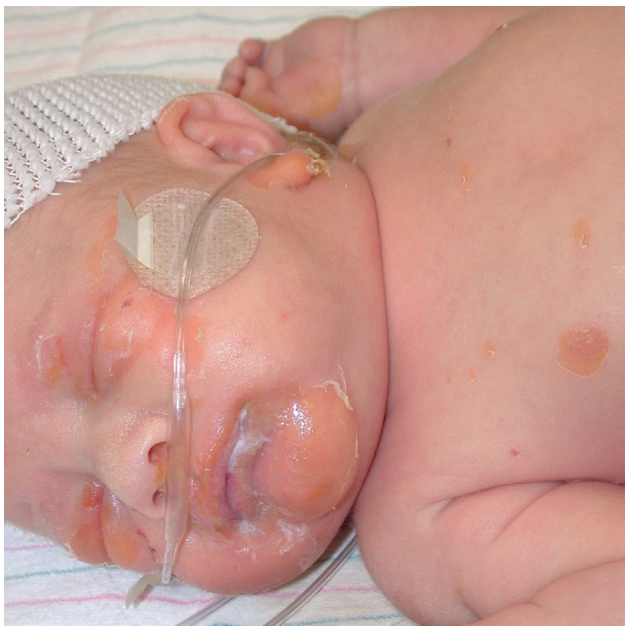


Figure 5: Superficial blister in neonate (lesion appears in the perioral region) (Fitzpatrick et al., 2018)

2.2.6.2. Exfoliative toxins

SSSS is caused by ET-producing *S. aureus* strains. At present, at least five serologically distinct toxins have been described. All toxins have been shown to exhibit exfoliative activity and to share a variable sequence similarity (Imanishi et al., 2019).

From a distribution point of view, the prevalence of toxins in producing strains significantly vary according to the geography (Imani Fooladi et al., 2015; Imanishi et al., 2019; Mariutti et al., 2017). ETs are protease toxins; most studies in mouse infection models have reported them to be produced in the late exponential growth phase. Their secretion is followed by local absorption, and passage into systemic circulation. The toxins, upon reaching epidermis zonula granulosa targets desmoglein-1, a molecule that plays an important role in cell-cell focal adhesion (Mariutti et al., 2017).

ETA is the first serotype to be described and purified, thus the first to have its crystal structure determined (Mariutti et al., 2017). Structural studies have highlighted the similarities between ETs and serine proteases. In fact, as much as 25% of serine proteases sequence is found identically in ETs, and that similarity also include the so called catalytic triad (Serine-Histidine-Aspartate) as shown in **Figure 6** (Y. Chen et al., 2021; Ladhani, 2003). In skin, ETs target desmoglein-1 (dsg-1). Dsg-1 is found ubiquitously in superficial layers of epidermis as major component of desmosomal junctions, ensuring integrity of stratum granulosum. Compared to its homologous desmoglein-3, the relatively low abundance of dsg-1 in deep skin layers (**Figure 7**) is thought to be the reason why SSSS is only limited to superficial skin layers (Bukowski et al., 2018; Fitzpatrick et al., 2018).

It is reported that the production of either of the toxin serotypes in ET-producing *S. aureus* (or staphylococci) follows a host-specific pattern. Therefore, as mentioned previously, clinical *S. aureus* strains mostly produce ETA and ETB; although ETD has also been associated with human infection, its contribution to SSSS has not been clearly evidenced; rather, studies have reported ETD from strains causing skin abscesses and furuncles (Bukowski et al., 2018; Yamaguchi et al., 2002; Yamasaki et al., 2006). Their homologous ETC, and the recently described ETE have been shown in association with animal infection, respectively in horses and ruminants

(Bukowski et al., 2018; Imanishi et al., 2019). Moreover, ETs have been studied considering a potential super antigenic activity, although the function remains under debate (Bukowski et al., 2018).

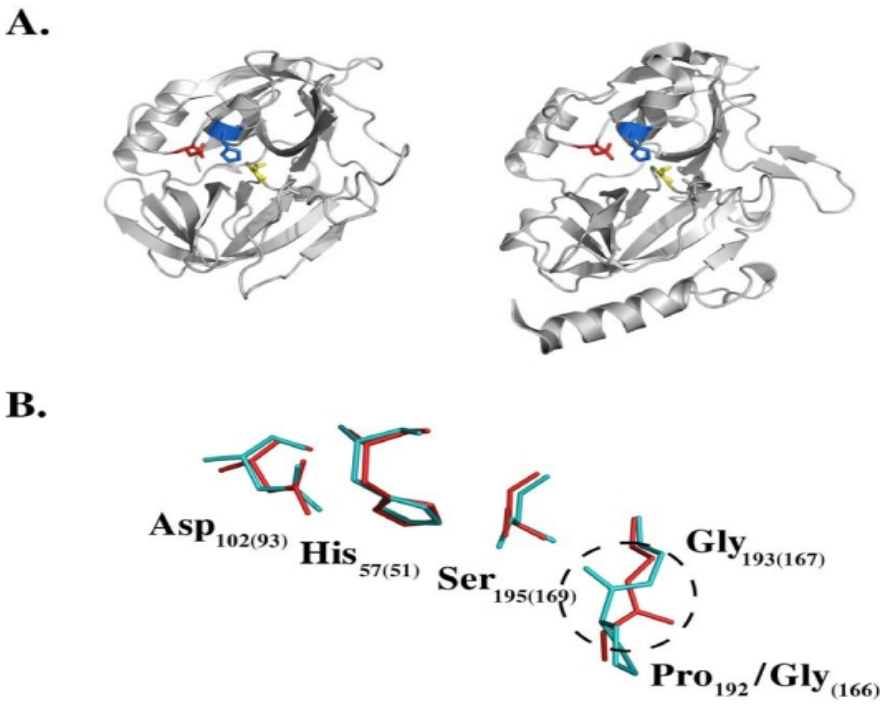


Figure 6: Crystal structure comparison of whole molecules (A) and catalytic triad (B) (Bukowski et al., 2010)

