TURKISH REPUBLIC OF NORTHERN CYPRUS
NEAR EAST UNIVERSITY
HEALTH SCIENCE INSTITUTE

FREQUENCY OF ANTIBIOTIC SUSCEPTIBILITY TO MRSA IN PERIODS OF TIME

MATEEN ESSMAT HADI HADI

MASTER OF SCIENCE THESIS

DEPARTMENT OF MEDICAL MICROBILOGY AND CLINICAL MICROBILOGY

SUPERVISOR

PROF. DR. NEDIM CAKIR

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DECLARATION

I hereby declare that this research solely belongs to me and there was no unethical behavior observed in all stages of the research from planning and writing of the thesis. All data and information was obtained in accordance to the academic and ethical rules instituted by Near East University.

Mateen Essmat Hadi HADI

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ABSTRACT

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Background: *Staphylococcus aureus* (*S aureus*) is one of the major human pathogen that is responsible for broad spectrum of infectious conditions in both community and nosocomial settings. It is one of the main antimicrobial resistant microorganisms that have the ability to acquire resistance to various types of antimicrobials. Also, since the emergence and spread of Methicillin-resistant *Staphylococcus aureus* (MRSA) which are majorly drug resistant pathogens in both hospitals and community settings. MRSA also described as "Healthcare-associated MRSA" (HA-MRSA) mostly infect elderly patients within healthcare facilities. Till date, the epidemiology of MRSA has been evolving overtime and since the emergence of the "community-associated MRSA" (CA-MRSA) a new strains in patients with no previous history of hospitalization, MRSA and its distinct clones have become microorganisms of interest in scientific researches.

Method: The study was conducted at the Near East Hospital laboratory in which 80 strains of *S aureus* samples were collected from all participants. All samples were examined based on a specific microbiological standard for MRSA, samples were sub-cultured on a Mueller-Hinton Agar containing 6 μg/ml oxacillin and 4% NaCl according to "CLSI MRSA identification methods". After the isolation and

identification of MRSA strains, antibiotic susceptibility tests were performed according to Kirby Bauer methods.

Statistics: Susceptibility tests have been evaluated by the measuring radius of inhibition zones and comparing them to each other. In our study the Statistical Package Social Sciences (SPSS) was used to manipulate each parameter with other, the data pertaining non-parametric and there are some quality (category) data, the Chi-square test was used to solve the relationship between the first and second group of antibiotic.

The classification of the antibiotic into two groups:

The antibiotics used for this study were classified into two groups, according to the drug of choice preferences in daily clinical application.

1- The First group of the antibiotics is the first drug of choices: vancomycin (VA), and teicoplanin (TEC).

2- Second group of the antibiotics is the second drug of choices: rifampsin (RA), tigecycline (TGC), trimethoprim-sulfamethoxazole (SXT), clindamycin (DA), and cefoxitin (FOX).

Results: The MRSA strain sensitivities results of the first group were as follows: Teicoplanin (TEC) 80 (100%), vancomycin (VA) 80 (100%), respectively. And for the second group of antibiotics were as follows: clindamycin (DA) 77 (96.3%), cefoxitin rifampsin (RA) 75 (93.7%), trimethoprim-sulfamethoxazole (SXT) 75 (93.7%), tigecycline (TGC) 76 (95%). Only 38 out of the 80 samples of our MRSA strains were sensitive to (FOX) (47.5%). A significant statistical difference was observed in the

antibiotics susceptibility of the first group and second group, also there was a significant difference only for FOX in the first group and second group.

Conclusion: Cefoxitin (FOX) is not as effective as other antibiotics used in this study.

***Keywords*:** MRSA, antibiotic culture, susceptibility test, type of antibiotics.

ÖZET

Mateen Essmat Hadi HADI

Sağlık Bilimleri Enstitüsü, Tıbbi ve Klinik Mikrobiyoloji Programı

Giriş: Staphylococcus aureus (S aureus), hem toplumda hem de nozokomiyal ortamlarda bulaşıcı koşulların geniş spektrumundan sorumlu olan başlıca insan patojenlerinden biridir. Çeşitli antimikrobiyal türlerine direnç kazanma yeteneğine sahip ana antimikrobiyal dirençli mikroorganizmalardan biridir. Ayrıca, hem hastanelerde hem de toplum ortamlarında büyük ölçüde ilaca dirençli patojenler olan Metisiline dirençli Staphylococcus aureus'un (MRSA) ortaya çıkması ve yayılmasından bu yana. MRSA ayrıca "Sağlıkla ilişkili MRSA" (HA-MRSA) olarak da tanımlanır, çoğunlukla sağlık tesislerindeki yaşlı hastaları enfekte eder. Bugüne kadar, MRSA'nın epidemiyolojisi fazla mesai gelişmektedir ve "toplumla ilişkili MRSA" nın (CA-MRSA) ortaya çıkmasından bu yana, daha önce hastaneye yatış geçmişi olmayan hastalarda yeni bir suş, MRSA ve onun farklı klonları ilgi konusu mikroorganizmalar haline gelmiştir. bilimsel araştırmalarda.

Yöntem: Çalışma, tüm katılımcılardan 80 S. aureus suşunun toplandığı Yakın Doğu Hastanesi laboratuvarında gerçekleştirildi. Tüm numuneler MRSA için spesifik bir mikrobiyolojik standarda göre incelendi, numuneler "CLSI MRSA tanımlama yöntemlerine" göre 6 μg / ml oksasilin ve% 4 NaCl içeren bir Mueller-Hinton Agar

üzerinde alt kültürlendi. MRSA suşlarının izolasyonu ve tanımlanmasından sonra, antibiyotik duyarlılık testleri Kirby Bauer yöntemlerine göre yapıldı.

İstatistik: Duyarlılık testleri, inhibisyon bölgelerinin ölçüm yarıçapı ile ve birbirleriyle karşılaştırılarak değerlendirilmiştir. Çalışmamızda her bir parametrenin birbiriyle manipüle edilmesi için İstatistiksel Paket Sosyal Bilimler (SPSS) kullanılmış, veriler parametrik olmayan veriler ve bazı kalite (kategori) verileri mevcut olup, birincisi arasındaki ilişkiyi çözmek için Ki-kare testi kullanılmıştır. ve ikinci grup antibiyotik.

Antibiyotiğin iki gruba ayrılması:

Günlük klinik uygulamada antibiyotikleri ilaç tercihlerine göre iki gruba ayırdık.

1- İlk antibiyotik grubu, ilk tercih edilen ilaçtır: vankomisin (VA), teikoplanin (TEC).

2- İkinci antibiyotik grubu tercih edilen ikinci ilaçtır: rifampsin (RA), tigesiklin (TGC), trimetoprim sülfametoksazol (SXT), klindamisin (DA), sefoksitin (FOX).

Duyarlılık testleri, inhibisyon bölgelerinin ölçüm yarıçapı ile ve birbirleriyle karşılaştırılarak değerlendirilmiştir. Çalışmamızda her bir parametrenin birbiriyle manipüle edilmesi için İstatistiksel Paket Sosyal Bilimler (SPSS) kullanılmış, veriler parametrik olmayan veriler ve bazı kalite (kategori) verileri mevcut olup, birincisi arasındaki ilişkiyi çözmek için Ki-kare testi kullanılmıştır. ve ikinci grup antibiyotik.

Bulgular: İlk grubun MRSA suşu duyarlılık sonuçları şu şekildedir: Sırasıyla Teikoplanin (TEC) 80 (% 100), vankomisin (VA) 80 (% 100). İkinci grup antibiyotikler ise şu şekildeydi: klindamisin (DA) 77 (% 96,3), sefoksitin rifampsin (RA) 75 (% 93,7), trimetoprim-sülfametoksazol (SXT) 75 (% 93,7), tigesiklin (TGC) 76 (% 95). MRSA

suşlarımızın 80 örneğinden sadece 38'i (FOX) 'a (% 47,5) duyarlıydı. Birinci grup ile ikinci grubun antibiyotik duyarlılığında anlamlı istatistiksel fark gözlenirken, ayrıca birinci grup ve ikinci grupta sadece FOX için anlamlı fark vardı.

