

**A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY**

**PREVALENCE OF METHICILLIN-RESISTANT
STAPHYLOCOCCUS AUREUS ISOLATED IN IRAQ-WASIT/AL-
SUWAIRA HOSPITAL**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY**

**BY
MOHAMMED HAMEED AHMED AL-SABBAGH**

ÇANKIRI

2022

PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS
ISOLATED IN IRAQ-WASIT/AL-SUWAIRA HOSPITAL

Mohammed Hameed Ahmed AL-SABBAGH

October 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor : Assoc. Prof. Dr. ÖZGÜR KUZUKIRAN

Co-Advisor : Asst. Prof. Dr. Mohammed F. AL MARJANI

Examining Committee Members:

Chairman : Prof. Dr. Ayhan FİLAZİ
Pharmacology and Toxicology
Ankara University

Member : Assoc. Prof. Dr. Özgür KUZUKIRAN
Eldivan SHMYO
Çankırı Karatekin University

Member : Asst. Prof. Dr. Müge FIRAT
Eldivan SHMYO
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Mohammed Hameed Ahmed AL-SABBAGH

ABSTRACT

PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED IN IRAQ-WASIT/AL-SUWAIRA HOSPITAL

Mohammed Hameed Ahmed AL-SABBAGH

Master of Science of in Biology

Advisor: Assoc. Prof. Dr. Özgür KUZUKIRAN

Co-advisor: Asst. Prof. Dr. Mohammed F. AL MARJANI

October 2022

Staphylococcus aureus (*S. aureus*) that is resistant to methicillin has become a growing public health issue as a result of recent and outbreaks and reported fatalities, especially among people who have no recognized risk factors. It is the most virulent of the staphylococci species. It has a diverse set of virulence factors toxins, making it a frequent cause of toxin-mediated disorders. Further, methicillin-resistant *S. aureus* (MRSA) is a leading source of both acute and chronic infections, and the ability of MRSA to adhere and form biofilms is a marker of increased virulence among MRSA isolates. To detect the resistance of local isolates to methicillin and other antibiotics, as well as to assess the utility of amplifying the *mecA* gene and its reliability in identifying MRSA strains. 100 samples were collected from different surgical sites and initially diagnosed based on their phenotypic characteristics in the hospital. Of the 100 samples, only 37 were positive, while 44 samples showed different types of bacteria. The other 19 samples did not show any bacterial growth. A standardized Kirby-Bauer disk diffusion method employing the Muller-Hinton Agar plate technique and lethal inhibitory concentration by VITEC2 was performed on positive samples. Approximately %95 *S. aureus* isolates showed resistance to cefoxitin and methicillin. In comparison to other antibiotics, vancomycin showed a significantly higher level of inhibition of *S. aureus*. The diagnostic gene, 16srDNA, was used in this research to denote isolates belonging to *S. aureus*. All isolates were proven to be *S. aureus*, which included 35 resistant isolates and 2 sensitive isolates. These 35 isolates were identified by polymerase chain reaction (PCR) technique to notice the presence of the *mecA* gen. In between 35 isolates, a sample was negative for the *mecA* gene, whereas 34 were positive.

2022, 68 page

Keywords: *S. aureus*, Virulence, Pathogenesis, Methicillin-resistance, *mecA* gene