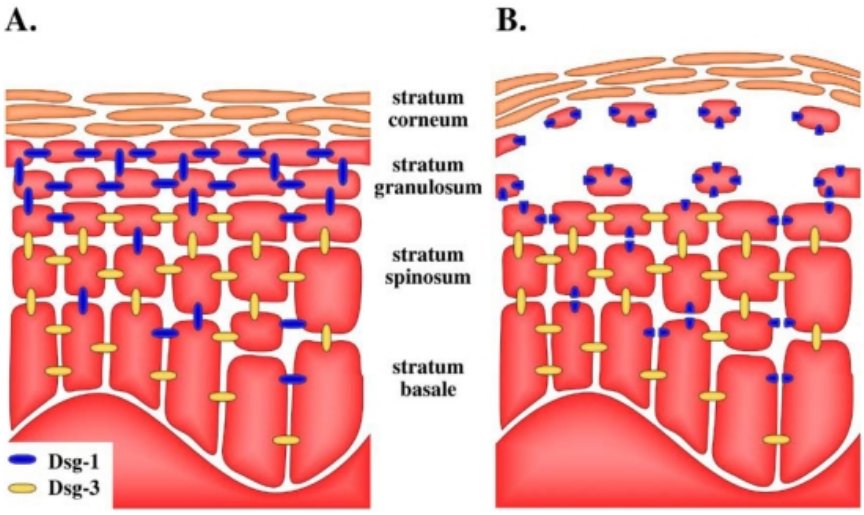


Figure 7: Differential distribution of desmoglein-1 and desmoglein-3 in skin layers (Bukowski et al., 2018)

CHAPTER III

MATERIALS AND METHODS

3.1. Sample collection

A total of 94 isolates were routinely collected at Near East University (NEU) Hospital between 2012 and 2021, representing a wide range of clinical conditions. All isolates were first identified as MRSA using Beckton-Dickinson Phoenix 100 antimicrobial susceptibility testing system and were subsequently sub-cultured to 5% blood agar; glycerol stocks were made from pure colonies obtained and stored at -80°c.

Isolates data such as patient demographics, clinical sites were retrieved from the NEU Hospital data system and stored in a database.

3.2. Coagulase tube test

All isolates were screened using confirmatory tests before being included in the core of the study. In this regard, isolates were confirmed as *S. aureus* by coagulase tube test. The test was performed as follows: individual colonies obtained from each sub cultured isolate were mixed in tubes containing 1 ml of human plasma. The tubes were incubated at 37°c for four hours. The test was scored after the incubation period and samples were reported as positive or negative based on whether or not there was clots at the bottom of the tube.

3.3. Antibiotic susceptibility testing

Susceptibility tests were performed by disc diffusion on Mueller-Hinton (MH) agar using cefoxitin discs (30 μg). The isolates were defined as MRSA based on resistance to cefoxitin.

Briefly, a bacterial suspension (one for each isolate) was prepared and set to McFarland 0.5 turbidity standard. The suspension was then spread onto MH agar plates using a sterile cotton swab. The cefoxitin (30 μg) disc was immediately applied on each plate; and the plates were incubated at 35°c for 24 hours. After incubation,

inhibition zone were measured and interpreted as per EUCAST guidelines (European Committe on Antimicrobial Susceptibility Testing, 2020).

i. zone of inhibition ≤ 22 mm: resistant to cefoxitin, thus MRSA
ii. zone of inhibition > 22 mm: sensitive to cefoxitin, thus methicillin-sensitive *S. aureus*

After the final test, all isolates confirmed as MRSA were stored for molecular screening. The non-MRSA isolates were excluded from the study.

3.4. Molecular investigation

3.4.1. DNA extraction

Genomic DNA was extracted using boiling method described by Shin et al, with some modifications (Shin et al., 2021). Briefly, each isolate sub-culture was suspended in 500 µl of sterile water contained in sterile Eppendorf tubes. The tubes were incubated in a heat block at 100°C for boiling for 10 minutes and were consequently centrifuged at 13000 rpm for 5 minutes to sediment cell debris. The supernatant fluid was then transferred into new Eppendorf tubes and stored at -20°c until use.

3.4.2. Amplification of etb and etd genes

For each 25µl PCR reaction, the reaction mixture was made of: 12.5µl of PCR master mix, 1 µl of both forward and reverse primers relevant to each gene, 6.5 µl of nuclease free water and 4 µl of DNA template. Each isolate was then amplified via single PCR for each gene using the cycling procedure described in Table 2. Primer sets are given in Table 3.

Table 2: Cycling parameters for *etb* and *etd*

Genes	Steps	Temperature	Time	Cycles
etb	Initial denaturation	95°c	5 mins	1
	Denaturation	95°c	1 min	35
	Annealing	50°c	1 min	
	Extension	72°c	1 min	
	Final extension	72°c	10 mins	1
	Hold	4°c		
etd	Initial denaturation	95°c	5 min	1
	Denaturation	95°c	1 min	35
	Annealing	47°c	1 min	
	Extension	72°c	1 min	
	Final extension	72°c	5 mins	1
	Hold	4°c		

Table 3: Sequence of the primers used for PCR amplification

Gene	Primer sequence (5′ to 3′)	Product size (bp)
etb-F	ACAAGCAAAAGAATACAGCG	226bp
etb-R	GTTTTTGGCTGCTTCTCTTG	
etd-F	AACTATCATGTATCAAGG	376bp
etd-R	CAGAATTTCCCGACTCAG	

3.4.3. Gel electrophoresis

Electrophoresis was carried out using 2% gel prepared by adding 2 g of agarose to 100 ml of Tris-Borate EDTA (TBE) buffer. The mixture was heated to dissolve and

10 µl of ethidium bromide at 0.5µg/ml concentration was added before the gel was cast into tray with appropriate comb.