Sonuçlar: Sefoksitin (FOX), bu çalışmada kullanılan diğer antibiyotikler kadar etkili değildir.

Anahtar Kelimeler: MRSA, Antibiyotik Kültürü ve Duyarlılık testi, antibiyotik türü

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LIST OF ABBREVIATIONS

MRSA	**Methicillin Resistant *Staphylococcus aureus***
MSSA	**Methicillin Sensitive *Staphylococcus aureus***
S aureus	***Staphylococcus aureus***
CoNS	**Coagulase Negative *Staphylococcus***
MLST	**Multilocus Sequence Typing**
PCR	**Polemare Chain Reaction**
MGE	**Mobile Genetic Elements**
TAT	**Turnaround Time**
HA	**Hyaluronic Acid**
Pc	**Penicillin G**
VRSA	**Vancomycin Resistant *S aureus***
MHC	**Major Histocompatibility Complex**
TSST-1	**Toxic Shock Syndrome Toxin 1**
SSSS	**Staphylococcal Scalded Skin Syndrome**
AD	**Atopic dermatitis**
SEB	**Staphylococcal Enterotoxin B**
Th2	**T-helper type 2**

SPSS	**Statistical Package Social Sciences**
CLSI	**Clinical and Laboratory Standards Institute**
BA	**Blood Agar Base**
CA-MRSA	**Community Associated MRSA**
HA-MRSA	**Healthcare Associated MRSA**
VA	**Vancomycin**
TEC	**Teicoplanin**
DA	**Clindamycin**
FOX	**Cefoxitin**
RA	**Rifampsin**
TGC	**Tigecycline**
SXT	**Trimethoprim-Sulfamethoxazole**

SECTION ONE: INTRODUCTION

1.1. INTRODUCTION

Staphylococcus is one of the Gram-positive bacteria belonging to the family of *staphylococcaceae*. The name *"staphylococcus"* was originally coined by Alexander Ogston a Scottish bacteriologist and surgeon in the 1980s. The bacterium is one of the major types of micro-biota found in animals and humans, and is invisible to the human eye. However, under the microscope, they appear to be spherical shape and arranged in a grape-like clusters. *S. aureus* can grow in up to 10% salt and are facultatively anaerobic in nature, usually because of their ability to grow in both aerobic and anaerobic environments at about 18 to 40 °C. There are at least 45 different species of staphylococcus of which most do not cause diseases in host. Nine out of the 45 species have two subspecies which preferentially cohabitate the human body. And till date, the two most studied strains of this bacterium are the *Staphylococcus aureus* (S aureus) and *Staphylococcus epidermidis* (S epidermidis) which in terms of pathogenicity, most are harmless and are usually found in the mucosal membranes and skin of both humans and animals and in the soil.

In humans, most of this *Staphylococcus* do not pose any danger to the skin or mucosal membrane, however, with opportunistic penetrations (for instance, a cut or damage to the skin can give them selective advantage of penetrating into the blood streams) and as such can cause wide range of severe infections. *S. aureus* can be identified from other types of *Staphylococcus species* by various biochemical tests, for example; the coagulase positive test is used to distinguish *S aureus* from other *Staphylococcus species*, mannitol fermentation positive test is used to identify *S aureus* from *S*

Epidermidis and, the novobiocin sensitive test is used to differentiate *S aureus* from S saprophyticus. S aureus infections can affect any individual however; the risk is higher in new born infants and patients with other conditions such as diabetes and cancer. Till date, the multi resistant phenotype of MRSA strains to antimicrobial drug alongside their intrinsic Beta-lactamase resistance makes them difficult and costly to manage, as such, they are of major concern to public health specialist.

1.2.1. AIM OF THE STUDY

1. The first purpose of the research was to detect the antibiotic susceptibility of MRSA to five different anti-staphylococcal antibiotics, by comparing the antibiotics results of two groups.

2. The second purpose of the study was to determine the prevalence of MRSA in the *Staphylococcus aureus* isolates of both the first and second group.

1.2.2. SCOPE OF THE STUDY

This study was designed to investigate different types of antibiotic susceptibilities to *MRSA* pathogens over a period of time. All samples were retrieved from Near East University Hospital in Nicosia Northern Cyprus. Upon, *S aureus* (MRSA) identification from the isolated samples, the susceptibility of each antibiotic to certain bacteria was compared between the first and second group.

2. GENERAL INFORMATION

2.1. History and Epidemiology

Staphylococcus aureus (S aureus) is one of the major pathogenic agents of nosocomial and community acquired infections worldwide. They are group of ovoid bacteria commonly found on the skin and mucous membrane of humans and other warm blooded animals. The term *staphylococcus* is generally used to describe this type of bacteria cells usually because; they aggregate in grape like clusters under the microscope. Microbiologically, in young cultures they are characterized as gram positive, non-motile, non-spores forming and facultative anaerobes (can grow with or without oxygen). Of significance importance among various species of *staphylococcus* are the *S aureus* and *S epiderdimis* strains. Although, the *S epidermidis* strains have mild pathogenic effects, hence, it only causes infections in people with lowered immune responses. On the other hand, *S aureus* is more pathogenic and is responsible for wide range of infectious condition including skin infections and food poisoning. They can also cause urinary tract infections, pneumonia and toxic shock syndrome (Rocchetti et al., 2018).

In the early 1960s, the emergence and spread of β-lactamase producing plasmid decreased the efficacy of penicillin in treating *S aureus* infections. And as a result, in 1959 a newly modified form of penicillin called methicillin was designed, a drug that has the ability to resist the destructive actions of staphylococcal β-lactamase. Unfortunately, in 1961, they were reports of *S aureus* isolates with resistance to methicillin in the United Kingdom (Jevons, 1961). And in subsequent years, methicillins resistant S aureus *(MRSA)* strains were later retrieved from Australia, Japan, United States and other European countries (Enright et al., 2002). At first,

MRSA infections were initially restricted to nosocomial settings. However, in recent years, there were reports of MRSA isolates in rural and urban regions (community settings), often referred to as the community acquired methicillin resistant *S aureus* (CA-MRSA) infections. These CA-MRSA strains also belong to the clonal lineage similar to that of the small staphylococcal cassette chromosome mec (SCCmec) element types of the MRSA (IV or V) (Strauß et al., 2017).

Currently, the MRSA strains are of great concern to public health specialist due to their ability to cause pre-eminent hospital acquired infections that are fashionably enigmatic to tackle, cause of their ability to resist antimicrobial drugs.

MRSA was first described in the 1960s in a hospital in Britain. By late 1960s, there were reports of an outbreak in other European countries (Ostojić & Hukić, 2015). Correspondingly, in 1990s, there were reports about the emergence of the community associated MRSA (CA-MRSA) strains with different characteristics compared to those seen in HA-MRSA isolates.

Data from center for disease control and prevention (CDC) recorded an estimate of 1.7 million HMA-MRSA infections and 99,000 MRSA associated deaths in the United States in 2002 (Klevens et al., 2007).

Recent data from the European Antibiotics Resistance Surveillance System (EARSS) evidently showed records of an increment in the prevalence of MRSA in 1990 to 2005 with varying proportions (1% and 50%) in Northern and Southern European countries, respectively (Ostojić & Hukić, 2015). The difference in this frequency could probably as a result of different antibiotic used in treating MRSA infections. Nevertheless, evidence on the prevalence of MRSA infections vary yearly, however, previously

published data shows an increment in the spread of the infection worldwide. Jernigan et al (2006) conducted a review in three communities in the US. They reported 18 to 25.7/100,000 annual incidence of CA-MRSA between 2001-2000 and most of the CA-MRSA isolates were associated with other clinical infections (Jernigan et al., 2006).

In the United States, approximately 95 million peoples have been reported to be carriers of *S aureus* bacteria and 2.5 out of 95 million are carriers of MRSA strains(Graham et al., 2006). In a cohort study, Fritz et al (2008) reported that, about 2.4% healthy children between the ages of 17 and 18 are asymptomatic carriers of the MRSA strains in the US (Fritz et al., 2008).