ÖZET

IRAK VASİT HASTANESİNDE İZOLE EDİLEN METİSİLİNE DİRENÇLİ STAPHYLOCOCCUS AUREUS PREVALANSI

Mohammed Hameed Ahmed AL-SABBAGH

Biyoloji, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Özgür KUZUKIRAN

Eş Danışman: Dr. Öğr. Üyesi Mohammed F. AL MARJANI

Ekim 2022

Metisiline dirençli olan *Staphylococcus aureus* (*S. aureus*), özellikle bilinen hiçbir risk faktörü olmayan kişilerde son zamanlarda ortaya çıkan salgınlar ve bildirilen ölümlerin bir sonucu olarak büyüyen bir halk sağlığı sorunu haline gelmiştir. Çeşitli virülans faktörleri ve toksinlerine sahiptir. *Staphylococcus* türleri içinde en virulent olanıdır. Bu da onu toksin aracılı bozuklukların sık karşılaşılanı yapar. Ayrıca, metisiline dirençli *S. aureus* (MRSA), hem akut hem de kronik enfeksiyonların önde gelen bir kaynağıdır ve MRSA'nın yapışma ve biyofilm oluşturma yeteneği, MRSA izolatları arasında artan virülansın bir belirticidir. Lokal izolatların metisilin ve diğer bazı antibiyotiklere direncini saptamak ve *mecA* geninin amplifikasyonunun yararlılığını ve MRSA suşlarının tanımlanmasındaki güvenilirliğini değerlendirmek için farklı bölgelerden 100 örnek toplandı. İlk olarak fenotiplerine göre teşhis edildi. Bu örneklerden sadece 37'si *S. aureus* pozitif, 44'ünün farklı bakteri türleri olduğu, kalan 19 numunede ise herhangi bir bakteri üremediği görüldü. VITEC 2 ile *S. aureus* olduğu tespit edilen pozitif numuneler Muller-Hinton Agar plate tekniği ile antibiyotik direnci araştırıldı. Dirençli izolatlara Kirby-Bauer disk difüzyon yöntemi uygulanarak minimum inhibitör konsantrasyonlar belirlendi. *S. aureus* izolatlarının yaklaşık % 95'i sefoksitin ve metisiline dirençli olduğu belirlendi. Diğer antibiyotiklerle karşılaştırıldığında, vankomisin duyarlılığının daha yüksek olduğu tespit edilmiştir. Bu çalışmada *S. aureus*'a ait izolatları belirtmek için tanısal gen olan 16srDNA kullanılmıştır. 35 dirençli izolat ve 2 hassas izolat dahil tüm izolatların *S. aureus* olduğu doğrulandı. Bu 35 numune, *mecA* geninin varlığını tespit etmek için polimeraz zincir reaksiyonu (PCR) tekniği ile tanımlandı. 35 örnekten 34'ü *mecA* geni için pozitif, biri negatifti.

2022, 68 sayfa

Anahtar Kelimeler: *S. aureus*, Virulans, Metisilin direnci, *mecA* geni

PREFACE AND ACKNOWLEDGEMENTS

My sincere thanks and gratitude to my thesis advisor, Assoc. Prof. Dr. Özgür KUZUKIRAN, for his great efforts, scientific guidance and valuable advice. My thanks and gratitude co-advisor, Asst. Prof. Dr. Mohammed F. Al MARJANI for his continuous scientific guidance and valuable advice. And I would like to thank my family in addition to the staff of laboratory who are helped me in this study.

Mohammed Hameed Ahmed AL-SABBAGH

Çankırı-2022



CONTENTS

ABSTRACT	iv
ÖZET.....	v
PREFACE AND ACKNOWLEDGEMENTS	vi
LIST OF SYMBOLS	ix
LIST OF ABBREVIATIONS	x
LIST OF FIGURES	xi
LIST OF TABLES	xii
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 General Characteristics of <i>S. aureus</i>	3
2.2 Virulence Factors	4
2.2.1 Cell surface factors	4
2.2.2 Secreted factors (exotoxins)	5
2.3 Pathogenesis.....	6
2.4 Methicillin Resistant <i>S. aureus</i>.....	7
2.4.1 Methicillin resistance mechanism	8
2.4.2 Penicillin-binding proteins.....	9
2.4.3 <i>MecA</i> gene and staphylococcal chromosome cassette	11
2.4.4 Epidemiology of MRSA.....	13
2.4.5 Treatment of MRSA	14
2.4.6 Preventative actions.....	16
3. MATERIALS AND METHODS.....	18
3.1 Materials	18
3.1.1 Instruments and equipments	18
3.1.2 Culture media.....	19
3.1.3 Chemicals and antibiotic agents	19
3.2 Methods.....	21
3.2.1 Patients and sample collection.....	21
3.2.2 Sterilization	21
3.2.3 Preparation of culture media.....	21