After set, the gel was placed into electrophoresis tank and loaded with the samples and 50 bp DNA ladder. The gel was run for 60 minutes at 120V, and amplified fragments were visualized using UV transilluminator.

CHAPTER IV

RESULTS

4.1. Population characteristics

A total of 94 samples were initially collected at NEU Hospital Microbiology laboratory. After confirmatory tests, 80.85% (76 out of 94) were confirmed as MRSA thus included in the core study. Of these 76 samples investigated in the study, 44 (57.9%) and 32 (42.1%) were obtained from male and female patients, respectively. Irrespective to the gender, inpatients subgroup was the predominantly affected group, accounting for 75% (n=57) of the total number of isolates (

Figure *8*).

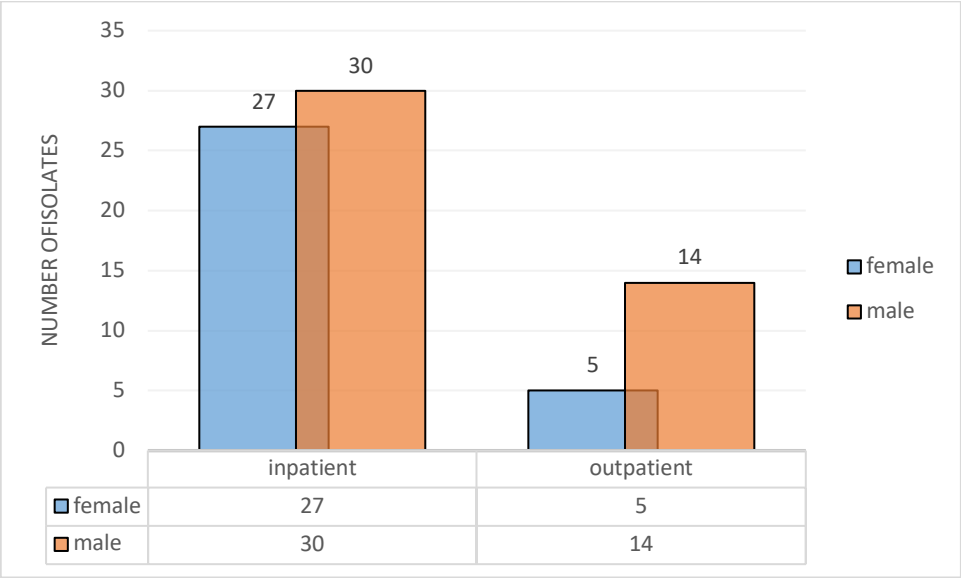


Figure 8: Distribution of population by gender and patient subgroup

Patient age ranged from 1 to 99 years, with a mean age of 60 years. Based on the age group analysis, patients between 51-80 years age groups were mostly affected by MRSA (n=48; 63.15%), irrespective to the gender (

Figure *9*).

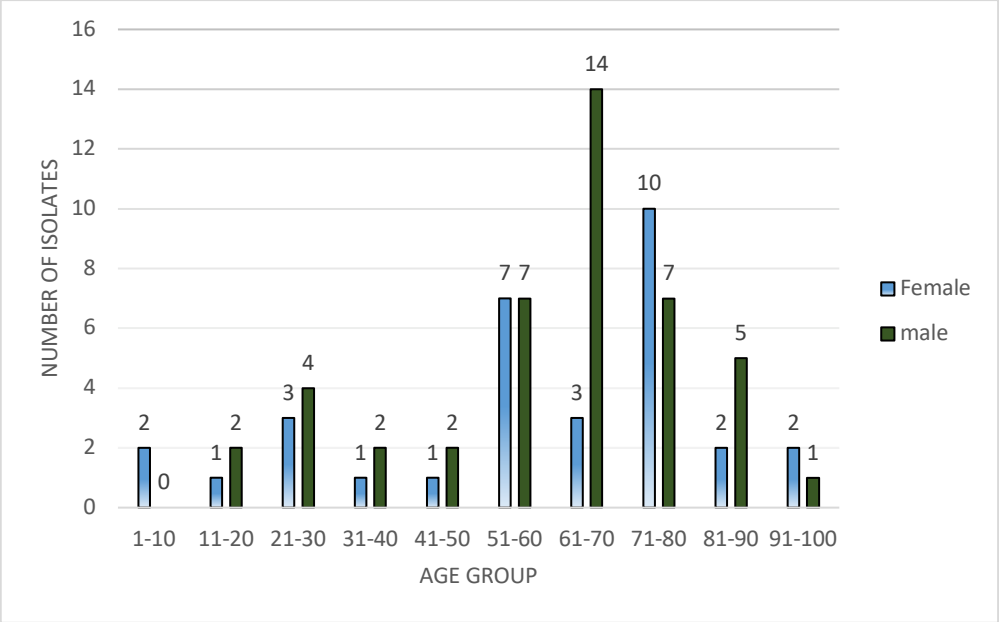


Figure 9: Age group frequency of infections (based on the gender)

4.2. Distribution of clinical samples

The frequency of isolation of MRSA from patients admitted to the hospital has been analyzed with respect to the hospital ward. Results of the analysis are summarized in

Figure *10*.