More recently, SENTRY antimicrobial surveillance program have reported 35.9% increment in the incidence of bloodstream MRSA infections in North America, 29% in Latin America, 22.8% in European (Yue et al., 2016). Also, in another recent studies by Gopal and Divya (2017), a new strain of MRSA was reported in India, Brazil, Malaysia, Korea, Denmark, United Kingdom and China (Gopal & Divya, 2017).

HA-MRSA clones mainly cause infectious conditions in individuals with weak immune system after a long term use of antibiotic or hospitalization (Ostojić, 2008). And are multi drug resistance (MDR), usually classified either into the SCCmec type I, II or III (Otter & French, 2010). Contrastingly, CA-MRSA majorly infects individuals without previous visits to nosocomial environment. In addition, CA-MRSA clones are usually panton valentine leucocidin (PVL) positive with smaller fitness alongside increased transmissibility and virulence, and are more susceptible to non-beta lactam antibiotic agents (Gordon & Lowy, 2008).

2.2. Genetic Component of MRSA

MRSA are genetically distinctive from other *S aureus* species due to the presence of the small staphylococcal cassette chromosome mec (SCCmec) locus carried on a mobile genetic element (MGE) which contains a methicillin resistance gene called mecA (Hanssen & Ericson Sollid, 2006). The mecA gene in MRSA play a crucial role in the synthesis of a 78-kDa protein penicillin binding protein 2a (PBP2a), a membrane bound enzyme that activates a transpeptidation reaction that is required for cross-linking of peptidoglycan chain(Lim & Strynadka, 2002; Powers & Wardenburg, 2014), but is absent in other *S aureus* susceptible strains. Till date, five class of *SCCmec* elements have been identified and grouped into SCCmec (type I-VI) (Gopal & Divya, 2017). The SCC locus is a region where genetic exchange among different staphylococcal species takes place (Katayama et al., 2003). Although, the process of genetic recombination among different *S aureus* species is not properly understood, evidence from a study by Ito et al (2001) indicate that the process is usually sequence specific and the presence of some repeated sequences that are only recognized by the SCCmecA-specific-recombinases during the integration and excision of mecA gene in and out of the SCC chromosome is required (Ito et al., 2001). In general, PBPs have similar activities to that of the serine protease (an enzyme that cleave peptide bonds at the active sites of nucleophilic amino acid). However, in MRSA isolates, PBP2a is substituted for other PBPs; hence their low affinity for all beta-lactam antibiotics allows them to survive higher concentration of antibiotics agents such as cephalosporins. Lim and Strynadka (2002) showed that the crystal structure of PBP2a differs from other PBPs and as such it blocks the binding of all Beta-lactam but allows transpeptidation at

the sites (Lim & Strynadka, 2002). Additionally, the *SCCmec* region encodes cytolysin gene (PSM-mec) and dependent regulatory RNAs (PSM-mec-RNA) that are responsible for suppressing the virulence in hospital acquired MRSA strains. The locus (SCCmec locus) also contains the *crr* A and B and *ccrc* genes which encode different recombinase enzymes belonging to the invertase and resolvase family that is responsible for regulating a site specific incorporation and removal of SCCmec element from S aureus chromosomes (Hanssen & Ericson Sollid, 2006). Furthermore, These SCC elements have also been found in other staphylococcus species including S Sciuri, S. hominis, S. epidermidis and S. haemolyticus (Gopal & Divya, 2017).

Also, other evidence suggests that lack of proper antibiotic usage and dosage could probably provide a selective mechanism by which *MRSA* acquires antibiotic resistance (Ostojić, 2008).

2.3. Classification of *S aureus*

Staphylococcus and *Micrococcus* were initially placed under the genera Stamatococcus and Planococcus under the same family *Micrococcaceae*, however, they were latterly re-classified in to the family *Staphylococcaceac* based on phylogenetic and molecular analysis (Stackebrandt et al., 1997). One of the oldest methods used for classifying *Staphylococci* was based on their ability to clot rabbit plasma, although, it is currently considered artificial (Becker et al., 2014).

Currently, there are 45 *Staphylococcal* species and 24 subspecies of staphylococcal, however in this study; emphasis would be made on the basis of their significance in causing human infectious diseases. Concurrently, *Staphylococci* are subdivided into

three groups based on the presence or absence of catalase enzymes on their cell wall; (i) the first group is the coagulase positive staphylococci (COPs), which comprises of *S intermedius, S pseudintermedius, S aureus, S anaerobius* and *S delphini*. (ii) The second group consists of coagulase negative staphylococci (CoNS) among which are *S pasteuri, S saprophyticus* and *S epidermidis.* (iii) The third group is the *Staphylococcus* intermidius group consisting of *S intrae, S schleifer ssp,coagulans, S agnetis* and *S hyicus* (Bonar et al., 2018).

Of significance importance to human is the *CoNs* Staphylococci group usually because of their ability to acquire resistance to antimicrobial agents (for example methicillin resistance) due to the presence of the mecA reservoir which confer them with the capacity to transfer Methicilin resistance to susceptible staphylococcus isolates including S aureus species. And as such, this study will focus on finding a possible treatment for the Staphylococci infections (Becker et al., 2014).

2.4. The Morphology of *S aureus*

The name ‘’*Aureus”* means golden and is used to describe the appearance of a certain type of *Staphylococcus* colonies after been grown on a solid media. Notably, other CoNS *Staphylococci* forms different colors such as translucent, pale and white on solid media (Kloos et al., 1998). The size of *S aureus* bacteria is 0.5 -1.5 in micrometer with an average genome size of 2.8mb as reported in 2001 (Kuroda et al., 2001). The cell wall is approximately 20-40nm thick (Shockman & Barren, 1983) and beneath the cell wall is a cytoplasm that is enclosed by a cytoplasmic membrane (Harris et al., 2002). Other cell wall constituents of *S aureus* are peptidoglycan and teichoic acids (also known as phosphate containing polymers) (Knox & Wicken.,

1973) which account for 40 and 50% of the cell wall weight, respectively. Another important constituent of the cell walls includes exoproteins, peptidoglycan hydrolases and surface protein which play crucial roles in attaching the wall of the *S aureus* to other surfaces as well as determining their virulence factor (Harris et al., 2002).

2.5. Culture

One of the oldest and effective techniques for detecting staphylococci species is the blood culture systems which for the past decades have reduced the time taken in identifying positive blood cultures. The technique require continuous monitoring of blood cultures over a period of time and once a positive blood culture broth is identified, presumptive detection using Gram stain is used to identify the type of microbial organism present in the isolated blood samples. Upon culturing, the appearance of Gram positive cocci in grape like clusters on the Gram stain indicates the presence of S species. In addition, further analysis is required to differentiate S aureus species from other CoNS. Species. The differentiation is necessary due to the difference in the virulence factors and because CoNS. Species are mostly isolated as contaminant (Murdoch & Greenlees, 2004). Other techniques for identifying *Staphylococci* in blood cultures include the following; (i) direct tube coagulase test (TCT), (ii) the Api RAPIDEC Staph systems (APi, (bio meriux Durham, N.C)) and, (iii) peptide nucleic acid (PNA) fluorescence insitu hybridization (FISH, (Advan Dx, Woburn, mass)) (Chapin & Musgnug, 2003). Currently, the fastest and easier molecular testing methods includes polymerase chain reaction (PCR) –restriction

fragment length polymorphism analysis, whole genome DNA-DNA hybridization analysis, 16rRNA gene and MTLS (Bonar et al., 2018).