3.2.4 Preparation of buffer and solutions	21
3.2.5 Culture techniques and characteristics	22
3.2.6 Antimicrobial sensitivity test	23
3.2.7 Identification and antibiotic susceptibility test	24
3.2.8 Molecular techniques.....	24
4. RESULTS AND DISCUSSIONS	29
4.1 Collection and Identification of Isolates	29
4.2 Microscopic Characterization.....	31
4.3 Antibiotic Susceptibility	32
4.4 Antibiotic Susceptibility Test of <i>S. aureus</i> Using VITEC 2 System	34
4.5 Effect of Gender on the Infection Rate with <i>S. aureus</i>	35
4.6 Effect of Age on the Infection Rate of <i>S. aureus</i>	36
4.7 Identification of Bacterial Species by 16s rDNA.....	37
4.8 Detection of the <i>mecA</i> gene in <i>S. aureus</i> using PCR technology.....	38
4.9 Sequence Analysis of 16s rDNA and <i>mecA</i> Gene.....	39
5. CONCLUSIONS AND RECOMMENDATION	41
5.1 Conclusions.....	41
5.2 Recommendations	41
REFERENCES.....	41
APPENDICES	59
CURRICULUM VITAE.....	68

LIST OF SYMBOLS

%	Percent
°C	Celcius degree
µg	Mikrogram
µL	Mikroliter
µm	Micrometer
bp	Base pair
cfu	Colony forming unit
cm	Centimeter
g	Gram
h	Hour
inch	Inch
Kb	Kilobase
KDa	Kilodalton
L	Liter
lb	Libre
M	Molar
mA	Milliamperes
mg	Milligram
mL	Milliliter
mm	Millimeter
mmol	Millimol
ng	Nanogram
nm	Nanometer
nm	Wavelenght
pmol	Picomole
rpm	Revolutions per minute
v	Volume
w	Weight

LIST OF ABBREVIATIONS

CA-MRSA	Community associated methicillin resistance <i>S. aureus</i>
CCR	Cassette chromosome recombinases
CLSI	Clinical and Laboratory Standard Institutes
DNA	Deoxyribonucleic acid
EMRSA	Methicillin-resistant <i>S. aureus</i> epidemic
HA-MRSA	Hospital acquired methicillin resistance <i>S. aureus</i>
HMM	High molecular mass
LMM	Low molecular mass
MRSA	Methicillin-resistance <i>S. aureus</i>
MSSA	Methicillin-sensitive <i>S. aureus</i>
NCBI	National Center for Biotechnology Information
NCCLS	National Committee for Clinical Laboratory Standard
PBP2a	Penicillin binding protein 2a
PCR	Polymerase chain reaction
PG	Peptidoglycan
PVL	Panton-valentine leukocidin
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SCCmec	Staphylococcal cassette chromosome <i>mec</i>
TG	Transglycosylation
TP	Transpeptidation
TSS	Toxic shock syndrome

LIST OF FIGURES

Figure 4.1	<i>S. aureus</i> colonies on Blood Agar and the appearance of clear areas indicate hemolysis due to the production of hemolysin by the isolates	30
Figure 4.2	<i>S. aureus</i> appears in golden color on Mannitol Agar	31
Figure 4.3	Catalase test.....	31
Figure 4.4	<i>S. aureus</i> under light microscope	32
Figure 4.5	Antibiotic susceptibility test of <i>S. aureus</i>	34
Figure 4.6	Diagnosis of staphylococci by using PCR. The appearance of bundles defining the 16s rDNA gene across an agarose gel was documented using a UV.....	38
Figure 4.7	Amplification of the <i>mecA</i> gene for <i>S. aureus</i> on agarose-stained gel	38