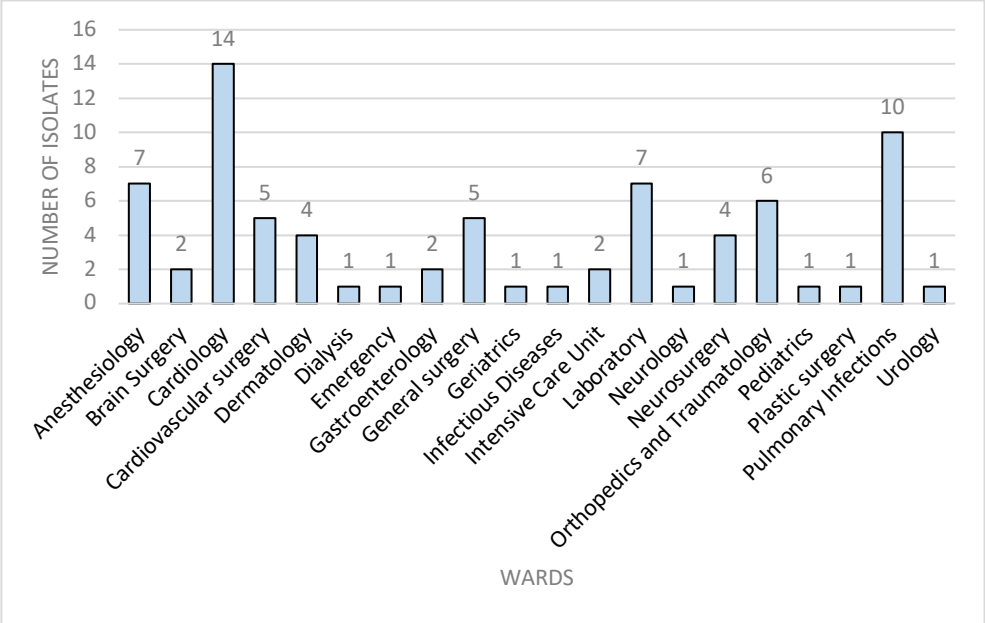


Figure 10: Frequency of MRSA isolation in the different hospital wards

The samples were obtained from a wide range of clinical sites (

Figure *11*). Predominantly, isolates were obtained from abscess/wound (25%; n=19), blood (22.4%; n=17), tracheal aspirates and nasal swabs (both 17.1%; n=13). The remaining strains were obtained from sputum samples (6.6%; n=5), bronchi-alveolar lavage and urethral samples (both 1.3%; n=1).

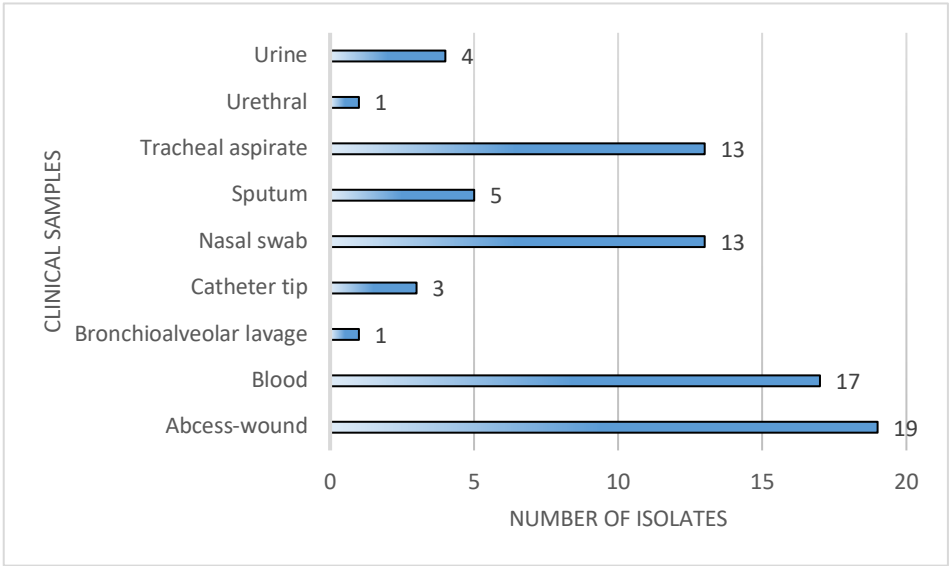


Figure 11: Distribution of samples from clinical sites

4.3 Results of molecular investigation

The presence of exfoliative toxin genes in clinical strains was determined by PCR. *Etb* was found in 1.3% (n=1/76) of the MRSA isolates while no *etd* positive isolate was detected in the PCR screen. Respective gel images can be found in **Figure 12** and **Figure 13**.