2.6. Biochemical Properties

Staphylococcus species are clinically identified using various biochemical and physiological properties they express which includes the following; their ability to form a colonial pigment, free coagulase, stable heat nuclease enzyme, protein-A, clumping factor and to produce lipase acid from mannitol during growth(Karmakar et al., 2016), the presence or absence of these biochemical substances aid in detecting the positive isolates with *S.Spp*. These chemical properties are presented in Table 1.1

Table 1.1.*S aureus* Chemical Properties

Characteristics	**Properties**
Catalase	Positive
Citrate	Positive
Coagulase	Positive
Gas Formation	Negative
Gelatin Hydrolysis	Positive
H_2S	Negative
Beta-Hemolysis	Positive
Indole	Negative
Methyl Red	Positive
Nitrate Reduction	Positive
Oxidase	Negative
Urease	Positive
Fructose	Positive
Galactose	Positive
Glucose	Positive
Lactose	Positive
Maltose	Positive
Mannitol	Positive
Mannose	Positive
Sucrose	Positive

This table was adapted from (Mamza, S. et al 2016).

2.7. Capsule

In general, most micro-organism involved in the pathogenesis of various infectious diseases produces a substance called extracellular capsular polysaccharides (O'Riordan & Lee, 2004). The exact function of the extracellular capsules in *S aureus* is not fully understood, however, they are classified as substance that enhances microbial virulence, which in turn makes them resistant to phagocytosis. Capsule productions by S aureus were first described in mice by Isabelle Gilbert in 1931 (Gilbert, 1931). After which, they were typified and graded into two different class; the Smith diffuse and Strains M, based on their virulence factors and capacity to resist phagocytosis as well as the ability to produce mucoid colonies (O'Riordan & Lee, 2004). Later in 1980s, Arbeit et al (1984) introduced a new scheme for grouping these extracellular capsular polysaccharides identified in *S aureus* based their ability to absorb rabbit antiserum prototype strains (Arbeit et al., 1984). In this study, the author's grouped *S aureus* capsule in to eight serotypes; the previously identified capsule (Strain M and Smith diffuse) were assigned in to serotype 1 and 2 based on their ability to produce mucoid colonies on solid media while the remaining isolates produced non-mucoid colonies and were assigned into serotype 3 to 8. Currently, different capsules from about eighteen different *S aureus* strains have been identified and classified based on their biochemical properties (Lee, 1995). Each of these capsules from *S aureus* strains contains a unique hexosaminuromic acids and polysaccharides which makes them easier to be identified blood cultures. For instance, the Smith diffuse and Strains M in

serotype 1 and 2 expresses the following chemical structure: (-4)- β-D-GlcNACA-(1-4)- β-GlcNACA-(1-4)-β-DGlcNACA-(l-alanyl)-(1-)n (Hensen et al., 2000) and (-4)-α-D-GalNACA-(1-4)-α-D-GalNACA-(1-3)-a-d-D-fucNAC-(1-)n (Murthy et al., 1983).

2.8. Virulence Factors

All *S aureus* species harbor an arsenal of virulence factors that aid in invading and bridging host immune systems, host cell injury and tissue adhesion. Also, these virulence factors are responsible for compromising vascular integrity, causing inflammations, altering blood coagulation and impairing immune cell function in the affected host (Powers & Wardenburg, 2014). A clear explanation of virulence in *S aureus* is showed in the clinical manifestation of infection called Sepsis, in which systemic inflammation and vascular issues caused by *S aureus* impaired blood pressure and cardiac function leading to impaired oxygen delivery to tissues which results to organ failure in host (Powers & Wardenburg, 2014). Data from published studies have reported the involvement of multiple *S aureus* proteins and cell wall components in pro-inflammation, eliciting host immune responses in relatedness to that caused by Gram-negative lipopolysaccharide (LPS) bacteria (Salomao et al., 2012). Also, Kimpe et al (1995), showed how blood streams exposure of rat to lipoteichoic acids and peptidoglycan from *S aureus* led to the induction of cytokines such as *IL-1* and *IFN-γ* (De Kimpe et al., 1995). Similarly, the action of lipoproteins from *S aureus* produced cytokines such as, *IL-6* and *TNF-α* on mononuclear phagocytes through the activation of the *TLR-2* pathway (Hashimoto et al., 2006). These findings suggest that many virulent factors alongside other genetic regulatory control activities which mediate the

production of phenonol soluble modulins (PSM) and toxin-alpha hemolysin (alpha-toxin or Hlα) might partly be responsible for host inflammatory response to severe *S aureus* infection.

2.9. Catalase

Catalase enzyme is one of the enzymes produced by *S aureus* which plays a major role in the hydrolysis and disintegration of hydrogen peroxide H_2O_2 in oxygen and water. The molecular weight of catalase is 250 kDa and embedded in it are four class of hemoprotein (Hadwan, 2018). In correlation to other anti-oxidant enzymes, catalase enzymes are produced by both hepatic and renal cells of animals. The enzyme is one of the most common intracellular substances that are secreted by a large number of facultative anaerobes including *S aureus* and the second most produced antioxidant enzyme after superoxide dismutase. In humans, catalase enzyme attenuates the level and concentration of reactive oxygen species that are mostly associated with certain human diseases (e.g. cancer and ageing). It is proposed that enzyme is secreted by cell wall of *S aureus* and might partly play a role in facilitating their escape from host immune responses through impaired oxidation of H_2O_2, and increased oxidative stress. Furthermore, the enzyme is commonly used to identify and differentiate Staphylococcus bacteria from *Streptococcus* (Hadwan, 2018; Powers & Wardenburg, 2014).

2.10. Coagulase

Coagulase is also another enzyme that has been associated with *S aureus* infections, although, it is also found in both humans and animals. In humans, the enzyme is responsible for changing blood fibrinogen to fibrin. It is used in the laboratory to distinguish between different staphylococcal isolates (Harris et al 2002). More importantly, *S aureus* are usually positive for coagulant, therefore, a positive clotting test likely indicate the presence of *S aureus* strains. Contrastingly, negative clotting tests more likely indicate the presence of a negative clotting organism. Generally, coagulase enzymes is considered as virulent factor usually because, the protein play a major role in blood aggregation by binding to prothrombin factor in the host organism. The process is called staphylothrombin, upon binding with the host thrombin factor, a protease enzyme is secreted which forms a complex that facilitates the transformation of fibrinogen to fibrin (Chauhan et al., 2013).

Another sufficient method that is used to identify *S aureus* from other bacterial species is the coagulase tube test which is based on the formation of blood clotting when a drop of host infected plasma is mixed with *S aureus* strains. The presence or absence of clotting determines the micro-organisms present in the isolated sample. However, further analysis using gram stain is required to confirm whether the causative agent is *S aureus*. Also, a thermo stable-Deoxyribonucleases test can be done to identify the presence of *S aureus* in isolated sample (Kateete et al., 2010; Lowy, 2003).

The coagulation occurs when the clotting factor fibrinogen and thrombin aggregated outside the cell (extracellular), Also there are many study mention as

genetically and it is obviously exposed the separate entities of both clumping factor and coagulase, in addition, it has been clarified that the occurrence of any mutation will cause the clotting causative action and maintain coagulase clumping while it is direct express normally. The pathogenic gene which associated with Staphylococcus in human body is conventionally separated according to the capability of clot formation of plasma into two groups and the causative agent formation of the clotting to host are *S aureus* but other *Staphylococci* are considered tube the CoNS which less causative pathogenicity to the skin and certain species able to forming inflammation when comparing to the *S aureus*, there for the bacterial Staphylococcus microbiological separate into coagulase +ve and –ve (Subramanian et al., 2017).

2.11. Lipase

The hydrolysis of lipid substance in *S aureus* is mainly conducted by lipase enzymes a subclass of protein called esterase. Lipase are water-soluble substance that modifiable ester bonds in inexplicable acylglycerols and lipases élite acyl group from glycerides creating lipase-acyl complex then transfers OH group of water. *Staphylococcal* lipase enzyme is used as a biocatalyst marker to spot and split trans-esterification, alcoholics, and alcohols esterification in non-aqueous media (Chauhan et al., 2013). Moreover, the activity of the lipase enzymes has been associated with lipid hydrolysis in relative to high temperature and PH range. Furthermore, the virulence's enzymatic have some specific characteristic relate to the bacteria such as biocatalytic possessions associated to specificity, steadiness, temperature, and ph. Nowadays the lipase enzyme that associated with bacterial activity was recently having

great position adaptability which leads it more interesting in the medical field currently in order to study extremophiles because it is characteristic to have high ability to make resistance to the hemophilic homologs in addition to chemical agents and PH range (Ben Bacha et al., 2018).