LIST OF TABLES

Table 3.1 Instruments and equipments	18
Table 3.2 The culture medias	19
Table 3.3 Solutions, chemicals and their origins	19
Table 3.4 Antibiotic discs and their origins	20
Table 3.5 Diagnostic kits and their origins	20
Table 3.6 Primers used throughout the current research.....	20
Table 3.7 The component of PCR reaction.....	26
Table 3.8 PCR program.....	27
Table 4.1 <i>S. aureus</i> according to the surface of samples	30
Table 4.2 Sensitivity rate of <i>S. aureus</i> isolates different antimicrobials.....	34
Table 4.3 Antibiotic susceptibility test of <i>S. aureus</i> using VITEC 2.....	35
Table 4.4 MRSA distribution according to gender	36
Table 4.5 Prevalence of MRSA by age.....	37
Table 4.6 Comparison between 16sDNA sequences of <i>S. aureus</i> strains with gene bank by using BLAST software.....	40
Table 4.7 Comparison between <i>mecA</i> sequences of <i>S. aureus</i> strains with gene bank by using BLAST software	40

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a prevalent infection, despite advances in medical and diagnostic technology. *S. aureus* dwells in 20–30 % of the general population without producing any clinical signs, however it remains a versatile and serious pathogen for people (Gajdács 2019).

S. aureus is a non-spore forming, nonmotile, facultative anaerobic, Gram-positive, coccal-shaped bacterium that grows in clusters that are described as "grape-like," measuring 0.5 to 1 μm in diameter. It is the most virulent of the staphylococci species. It takes 20-30 minutes to generate and can develop in high-salt environments at temperatures ranging from 10 to 46 °C. *S. aureus* has a various set of toxins and virulence factors, making it a frequent cause of toxin-mediated disorders such as toxic shock syndrome, staphylococcal food borne infections, and scalded skin syndrome. *S. aureus* may also respond to challenges posed by the human immune system by using these virulence factors and toxins (Lakhundi and Zhang 2018).

Methicillin-resistant *S. aureus* (MRSA) are bacterial strains that have acquired the *mecA* gene, which allows them to withstand the effects of methicillin and numerous other β -lactam antibiotics. *MecA* gene, which is present in all MRSA strains and is known to encode penicillin binding protein2a (PBP2a, PBP2'), which has a low tropism to all β -lactam antibiotics, is thought to be the linchpin of the MRSA phenomenon, according to the majority of studies on the subject (Ma *et al.* 2020). MRSA classified into two sub-categories: Hospital acquired methicillin-resistance *S. aureus* (HA-MRSA) (patient acquires an infection 48 hours after being admitted to the hospital) and community associated methicillin-resistance *S. aureus* (CA-MRSA) (patient get infection from community). These are genetically varied and notable (Butler *et al.* 2019). CA-genetic MRSA's content seems to differ in several domains and regions when compared to HA-MRSA, allowing it to be recognized and monitored by genotyping (Lee *et al.* 2018). These divisions are linked to repeated staphylococcal cassette chromosome *mec* (SCC*mec*) insertions into methicillin-sensitive *S. aureus* (MSSA) lineages, and MSSA

acquired resistance is linked to the acquisition of the methicillin-resistance gene *mecA* (Doulgeraki *et al.* 2017). Because bacteria's capacity to attach and create biofilms is indicative of heightened virulence among isolates of MRSA, which causes acute and chronic illnesses. MRSA has become a growing public health issue as a result of recent outbreaks and reported fatalities, especially among people who have no recognized risk factors (MacIntyre and Bui 2017). Because of the bacteria's complicated processes of antibiotic resistance and epidemiology, which have become a significant source of systemic infection in the community and hospitals, MRSA causes mortality among people with no recognized risk factors, posing a therapeutic challenge for clinicians (Alani *et al.* 2017).

After methicillin was licensed for the treatment of penicillin-resistant staphylococci in humans in 1961, this resistant strain was first identified (Rahman *et al.* 2018). MRSA has shown the potential to spread across and inside hospitals, and is known as epidemic MRSA or methicillin-resistant *S. aureus* epidemic (EMRSA) (Boswihi and Udo 2018). Of the two billion persons worldwide who have *S. aureus*, 53 million (2.7 %) are MRSA carriers (Bentum 2019). It has recently been discovered that the number of MRSA infections in the community is increasing, and that these infections are not transmitted from hospital surroundings (Negm *et al.* 2019).