4.3.1. Gel images for etb amplification

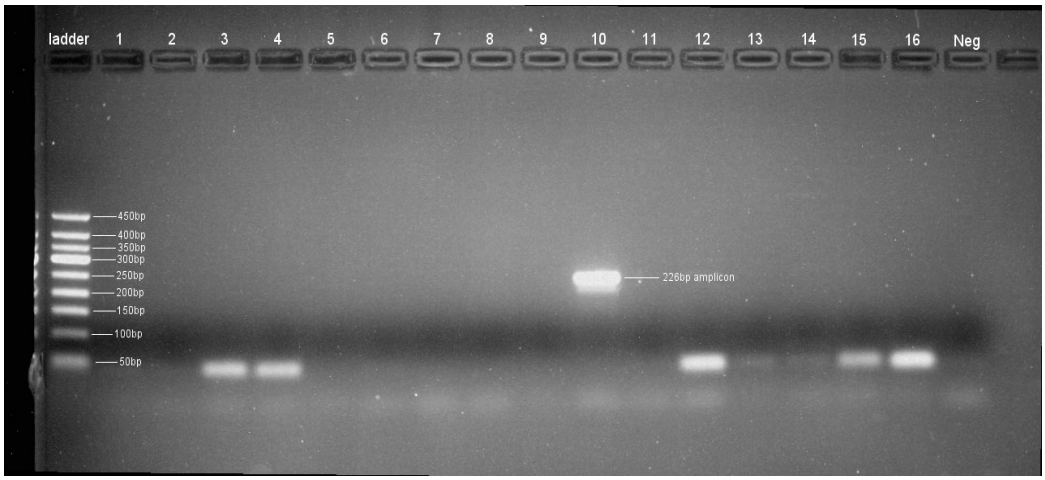
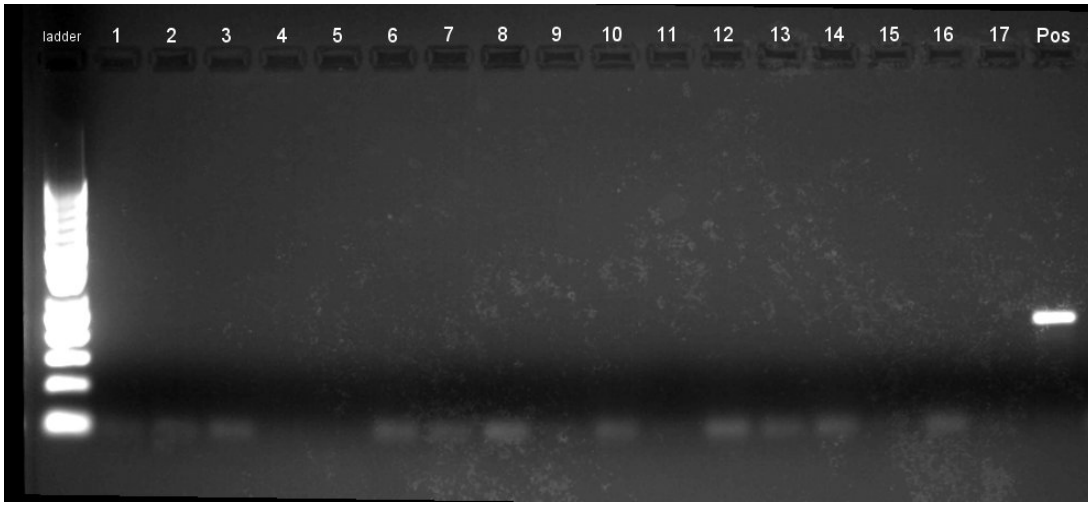
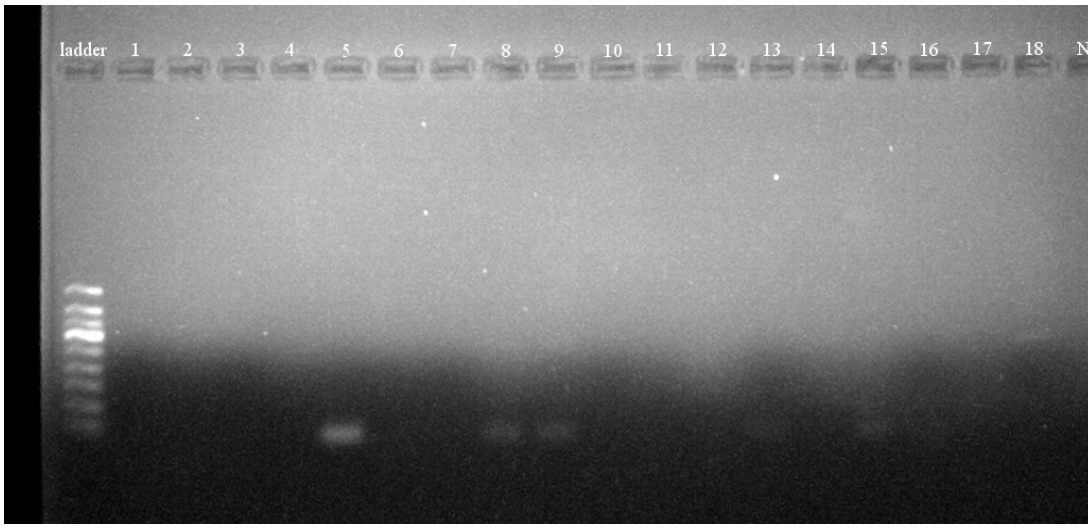
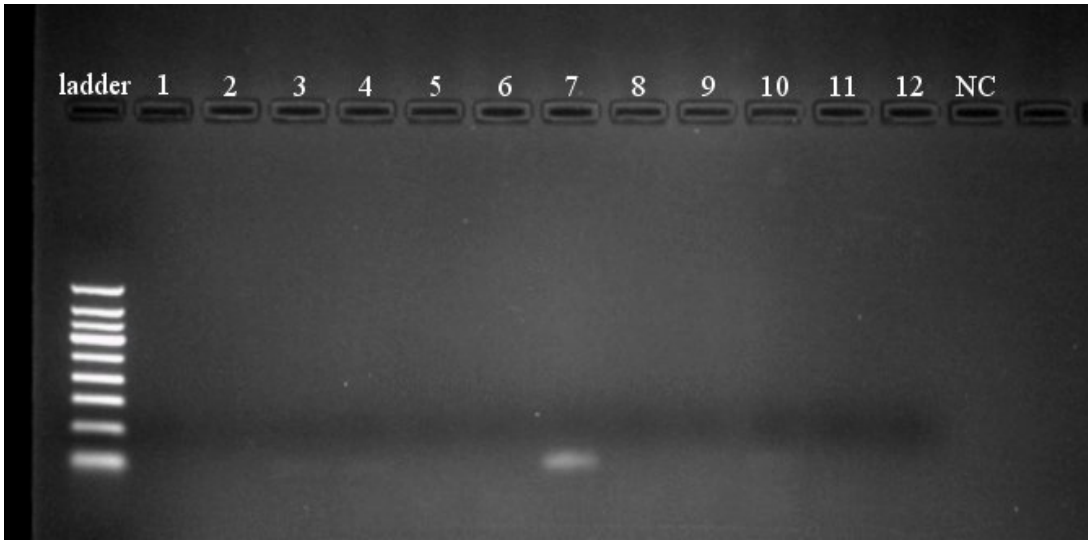