2.12. Hyaluronidase

Staphylococcus aureus contains a wide range of proteins, including surficial and inter-secreted proteins that are involved in the pathogenesis of infectious diseases. For instance, molecular hyaluronidase (also known as hyaluronidase) are enzymes which breaks down hyaluronic acid (HA) and it is involved in immunomodulation. Hyaluronidase also play an important role in inflammation and fluid homeostasis. In S aureus, HA have been reported as enzymes that are involved host immune invasion enabling in tissue penetration (Abdelkader et al., 2018; Biedenbach et al., 2007).

The active role of hyaluronidase enzymes in *S aureus* have been associated with HA Nanocapsules activation which contains polyhexanide, and HA amoxicillin-loaded mesoporous silica nanoparticles coated in infections. This enzyme enables S aureus into the host cell membrane by electrostatic repulsion. Also, HA actively accelerate wound healing process by activating inflammation. Till date, five different types of hyaluronidase have been identified in humans, and are involved in various biochemical, physiological, and pathological actions such as, the degradation of hyaluronic acid, embryogenesis, transmembrane diffusion of drugs and toxins, an inflammatory and allergic response to antigens, healing of wounds, bacterial meningitis, bacteremia, and pneumonia (Ji et al., 2016).

2.13. Staphylokinase

Staphylokinases also referred to as staphylococcal fibrinolysis or Müller's factor is an enzyme that is secreted by *S aureus.* Structurally, the enzyme has 136 amino acids and is 15kDa in weight. It functions in the activation of the plasminogen and in the alteration and slicing of immunoglobulin G and C3b, which in turn inhibit phagocytosis. Staphylokinase have also been associated with lysogenic bacteriophages as well as plasmin proteolysis which inhibited the activity of the fibrin clots in host (Gillaspy et al., 2019).

2.14. Deoxyribonucleases

There are some bacteria that contain DNase which acting between the materials of nucleic acid hydrolyses to oligonucleotides production. Moreover, the mechanical action of these enzymes is described in the works for numerous periods which are the extracellular DNA destruction by DNase I on characteristics of forming bacterial biofilms At the same time as these enzymes are involved in diagnostic bacteria that can produce these types of enzymes such as specific extracellular DNase countenance characteristic, like *s aureus*, since there are some of the clinical studies performed to recognize the function of DNase enzyme but until now the issues of this protein are r continued to be indistinct. DNase production by certain bacteria have some physiological characters which involve in the deliverance of nucleotides that have benefit in an improvement deliberate development also the DNase have come feature toward the decreases of the infected exudate viscosity, also later potentially allow moving bacteria toward cells to invade to inside tissue of the host then bacterial distribution, and it may cause in risen neutrophil extracellular later on stopping

promoting the development of bacteria and later on result in murder bacteria. Further, the DNase extracellular enzyme leads bacteria to escape and survive it from the antibacterial therapeutic agent and it may bacteria more stable toward causing wide infection to host cells (Tetz, V.,&Tetz, 2010; Palmer, et al, 2012).

2.15. Penisilinase (Beta-lactamase)

The introduction of penicillin (PC) in the early 1940s dramatically improved the prognosis of patients with Staphylococcal infection Some S aureus strains remain susceptible to penicillin G (PC), However, in the early 1942, penicillin resistance staphylococci emerged in hospitals and later spread in community settings (Lowy, 2003). The two proposed mechanism which contributes to PC resistance in S aureus includes the following processes; firstly, the production of an extracellular enzyme named penicillinase in S aureus which activates PC by hydrolyzing the Beta-lactam ring that is encoded by the blaZgene. The blaZ gene in S aureus is genetically regulated by two genes, the repressor (bla1) antirepressor (blaR1) gene (Kernodle, 2014). Data from previously published studies indicates that the signaling pathway which activates the Beta-lactamase requires the sequential cleavage of both bla1 and blaR1 regulatory genes, notably, blaR1 (a trans-membrane sensor transducer cleaves itself when it is exposed to B-lactam agents (Lowy, 2003). Secondly, the production of an altered PC binding protein also known as the PB2a that is encoded by the mecA gene which leads to methicilin resistance strains. Methicillin was later introduced in 1961 as the first semisynthetic penicillinase resistance pemicillins. Unfortunately, reports about methicillin resistances *S aureus* isolates (MRSA) later emerged in hospitals in the

United Kingdom (Gopal & Divya, 2017). Just like the PC resistance isolated strains, *MRSA* isolates requires a gene named *mecA* which is responsible for synthesizing PBP2a. These PBPs are membrane bound enzymes that catalyzes a transpeptidation reaction necessary for cross-linking peptidoglycan chains. They act as serine protease enzymes in *S aureus*, by substituting PBP2a for other PBPs and due to their low affinity to Beta-lactam antibiotic agents allowing them to survive even after exposure to beta- lactam drugs (Hanssen & Ericson Sollid, 2006).

Additionally, another strain with antimicrobial agents is the Vancomycin resistance *S aureus.* vancomycin was later used as antibiotic agent to treat infection caused by *MRSA*, and similar to (PC and methicillin), they were reports of a newly emerged vancomycin resistance in *S haemolyicus* strains (Schwalbe et al., 1987). Till date, only two forms of *S aureus* isolates that are resistance to vancomycin have been identified. First, the pre-VISA and VISA strains which were resistance to 8-16 ug/ml dose of vancomycin (Lowy, 2003). Hanaki et al (1998) proposed that reduced susceptibility to vancomycin is due to changes in biosynthesis of peptidoglycan (Hanaki et al., 1998). Notably, the VISA strains unlike other S aureus strains synthesizes more number of peptidoglycan that the irregular in shape which contributes to an unusual thickening of *S aureus* cell wall. These VISA strains also have decrease levels of cross-linking peptidoglycan leading to them being exposed to more *D-Ala-D-Ala-residues*. The second type is the vancomycin resistance *S aureus* isolates (VRSA), acquired through the transfer of VanA operon from vancomycin resistant *E.Faecalis* and similar to the VISA strains, these VRSA isolates harbor changes in the biosynthesis of peptidoglycan

but contrastingly, the resistance in VRSA strains result from alterations of the terminal peptide of *D-Ala-D-lac* exposure rather than *D-Ala-D-Ala* as observed in VISA strains (Lowy, 2003).

2.16. Toxins

S aureus is classified as an infectious causing agents due to their ability to produce various virulent factors, among which secreted toxins play crucial role in (Oliveira et al., 2018; Dinges et al., 2000). These secreted toxins are grouped into three groups; (i) exfoliative toxins (ETs), (ii) superantigens (SAgs), and (iii) spore forming toxin (PFTs). Toxins are further grouped into four class; Hemolysin-alpha (a-toxins or Hla), phenol-soluble modulins (Psms), leukotoxins and Hemolysin-Beta(Gopal & Divya, 2017; Gordon & Lowy, 2008; Oliveira et al., 2018). Furthermore, aside severe infections, *S aureus* secreted toxins can cause other diseases including staphylococcal scalded skin syndrome (SSSS), deep seated skin infections (or necrotizing pneumonia) and toxic shock syndrome (TSS)(Oliveira et al., 2018). Data from previous studies shows that there are differences in the allocation of toxins between *S aureus* clones. Notably, in all *S aureus* infections and diseases, these toxins as well as other secreted enzymes play a major role in the pathogenesis of such conditions, usually by damaging the cell membrane of the host by modulating immune responses or degrading inter-cellular connections. The differential expression of toxins in *S aureus* clones is regulated by such core-genome encoded toxins genes and further understanding of these genes and their role on host immunity will provide means for preventing and managing *MRSA* infections (Harris & Richards, 2006).