This research was meant to accomplish the following goals; Isolation and identification of MRSA from different clinical samples from Iraq-wasit/AL-Suwaira Hospital. Detection of resistance of local isolates to methicillin and other antibiotics, as well as measurement of the minimum inhibitory concentration of antibiotics utilizing the VITEC 2 system. Detection of the methicillin-resistant *mecA* gene using a polymerase chain reaction (PCR) technique among local isolates and the investigation of isolates matched by nucleotide sequences through the site National Center for Biotechnology information (NCBI).

2. LITERATURE REVIEW

2.1 General Characteristics of *S. aureus*

S. aureus is a non-motile Gram-positive bacteria that does not produce spores. It is the most significant member of the micrococcaceae family. They are cocci-shaped and tend to be grouped in clusters that are characterized as "grape-like" in clinical terms. They may split along three clearly oriented planes that are at right angles to each other. Staphyle means grape cluster in Greek, while kokkos means fruit. At temperatures between 18 and 40 °C, these bacteria may grow both aerobically and anaerobically (facultatively). Colonies of these organisms are often golden or yellow (aureus means golden or yellow), and they can survive in media with up to 10% salt (Taylor and Unakal 2021).

S. aureus is always changing because of mutations and the procurement of mobile genetic elements that provide increased resistance and virulence. The bacteria cause illness by creating a number of cell-surface and secreted virulence factors, and their ability to acquire antibiotic resistance contributes to the spread of illness (Cheung *et al.* 2021). In both community-acquired and nosocomial infections, *S. aureus* is the most frequently isolated bacteria. Ogston found *S. aureus* in purulent pus from a leg abscess in the 1880s, and Rosenbach isolated it shortly afterwards. *S. aureus* is well-adapted to its human host and the healthcare environment. This bacteria is well recognized as a major human pathogen, and it may result in significant mortality and morbidity in patients, as well as hospital-acquired infections. However, they are not necessarily harmful; they dwell as a normal component of the flora on human skin and mucous membranes as commensals (Turner *et al.* 2019).

Early in the 1940 s, penicillin was employed for the first time to treat staphylococci infections, but within a short period of time, *S. aureus* developed a resistance to it. Methicillin was then developed, and it first provided relief from the problem. But two years after methicillin's release, resistance developed (Harkins *et al.* 2017). MRSA was

responsible for 20–35% of *S. aureus* infections in hospitalized patients in 1990. Due to MRSA's drug resistance, it has become a clinical issue (Ersoy *et al.* 2019).

2.2 Virulence Factors

S. aureus has a virulence factor repertoire that improves its capacity to survive and cause infections in humans. This is due to its great capacity to express a variety of variables that have a role in the spread of these illnesses. Despite advancements in healthcare treatment, most nosocomial infections in hospitals are still caused by *S. aureus* (Buchan 2018). This bacteria may stick to objects and bodily tissues, evade or penetrate the immune system, and damage the host. These elements are referred to as virulence determinants. Cell-surface associated (adherence) and secreted (exotoxins) components may be distinguished (Jajere 2019). Three traits distinguish *S. aureus* from the majority of other clinically important bacteria. It may be found in both hospital and community settings, can express a broad variety of virulence factors, and can acquire and disseminate resistance to a large variety of antimicrobial treatment classes. These varied traits help define the host-microbe relationship and contribute to its pathogenicity (González-Martín *et al.* 2020). Two other notable characteristics of staphylococci are that a virulence factor may perform numerous tasks in pathogenesis and that many virulence factors can fulfill the same function (Pérez *et al.* 2020).

2.2.1 Cell surface factors

The polysaccharide capsule enhances phagocytosis resistance, the key host defensive mechanism against *S. aureus*. Furthermore, capsular polysaccharide may have a role in bacterial adherence to medical device polymer surfaces (Nasser *et al.* 2019). Staphyloxanthin and catalase are produced by *S. aureus*, and these antioxidants aid intracellular survival following phagocytosis (Painter *et al.* 2017).

2.2.2 Secreted Factors (Exotoxins)

One of *S. aureus* most notable characteristics is its capacity to release poisons, which are involved in disarming host immunity. In fact, they damage host cells and tissues and suppress the host immune system in order to liberate nutrients and promote bacterial dispersion (Zhou and Yang 2019). There are four different types of secreted factors: super-antigens, cytolytic (pore forming) toxins, different exoenzymes, and other proteins are all examples of super antigens (Ukpanah and Upla 2017).