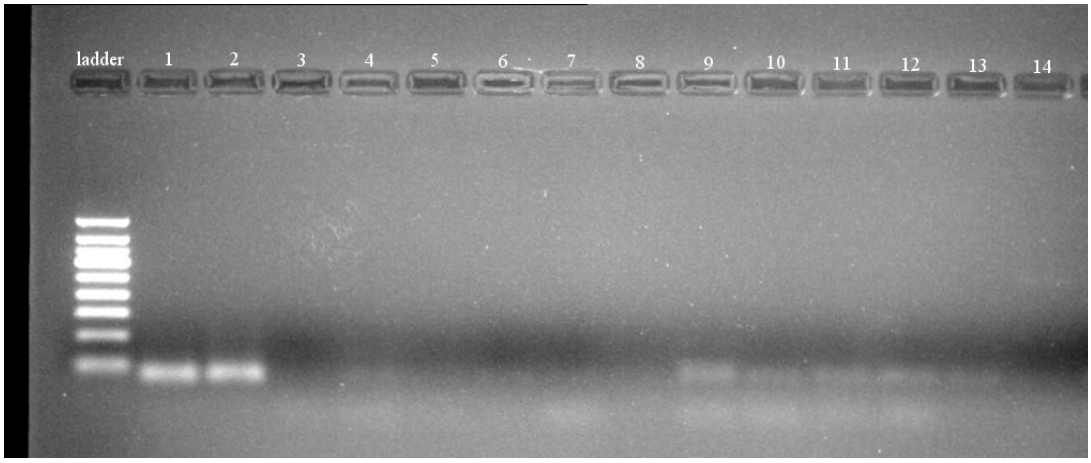
Figure 12: Gel image showing amplification of 226 bp fragment, specific for *etb* gene lines: "ladder", 50 bp DNA ladder; "lanes 1 to 9/11 to 16", negative samples; "lane 10", positive sample; "Neg" negative control

Following gel images show gel electrophoresis results for the remaining clinical samples:


ladder 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 Pos


ladder 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18


ladder 1 2 3 4 5 6 7 8 9 10 11 12 NC



4.3.2. Gel images for etd amplification

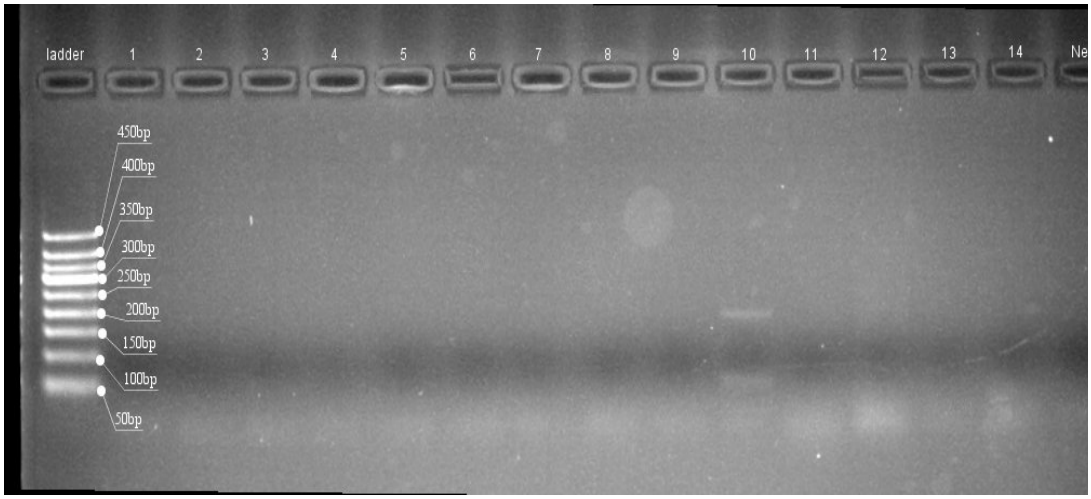
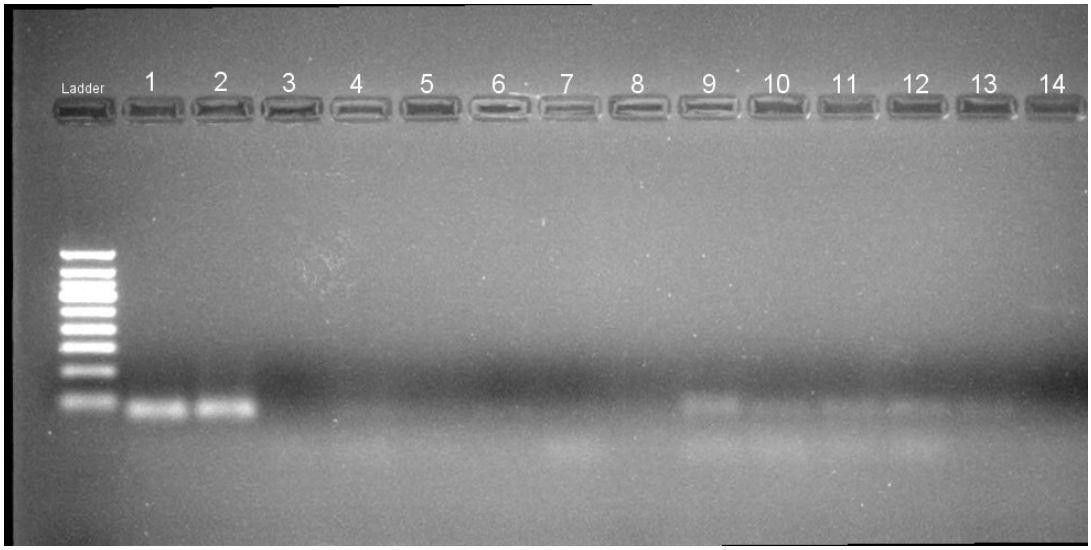


Figure 13: Gel image showing no amplification for *etd* gene, the expected amplicon size is 376 bp. Lines: "ladder", 50bp DNA ladder; "lanes 1 to 14", negative samples; "Neg", negative control