2.17. The Pathogenesis of *S aureus* Infections

S aureus is pathogenic agent of common human infections (e.g food poisoning and abscess). The pathogenesis of *S aureus* infections is categorized into five stages; (1) colonization, (2) local infection, (3) systemic dissemination or sepsis, (4) metastatic infections, and (5) toxinosis (Archer, 1998). In the first stage, colonization precedes infections, usually because about 30% healthy individuals are asymptomatic carries of *S aureus* species in their skin, mucous membrane and perianal regions (Diekema et al., 2001). But harboring these micro-organisms might not necessarily indicate the presence of infections. After colonization, they should be able to cause local infections. Localization occur when there is a damage to the skin and the micro-organisms get inoculated into the skin from the site of colonization for instance an injury or wound on the skin into the blood streams. Once in the bloodstreams they should be able to disseminate and spread to distant organs causing hematogenous systemic infections such as septic arithritis, osteomyelitis and endocarditis. Upon disseminating into bloodstreams or systemic syndromes such as food-borne gastroenteritis, they metastasize to other regions of the body and lastly, they should be able to secrete toxins that can invade host immune responses (Nakamura et al., 2012). Nevertheless, there are global regulatory elements (e.g. Sar and agr genes) that determines which virulent factors are produced during the development of *S aureus* infections and diseases in their host (Diekema et al., 2001; Nakamura et al., 2012).

2.18. Vaccination

The advent of multiple drug resistance *S aureus* such as *MRSA* and *VRSA* has triggered efforts towards developing vaccines that can prevent *S aureus* infections in both human and animals (Moellering, 2011). Data from previous studies have reported the effectiveness of vaccination with various staphylococcal antigens in Bovine mastitis (Archer, 1998). Unfortunately; there has been little success in human isolates. This prompted efforts towards developing a conjugate bivalent vaccine with *S aureus* type 5 and 8 capsular polysaccharide (CP) coupled to pseudomonas exotoxin. In 1993, the vaccine was reported to be safe and highly immunogenic in human (A. Fattom et al., 1993). Also, in a subsequent study, Fattom et al (1996) investigated the efficacy of capsular polysaccharide (CP) –specific antibodies composed type 5 and 8 *S aureus* CP linked to pseudomonas aeruginosa exoprotein with immune immunoglobin G (1-igG) obtain from vaccinated plasma donors in lethal and sublethal mouse (A. I. Fattom et al., 1996). The study further confirmed the role of conjugate vaccine with *S aureus* type 5 and 8 CP as protective antigen for managing *S aureus infections*. However, the effectiveness of this vaccine and antibody derived from plasma donors in human trails will validate their usefulness in treating *S aureus* infections.

2.19. SIGNIFICANCE OF THE STUDY

The outcome of this study will shed light on the antimicrobial resistance of five different drugs on MRSA strains and help discern which of the drugs are more appropriate and effecting for managing MRSA infections.

SECTION TWO: MATERIAL AND METHOD

2.0. Ethical Approval

The research proposal form project under the title (FREQUENCYOF ANTIBIOTIC SUSCEPTIBILITY TO MRSA OVER A PERIOD OF TIME) has been reviewed by the regulators of the Near East University Scientific Committee and Patients right consent and Confidential is respected according to Helsinki guidelines.

2.1. Material

2.1.1. Devices and Tools

The research material and tools used in this study are listed below:

1. Pipette
2. Yellow Tip
3. Blue Tip
4. Timer
5. Incubator
6. Autoclave
7. Microscope
8. Sterile Cup
9. Slide
10. Cover Slip
11. Petri dish
12. Blood agar Base
13. Ethanol ethyl
14. Povidone iodine

15. Sodium hypochloride

16. Swap

17. Hydrogen peroxide

2.1.2. Composition of Blood Agar

1. 0.5% Peptone
2. 0.3% beef extract/yeast extract
3. 1.5% agar
4. 0.5% NaCl
5. Distilled water
6. 5% Sheep Blood
7. PH should be from 7.2 to 7.6 (7.4)

2.1.3. Composition of the Mueller Hinton Agar

1. 2.0 g beef extract
2. 17.5 g casein hydrolysate
3. 1.5 g starch
4. 17.0 g agar
5. All of above mixed in 1 liter of D.W with PH adjusted to neutral at 25 °C

2.1.4. **Blood Agar Kits Company Name**

1. Blood Agar Base (LAB M)
2. LOT: 115835/153
3. www.labm.com
4. United Kingdom

3.0. Methods

3.1. Sample collection

This study was conducted at Near East University Hospital Laboratory in Nicosia Northern Cyprus. The study samples included 80 strains of *Staphylococcus aureus* that were isolated from two groups. Samples were obtained using different measures either through blood, nasal swabs, throat swab, nasopharyngeal swabs, sputum swabs, wound swabs, aspirate swabs, urethral discharge swabs and urine swabs. A culture examination was performed based on specific microbiological standards for *MRSA*. This step is essential, usually because some strains of *S aureus*, including many MRSA, may delay clotting which is rapidly lysed at 37°C by an enzyme named Staphylokinase. After sample collection, all samples were prepared in a culture media and placed on a blood agar. Samples were incubated at 37°C for 12-16 hours. After incubation, we detected growing MRSA on blood agar and give us smooth round raised and glistening *Saureus* usually from grey to deep golden yellow colonies. In this research, clinical testing was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines which recommend that, a plate containing 6 μg/ml of oxacillin in Mueller-Hinton agar should be supplemented with 4% sodium chloride should be used as an alternative method for testing of MRSA.

Upon sample screening on blood agar, all tubes containing the isolates were sterilized with 4ml of distilled water and were transferred into a petri dish containing the prepared Mueller-Hinton agar. After which, five different types of antibiotic agents were added to the petri dish containing the isolates and were incubated for 24hr in order to evaluate the resistance and bacterial susceptibility.

3.2. The experiment was designed to test the susceptibility of antibiotics drug on two groups:

We classified antibiotics into two groups according to their drug of choice preferences in daily clinical application.

1- The First group of the antibiotics is the first drug of choices: vancomycin (VA), and teicoplanin (TEC).

2- Second group of the antibiotics is the second drug of choices: rifampsin (RA), tigecycline (TGC), trimethoprim sulfamethoxazole (SXT), clindamycin (DA), and cefoxitin (FOX).

3.3. Culture

3.3.1. Blood Agar

The blood agar is a microbiological culture that is scientifically used to grow various types of bacteria. The blood agar is used as a different media in order to separate fastidious microorganisms, in addition, to distinguish either there are a hemolytic activity or not.

3.3.2. Preparation of Blood Agar

1. 28 g of nutrient agar powder has been suspended in 1 liter of DW.
2. The mixture was heated while stirring to fully dissolve all components.
3. We Autoclaved the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. When the agar has cooled to 45-50 °C, added 5% (vol/vol) sterile de-fibrinated human blood that has been warmed to room temperature and mix gently but well without bubble forming.
5. We dispensed the medium into sterile plates while liquid.

3.3.3. Preparation of Mueller Hinton agar MHA

A. 200ml of distilled water has been taken.

B. 6.8gm of powder media has been riches in D.W.

C. After mixing, we put it in the autoclave for two hours.

D. We avoid it from bubble formation.

E. Transferring to culture plates.

3.3.4. Catalase test

1- A drop from hydrogen peroxide was put and mixed with the smear of bacterial growth from the cultured on a slide.

2- After the procedure, if there was any bubble seen this means the *S aureus* is available and the test considers to be a positive result and if there was no bubble were available this means the test result is negative and there is no *S aureus* are available.

3.3.5. Coagulase test

1- 5 ml of plasma has been put into the test tube.

2- One loopful Sample microorganism has been added into the sample plasma.

3- The presence of clotting in the sample indicates the presence or absence of S aureus in the isolated sample.

The Coagulase test is used to differentiate *S aureus* (positive) from Coagulase Negative *Staphylococcus* (CoNS). Coagulase is an enzyme produced by *S aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin, the enzyme is absent in other types of Staphylococcus species.