Super antigens: Super antigens are a class of very potent immune-stimulatory proteins that harm host tissues and may cause a range of illnesses in humans, including toxic shock syndrome (Ukpanah and Upla 2017).

Cytolytic toxins: *S. aureus* produces a huge range of cytolytic toxins, all of which have a comparable impact on host cells despite their structural differences and target specificity. Panton-Valentine Leukocidin (PVL) is probably the most extensively studied toxin. This toxin is known to create necrotizing pneumoniae, a swiftly deadly infection (Gillet *et al.* 2018). In addition, it is a sign of CA-MRSA strains and is connected with severe infections (Teng *et al.* 2019).

Various exoenzymes: To acquire nutrients for bacterial development and promote bacterial spread, nearly all strains of *S. aureus* release a number of extracellular enzymes whose presumed goal is to damage host tissues and/or inactivate host antimicrobial defenses (e.g., lipids, defensins, antibodies, and complement mediators). These exoenzymes consist lipases, nucleases, proteases (serine, cysteine, aureolysin), hyaluronidase, and staphylo kinase (Reddy *et al.* 2017).

Miscellaneous proteins: Other particular proteins discovered in *S. aureus* may have a significant influence on the adaptive and innate immunity. Staphylococcal complement inhibitor is one of these proteins (Pietrocola *et al.* 2017). These proteins consist of staphylococcal complement inhibitor, extracellular fibrinogen-binding protein,

chemotaxis inhibitory protein of *S. aureus*, formyl peptide receptor-like inhibitory protein, and extracellular adhesion protein (de Vor *et al.* 2020).

2.3 Pathogenesis

A microorganism's capacity to cause illness is known as pathogenesis. This covers the microorganism's entrance, colonization, and growth within the host (Scudiero *et al.* 2020). The major form of *S. aureus* transmission is direct contact, often skin to skin contact with a colonized or ill person, as well as contact with contaminated items and surfaces, which may also play a role (Hiko 2019).

S. aureus is a well-known and widely spread bacterial pathogen that causes simple skin infections and hundreds of thousands to millions of serious invasive infections around the world every year (Dahlen *et al.* 2019). It is a common cause of pneumonia and other respiratory infections, as well as surgical site, prosthetic joint, and cardiovascular infections, as well as nosocomial bacteremia (Chalmers and Wylam 2020). *S. aureus* is the most prevalent source of infection and is responsible for a variety of illnesses, including impetigo, folliculitis, and carbuncles, which are often not life-threatening but may cause severe morbidity and pain (Karauzum *et al.* 2021).

S. aureus infections are troublesome owing to the prevalence of antibiotic resistance in *S. aureus* isolates, with MRSA being the most clinically significant strain (Dweba *et al.* 2018). Infections caused by MRSA are associated with greater mortality, morbidity, and length of hospital stay compared to infections caused by MSSA (Chatterjee *et al.* 2018).

In 2007, Tang and his coworkers discovered that all staphylococci species were recognized as pathogens in bloodstream infections. In addition to skin and soft tissue infections, post-operative wound infections, and eye infections cited by (Peker *et al.* 2018). Invasive illnesses, such as osteomyelitis, endocarditis, pneumonia, and toxin-mediated disorders such as food poisoning, toxic shock syndrome, and scalded skin syndrome (Fontana and Favaro 2018).

S. aureus infections are often acquired in community or hospital settings. In addition, only a percentage of bacteremia or local infections develop into sepsis. Old age, immunosuppression, chemotherapy, and invasive procedures are risk factors for sepsis one of the most prevalent causes of sepsis is *S. aureus* (Luzum *et al.* 2020). Despite the fact that *S. aureus* prefers the nose, it may also live for extended periods of time on the skin and in the environment (Rimawi 2020). Other mucosal surfaces that contain *S. aureus* include the throat, the vaginal wall, and the digestive tract. Nevertheless, nasal carriage is likely the most significant since nose-picking can successfully spread the bacterium to other body surfaces and hosts (Ajayi 2018). Thus, a fundamental biological trait of this bacteria is its capacity to asymptomatically colonize healthy persons who are considered to be high-risk infection carriers (Bencardino *et al.* 2021).