Ladder
1
2
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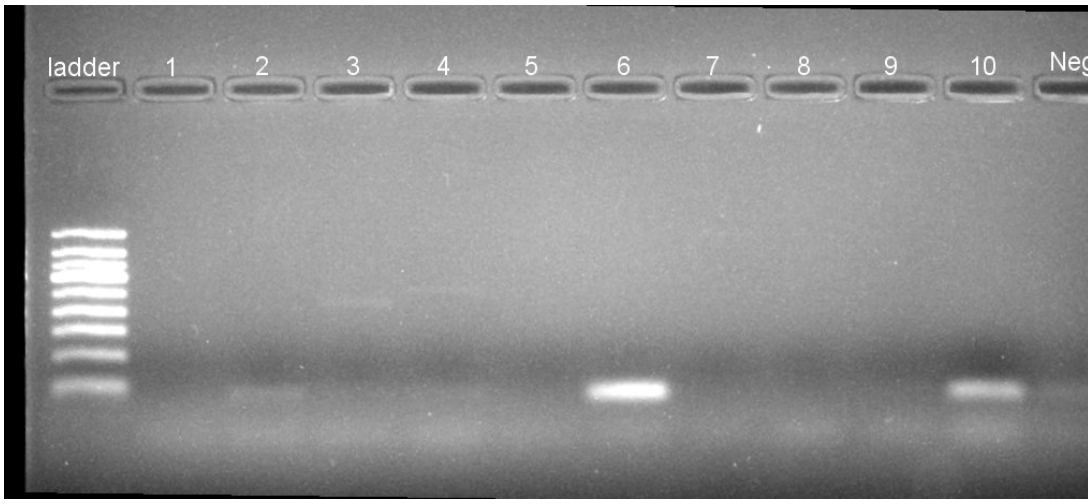
14


ladder
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CHAPTER V

DISCUSSION

Accurate insight of pathogen ecology and virulence characteristics in clinical environment is important to address prevention, treatment and control of infections in healthcare settings. The ultimate goal of biomedical research is to develop a general overview of risk factors pertaining the ability of a pathogen to prove itself as, or become more virulent. The global emergence of antimicrobial resistance and its impact on public health necessitates the unraveling of the molecular mechanisms behind infection pathogenesis. This study is directed towards that goal and represent the first attempt to characterize the presence of exfoliative toxin genes in MRSA isolates in Northern Cyprus.

This study was focused on *S. aureus* clinical isolates which are resistant to methicillin. Pal et al, have discussed the fact that MRSA is a trending and increasing cause of infections worldwide (Pal et al., 2019). In their study, Macmorran et al., also described as high as 60% of the *S. aureus* isolates investigated being resistant to methicillin (MacMorran et al., 2017). However, it is important to note that MRSA strains are not evenly distributed worldwide. Indeed, Tsige et al., have found significantly lower prevalence (9.8% vs 80.85%) (Tsige et al., 2020). Besides, and in accordance with our results, they have reported similar trend in the distribution of MRSA isolates in inpatients and outpatients group, and in gender distribution. The male-female ratio described in our study is 1.3:1. Male gender is an indicator that have been shown to be a strong predictor of high incidence (Inoue et al., 2018; Jayaweera et al., 2017; Lin et al., 2018).

The predominance of inpatients is correlated with extended exposure to medical setting. This group of patients is more likely to develop hospital-acquired infections (Haque et al., 2018; L. Wang et al., 2019). As expected and described by several other studies, another risk factor is the age of patients. In our study, the median age of our population is 60 years, and irrespective to the gender, as high as 63.15% of our patients aged between 51 and 80 year. Similar pattern have been described by Jayaweera et al. in 2017 (Jayaweera et al., 2017).

Moreover, the data presented in the current study indicate that most of the isolates were recovered from patients attending surgical departments. Being so, one could infer a strong association with the predominance of wound and abscesses as most frequent clinical foci in the study. *S. aureus* is a commensal of skin and mucosa. Its ability to cause skin and soft tissues infection is made through breach in skin and access to deep tissues. MRSA is the most common cause of skin and soft tissue infection (Olaniyi et al., 2016; Vella et al., 2021). From what precede, any trauma such as surgical incision evoked above may allow the bacteria to cause infection. Such scenario is also described in patients suffering from chronic skin condition (Mitevska et al., 2021; Wong et al., 2018). Following, blood samples were found to be the second most common sample from which MRSA was isolated, accounting for 22.4%. To establish a continuous link between *S. aureus* recovery from blood and primary trauma in skin, the literature was investigated regarding this scenario. Previous studies have recently investigated the prevalence of *S. aureus* bacteremia in injection drug users and have found its prevalence to be significantly high compared to non-drug injectors; although drug consumption is not the debate of our study, we found relevant the fact association bacteremia with increased likelihood of primary traumatic inoculation of the bacterium in the body. The findings also reported and additional cases of secondary infections such as endocarditis, associated with the scenario (Packer et al., 2019; Shoucri et al., 2021). Interestingly, and in relation with age above discussed risk factor, all the blood isolates were obtained from adults and elders (age range between 41 and 88 years). No statistical analysis was performed to establish relationship between age and bacteremia, however several studies have addressed and confirmed such relationship (Guillamet et al., 2018; Lam et al., 2019; Thorlacius-Ussing et al., 2019). A partial conclusion may suggest improved care for population, considering each risk factor identified above.

The contribution of virulence factors in the course of infections is a widely acknowledged phenomenon (Bae et al., 2021; Bennett & Thomsen, 2020; Pivard et al., 2021). The prevalence of the exfoliative toxins, serotypes B and D, involved in skin affection was examined. Of all the isolates, only one sample (1.31%) have been found to be positive for *etb* gene; whereas none of the isolates were positive for the *etd* gene. SSSS, the sole medical condition in which these investigated toxins cause skin desquamation in most commonly seen in neonates and infants (Liy-Wong et al., 2021;

Martinez & Jordan, 2019; Siegel & Lee, 2019). Interestingly, we describe here the case of a 2-year-old female infant admitted to the plastic surgery department. This isolate was the only sample tested positive for *etb* toxin in our study. This MRSA isolate was from a wound/abscess. The patient was admitted as an inpatient initially in the Pediatrics Department with multiple furuncles as a carbuncle in the right hand third finger. Patient had high fever on admission and was transferred to the Plastic Surgery Department upon initial assessment. The culture result was positive for MRSA. The carbuncle area was debrided and epidermis layer was excised. Patient was given amoxicillin/clavulanic acid and treated as an outpatient the following day. Based on the described geographical distribution which exfoliative toxins have been shown to depend on, our results are very similar to other studies (Botka et al., 2017; Hisatsune et al., 2013; Memariani et al., 2020).