3.3.6. Tube Coagulase Test Procedure

1. After preparing a 1-in-6 dilution of the plasma in saline (0.85% NaCl), then transferred to 1 ml volumes of the diluted plasma in small tube
2. Mix the colonies in 1 ml of diluted rabbit plasma to give a milky suspension
3. Incubate the tube at 35°C. In ambient air or a water bath for 4 hours
4. After a period of about one, two, and four hours investigations for clot formation by tilting the tube through 90°. Clots may liquefy after their formation
5. If the test gives a negative result, let the tubes at room temperature overnight and re-examine again.

3.3.7. Statistical Data Analysis

The Statistical Package Social Sciences (SPSS) application was used in order to manipulate each result parameter with other data in this study. Susceptibility tests were evaluated by measuring the radius of inhibition zones and comparing them to each other. The Chi-square test was used to solve the relationship between the first and second group of antibiotic.

SECTION THREE: RESULTS

3.1. Study Subjects

A total of 80 samples were retrospectively collected using previously stored stock at Near East University laboratory in Nicosia Northern Cyprus. Samples were graded according to the clinical records provided by the patients, among which (59.4 %) were males and (40.6 %) were females. Patients were further divided into three groups based on their age as children, Adults, or Elder (11.2 %, 31.8 %, and 57 %), respectively. As presented in (Table 3.1).

Table 3.1. The demographic characteristics of patient**s**

Patient's characteristics	**n (%)**
Children(less than 18y)	3 (11.2)
Adults (18-50y)	17 (31.8)
Elder (50y)	60 (57)
Gender	
Male	50 (59.4)
Female	30 (40.6)

Table.3.2. The distribution of sample types

Sample types	**n (%)**
Wound culture	20 (21.1)
Blood culture	19 (20.2)
Aspirate culture	11(13.3)
Sputum culture	8 (10)
Nasopharyngeal culture	7 (9)
Urine culture	5 (7.2)
Body fluids culture	4 (6.2)
Throat culture	3 (5.2)
Nose culture	2 (4.4)
Urethral discharge	1 (3.4)

Table.3.3. The distribution of samples that taken from different hospital department

Departments	n (%)
Cardiology	16 (16.4)
Other departments	15 (28.1)
Anesthesia	15 (15.4)
General surgery	8 (8.9)
Cardiovascular surgery	7 (7.9)
Lab	7 (7.9)
Orthopedic & traumatology	6 (7)
Infectious diseases	3 (4.2)
Urology	3 (4.2)

Table.3.4. The distribution of first and second group of antibiotics susceptibility according to gender

Gender	Susceptibility of the first group of antibiotics: n (%)		Susceptibility of the second group of antibiotics: n (%)				
	VA	TEC	DA	FOX	RA	SXT	TGC
Male	50 (100)	50 (100)	48 (96)	23 (46)	47 (94)	47 (94)	49 (98)
Female	30 (100)	30 (100)	29 (96.6)	15 (50)	28 (93.3)	28 (93.3)	27 (90)

Table.3.5. The distribution of first and second group of antibiotics susceptibility according to age

Age	Susceptibility of the first group of antibiotics: n (%)		Susceptibility of the second group of antibiotics: n (%)				
	VA	TEC	DA	FOX	RA	SXT	TGC
Children	3 (100)	3 (100)	2 (66.6)	3 (100)	2 (66.6)	3 (100)	2 (66.6)
Adults	17 (100)	17 (100)	17 (100)	11 (64.7)	17 (100)	15 (88.2)	17 (100)
Elder	60 (100)	60 (100)	60 (100)	24 (40)	56 (93.3)	57 (95)	57 (95)

According to the (Table3.5) expression, the study of the statistical analysis observation between the different stages of Age, which divided into three categories (children, Adults, and Elder) and looking for which of the stage are predominate more antibiotic susceptible in the second line of antibiotic. In addition, the result observation in the Children group as sensitivity to all types of antibiotic, which include DA 2 (66.6), FOX 3 (100), RA 2 (66.6), SXT 3 (100), and TGC 2 (66.6).Following, in the adult group the antibiotic result were found as DA 17 (100), FOX 11 (64.7), RA 17 (100), and SXT 15 (88.2), and TGC 17 (100).Finally, in the elder group result were showed as DA 60 (100), FOX 24 (40), RA 56 (93.3), SXT 57 (95), and TGC 57 (95).

Table.3.5 shows the statistical analysis difference between the dissimilar phase of the Age, which separated into three major classes (children, Adults, and Elder), and following up which one of these three classes are the more predominate first line of antibiotics.

Table.3.6. The distribution of antibiotics susceptibility among hospital department

Department	Susceptibility of the first group of antibiotics: n (%)		Susceptibility of the second group of antibiotics: n (%)				
	VA	TEC	DA	FOX	SXT	RA	TGC
Urology	3 (100)	3 (100)	3 (100)	2 (66.6)	3 (100)	2 (66.6)	3 (100)
Infection disease	3 (100)	3 (100)	2 (66.6)	3 (100)	2 (66.6)	2 (66.6)	2 (66.6)
Orthopedics & traumatology	6 (100)	6 (100)	4 (66.6)	4 (66.6)	6 (100)	6 (100)	6 (100)
Lab	7 (100)	7 (100)	7 (100)	4 (57.1)	7 (100)	7 (100)	7 (100)
KVC	7 (100)	7 (100)	7 (100)	2 (28.5)	7 (100)	7 (100)	7 (100)
General surgery	8 (100)	8 (100)	8 (100)	3 (37.5)	7 (87.5)	8 (100)	7 (87.5)
Anesthesia	15 (100)	15 (100)	15 (100)	7 (46.6)	13 (86.6)	13 (86.6)	15 (100)
Other sample	15 (100)	15 (100)	15 (100)	9 (60)	14 (93.3)	14 (93.3)	15 (100)
Cardiology	16 (100)	16 (100)	16 (100)	4 (25)	16 (100)	16 (100)	14 (87.5)

Table.3.7. Identifying dominates of susceptible antibiotics rendering to various samples.

Sample	Susceptibility of the first group of antibiotics: n (%)		Susceptibility of the second group of antibiotics: n (%)				
	VA	TEC	DA	FOX	SXT	RA	TGC
Aspirate culture	11 (100)	11 (100)	11 (100)	5 (45.5)	10 (90.9)	10 (90.9)	11 (100)
Blood culture	19 (100)	19 (100)	19 (100)	7 (36.8)	18 (94.7)	18 (94.7)	19 (100)
Body fluids	4 (100)	4 (100)	4 (100)	1 (25)	4 (100)	4 (100)	3 (75)
Nasal culture	7 (100)	7 (100)	7 (100)	3 (42.8)	7 (100)	6 (85.7)	7 (100)
Nose culture	2 (100)	2 (100)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)
Sputum culture	8 (100)	8 (100)	8 (100)	3 (37.5)	8 (100)	8 (100)	7 (87.5)
Throat culture	3 (100)	3 (100)	3 (100)	2 (66.6)	3 (100)	3 (100)	3 (100)
Urethral discharge	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.0)	1 (100)	1 (100)
Urine culture	5 (100)	5 (100)	5 (100)	3 (60)	4 (80)	5 (100)	5 (100)
Wound culture	20 (100)	20 (100)	17 (85)	13 (65)	19 (95)	18 (90)	18 (90)

Table.3.8. The Statistical difference between first and second group of antibiotics susceptibility

Group of antibiotic	TEC, VA	DA, FOX, RA, SXT, TGC	P. Value (0.05)
	Number of sensitive (%)	Number of sensitive (%)	
First and Second group of antibiotic without FOX	160 (100.0)	303 (94.7)	**0.0032 significant**
First and second group antibiotic only FOX	160 (100.0)	38 (47.5)	**<0.00001 significant**

* VA=Vancomycin * TEC= Teicoplanin * DA= clindamycin *FOX= cefoxitin

* RA=rifampsin *TGC= tigecycline *SXT= trimethoprim sulfamethoxazole

In this study, the different sample collects who identify as a *Staphylococcus aureus* (80strains were selected). The culture examination performed based on specific microbiological standards for MRSA depending on the Clinical and Laboratory Standards Institute (CLSI) which recommended the plate of Mueller-Hinton agar, following, performing susceptibility test based on two different lines of the antibiotic, the first group of antibiotic include the TEC and VA, the second group of the antibiotic contain DA,FOX, RA, SXT, and TGC, there were the different result of the antibiotic resistance and sensitivity was predominated between both groups of antibiotic. The statistical analysis of the susceptibility of the antibiotic according to MRSA showed as the following: First, the statistical analysis showed there is a significant difference

between the First group of the antibiotic and second group of antibiotic type among MRSA and our first P.value were ($P < 0.05$), which means there are signification differences between the first group and second group of the antibiotic without (FOX). Also, the second P.value was ($P < 0.05$) which means there are significant differences between the first group and the Second group (only FOX) of the antibiotic. Table 3.8, illustrates the significant analysis between the first and the second group of antibiotic susceptibility among bacteria *S aureus*.

SECTION FOUR: DISCUSSION

4.1. Discussion

This research was designed to investigate the efficacy of five antibiotic drugs in 80 strains of *S aureus* isolates obtained from 80 random patients. Samples were classified into two groups. The first group had VA and TEC as drug of choice while the second group had DA, TGC, RA, SXT and FOX antibiotic drugs. Till date, there are limited numbers of functional studies that have analyzed the efficacy of VA, TC, DA, TGC, RA, SXT and FOX in *MRSA* strains.

Generally, *Staphylococcus aureus* is a pathogenic bacterium that is responsible for wide spectrum of infectious condition and other systemic diseases (e.g. food borne illness, pneumonia, bloodstream infections, bone, and joint infections and toxic shock syndrome) (Archer, 1998; Gordon & Lowy, 2008; Ostojić & Hukić, 2015). The bacterium is one of the most prevalent infectious causing agents that have been associated with gastroenteritis and intoxication throughout the world (Samad et al., 2018). *S aureus* species of *Staphylococci* bacteria are known for their ability of producing coagulase and catalase enzymes and can survive been exposed to higher concentration of salt or dryness (Abdelkader et al., 2018; Samad et al., 2018). Simultaneously, the emergence of *MRSA* strains in hospitals and community settings over the past decades has prompted efforts toward finding treatments for *MRSA* caused infections. Evidence from previous studies shows that the presence of certain genetic regulator elements, such as *blaZ* and *mecA* genes play crucial roles in providing a mechanism by which *S aureus* acquire resistances against antibiotic agents including

penicillins, tetracyclins, streptogramins, methicillin, phenocols and folate inhibitors (Hanssen & Ericson Sollid, 2006; Kernodle, 2014). For methilincin susceptible S aureus (MSSA), antibiotic agents containing beta-lactam are the drugs of choice (Hanssen & Ericson Sollid, 2006; Moellering, 2011; Nakamura et al., 2012). While for *MRSA*, the recommended drug of choice is either daptomycin or vancomycin (Moise et al., 2013; Samad et al., 2018). In relation to this, several numbers of studies have investigated the efficacy of vancomycin and daptomycin in treating MRSA infections. However, to date contradictory result has been published. Han et al (2012) evaluated the effectiveness of vancomycin in treating MRSA infections. They reported that the drug was ineffective for treating MRSA infections in their data sets and they also observed low tissue penetration, increased resistance and slow bactericidal activity (Han et al., 2012). A number of studies have supported this findings, in such vancomycin was not effective for treating MRSA infections. Gould et al (2011) reported that vancomycin were ineffective in treating MRSA infections due to low penetrance in lung tissue (Gould et al., 2011) and in Blattella germanica (B.germanica) and periplanets Americana (p.americana) cockroaches (Abdolmaleki et al., 2019). Other evidence suggest that prior treatments using vancomycin can trigger daptomycin resistance in *S aureus* (Moise et al., 2013; Sakoulas et al., 2006). On the other hand, other studies have reported the success of daptomycin in treating MRSA infections (Kullar et al., 2013; Moore et al., 2012).

The choice of antibiotic treatments most often depend on the type of infection (whether or not if the treated infection is secondary to another). Other studies have evaluated

the efficacy of other alternative antimicrobial agents including ceftaroline (Zasowski et al., 2017), linezolid (Moise et al., 2013; Yue et al., 2016) and dalfopristin/quinupristin (D/Q)(Sander et al., 2002), in such all have proven to be successful in treating infection caused by MRSA bacteria, but none have been approved by the Food and Drug Administration (FDA)(Holland et al., 2014).

In this present study, multiple analyses were conducted. The result of the first investigation showed that there was a signification difference between the sensitivity of the antibiotic drug used in the first group (vancomycin and TEC) when compared to the second group of antibiotics (DA, RA, SXT and TGC) in this MRSA data sets (P-values<0.05). Also in the second investigation, a significant difference was observed between vancomycin and TEC (first group) and FOX (from second group) of antibiotics in these MRSA data sets (P-value<0.05). Previously published independent studies have supported the findings of this study, in which, the clinical impact of teicoplanin and vancomycin against 120 *Staphylococcus* isolates including MRSA and heterogenous vancomycin intermediate *S aureus* (h-VISA) showed that treatment with Teicoplanin is effective against h-VISA strains (Biedenbach et al., 2007). Also, Ruhe and Menon (2007) investigated the benefit of tetracycline as oral treatments against MRSA caused infections in soft tissues and community onset skin infections from October 2002 to February 2007; data was obtained from the archive of Central Arkansas Healthcare Systems in New York. They reported that tetracycline was beneficial in treating community onset skin MRSA (Ruhe & Menon, 2007). Contrastingly, tigecycline was reported to be less effective against S aureus infections compared to

other antibiotics with higher frequency of adverse effects and mortality rate (Shen et al., 2015). Furthermore, the clinical benefit of both Vancomycin and Trimethoprim-sulfamethoxazole treatments against severe MRSA infections were reported by (Gillaspy et al., 2019; Paul et al., 2015). Likewise, the benefits of ceftaroline and vancomycin treatments against prosthetic joint MRSA-caused infections in Rabbit (Gatin et al., 2014). The finding of this study indicates that our MRSA strains were sensitivity to VA, TEC, DA, TGC, RA, and SXT; however, they showed higher sensitivity towards VA and TEC compared to TGC, RA, SXT and DA. While on the other hand, they showed higher resistance towards FOX antibiotic drug.

SECTION FIVE: CONCLUSION

5.1. Conclusion

In summary, the methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that is associated with high morbidity and mortality worldwide. Up to date, there are contradictory evidence with respect to the most cost-effective methods for treating and controlling the spread of MRSA infections in both hospital and community settings.

In this study, the sensitivity of our MRSA strains to the drug of choice in the first group VA and TEC and those in second group including DA, RA, SXT and TGC were similar thus indicating that these antimicrobial agents can be used as medications for treating MRSA infections. On the other hand, when the sensitivities of VA and TEC from the first group was compared with only FOX drug from the second group, there was also a significant difference observed, thus indicating that the MRSA strains used in this study showed resistances to FOX drug, and as such, FOX is not effective against MRSA infections in these data sets. However, further studies using larger number of MRSA data sets will be required to validate these findings.

5.2. Limitations of the study

One of the limitations of this study is that there was a limited number of MRSA strains used included in the investigation and as such the results cannot be generalized. Therefore, this study only forms a basis for other future studies on the clinical use these tested antibiotic drugs in preventing MRSA infectious conditions.

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EDUCATION

(2013to 2017)
University of Zakho
Bachelor (B.SC.)Science Biology.

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DNA Mutation.

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Near East University /Medical Faculty/Cyprus

Master (M.SC.) Medical microbiology and Clinical microbiology.

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Frequency of antibiotic susceptibility to MRSA in periods of time.

WORK EXPERIENCE

- **INTERNSHIP 30 DAYS**
  - Working In Laboratory / Zakho General Hospital.
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- **CERTIFICATES**
  - Certification of (Nursing), NLP Organization for Training and Consultations, 2019.
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SKILLS

- Have capacity of working under pressure and manage personal stress level.
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- Have a good numerical and report writing skills.
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