With respect to geographic distribution alluded above, a Japanese study reported among MRSA isolates, a predominance of *etb* and a very low prevalence of *etd* carriers. These results are consistent with the trends recently reported by Bukowski et al. (Bukowski et al., 2010, 2018; Nakaminami et al., 2008). In France, similar trends in *etd* prevalence were described as in Japan, and the presence of *etd* was associated with milder skin infections. Authors have inferred that *etd* was associated with decreased clinical symptoms (Nakaminami et al., 2008; Yamasaki et al., 2006). More recently, a Chinese study (C. Liu et al., 2015) reported high prevalence for both toxins, 10.4% and 6.5% for *etb* and *etd* respectively; whereas a study from Brazil reported no *etb* positive strain (C. S. M. Souza et al., 2016).

This study was performed with a certain number of limitations. Due to the relatively small sample size and the nature of the study which was single-centered, the results presented above represent a limited perspective of the overall scope of the study and therefore may not be generalized. Also, the increasing blur between CA-MRSA and HA-MRSA strains as well as distinguishing characteristics between patients commensal strains and hospital strains on their molecular aspect in another limitation of this study which can be further addressed with the required research protocol. Thirdly, this study only relied on resistance to methicillin and did not screen for resistance to other commonly used antibiotics; one could infer that among MRSA strains isolated in the present study, a substantial amount of them might be multi-drug

resistant. Further studies are therefore required to comprehensively address the ultimate goal with reference to the current pioneering study.

CHAPTER VI

CONCLUSION

S. aureus is a leading cause of both hospital- and community-acquired infections worldwide, hence poses a great challenge to our healthcare systems. Moreover, increasing reports of resistance to antimicrobials further complicate the situation. In this study, the prevalence of a class of staphylococcal toxins responsible for the so-called staphylococcal scalded skin syndrome was investigated. The ultimate goal of the study was to characterize the presence of specific virulence factors - exfoliative toxins in clinical MRSA isolates. Investigations were conducted at Near East University Hospital. The study concluded that exfoliative toxin b is found at a low prevalence in strains circulating at the hospital, and that strains lacked exfoliative toxin d. The study yielded relevant information that will help medical practitioners as well as researchers to improve their knowledge and guide their decisions. Our results led to the conclusion that there are gender and age associations in the occurrence of MRSA infection. As there is a large body of literature discussing such aspect, existing and customized management policies may be easily developed and implemented on the field.

Despite the invaluable contribution of this work to literature, we acknowledge that further studies are required to comprehensively address the current challenge. In depth molecular and epidemiologic studies are indispensable. Future studies may include differentiation between CA-MRSA and HA-MRSA strains, therefore yielding relevant understanding on the dynamics governing distribution and characteristics of strains in both environments. Moreover, particular attention must be paid to populations with risk factors. Identification of virulence features in this populations may aid in infection management, reduce the overall incidence and mortality commonly seen in these subpopulations.

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APPENDICES

Appendix A

Turnitin Similarity Report

thesis final

ORIGINALITY REPORT

11%	%	11%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Shi Wu, Rui Pang, Jiahui Huang, Feng Zhang et al. "Evolutionary Divergence of the Novel Staphylococcal Species Staphylococcus argenteus", Frontiers in Microbiology, 2021 Publication	1%
2	"Staphylococcus aureus", Springer Science and Business Media LLC, 2017 Publication	1%
3	Christian Jenul, Alexander R. Horswill. " Regulation of Virulence ", American Society for Microbiology, 2019 Publication	1%
4	"13th European Congress of Clinical Microbiology and Infectious Diseases", Clinical Microbiology and Infection, 2003 Publication	1%
5	Jed F. Fisher, Shahriar Mobashery. " β-Lactams against the Fortress of the Gram-Positive Bacterium ", Chemical Reviews, 2020 Publication	1%

CURRICULUM VITAE

1. PERSONAL INFORMATION

NAME, SURNAME: BUSAYO OMOWUMI BAKARE DATE of BIRTH and PLACE: 16th November, 1995. FEDERAL CAPITAL TERRITORY, NIGERIA
CURRENT OCCUPATION: Masters Student Clinical and Medical Microbiology at Near East University ADDRESS of CORRESPONDENCE: ALI HOCA SOKAK, YARDIMCI 9 APARTMENT. DOOR 4. HAMITKOY LEFKOSIA, TRNC TELEPHONE: +905338212324 E-MAIL: busimama16@gmail.com

2. EDUCATION

YEAR	GRADE	UNIVERSITY	FIELD
2017	Second Class Upper	Bingham University, Karu, Nasarawa State. Nigeria.	Bachelor of Science (B. Sc Microbiology)

3. ACADEMIC EXPERIENCE

PERIOD	TITLE	DEPARTMENT	UNIVERSITY
2014-2017	BSc Microbiology	Biological Sciences	Bingham University
2013-2014	BSc Nursing 100level	Medical Sciences	Babcock University

4. FIELD OF INTERESTS

FIELDS OF INTERESTS	KEY WORDS
Food Microbiology Clinical Microbiology	Food poisoning, toxins, nosocomial infections.

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 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. Rüştü Onur

Yakın Doğu Üniversitesi

Bilimsel Araştırmalar Etik Kurulu Başkanı