2.4 Methicillin Resistant *S. aureus*

Prior to the invention of antibiotics, invasive staphylococcal disease was typically deadly, but the 1940s discovery of penicillin markedly improved survival rates (Guo *et al.* 2020). Antimicrobials have recently helped to put many serious infectious illnesses under control, saving the lives of millions of people and easing their suffering. Additionally, they have contributed to the notable increases in life expectancy that have been seen in the later part of the 20th century (Martens and Demain 2017).

Antimicrobial resistance is becoming a more widespread concern globally, particularly among nosocomial infections (Meade *et al.* 2020). *S. aureus* is perhaps the most concerning pathogen because to its innate virulence, tendency to produce a broad variety of life-threatening infections, and adaptability to several environmental situations (Lamut *et al.* 2019). The widespread and sometimes improper use of antibiotics, their broad use as growth promoters in animal feed, and the increase in domestic and worldwide travel are only a few of the causes of the formation of more antimicrobial-resistant bacterial species (Musini *et al.* 2021).

Penicillin was used to treat infections caused by *S. aureus*. Resistance to penicillin soon emerged when strains gained a genetic element carried by plasmid coding for the

development of lactamase (penicillinase), an enzyme that cleaves the β -lactam ring of the penicillin molecule, leaving the antibiotic useless (Bush 2018, Bush and Bradford 2020).

The staphylococcal cassette chromosome *mec* contains the *mecA* gene, which mediates methicillin-resistance especially SCCmec, which encodes an altered PBP2a with a decreased affinity for β -lactams (cephalosporins, penicillins, and carbapenems) binding (Oniciuc 2017). This permits resistance to all β -lactam antibiotics and eliminates their clinical utility in infections caused by MRSA (Makinde *et al.* 2019).

Finally, systemic antimicrobials including ciprofloxacin, trimethoprim, and rifampin have been utilized to eradicate *S. aureus* colonization in different populations. Rifampin has probably been the most extensively researched medication for stopping the colonization of *S. aureus*. The target enzyme modification causes resistance to certain antimicrobials (Jamilatun 2019).

2.4.1 Methicillin resistance mechanism

Before the 1950s, benzylpenicillin, β -lactam antibiotic, was used to treat *S. aureus* infections can impede the production of bacterial cell walls (Van den Honert 2019). Peptidoglycan is the primary structural element of the cell wall, by the late 1950s, however, *S. aureus* strains resistant to benzylpenicillin became a major cause. Resistant bacteria usually developed an enzyme called β -lactamase, which inactivated antibiotics with a lactam ring (Edowik 2020). The synthesis of penicillin derivatives resistant to β -lactamase hydrolysis was attempted. This was accomplished in 1959 with the synthesis of methicillin, in which two methoxy groups were attached to the phenol group of benzylpenicillin (Zhan and Zhu 2018). The amide bond's attraction for staphylococcal lactamases was decreased by the methoxy groups' creation of steric hindrance around the amide bond (Mikłasińska-Majdanik *et al.* 2018).

MRSA strains were found as soon as methicillin was placed in clinical settings. Resistance was caused through the formation of an additional PBP2a rather than by the production of β -lactamases (Paterson 2020). The molecular foundation of methicillin resistance is the acquisition of a gene that is termed a homologue of PBP2 called PBP2a or PBP2' produces a drug-resistant (Harrison *et al.* 2019). Unlike resistance to penicillin, methicillin resistance in *S. aureus* is not mediated by plasmid-borne β -lactamases (Armin *et al.* 2017).

In 1985, Utsui and Yokota confirmed that an altered PBP confers methicillin and cephem resistance in *S. aureus*. This new PBP was referred to as PBP2' (Panchal 2018). The gene that gives *S. aureus* its methicillin resistant phenotype was later shown to be located on the chromosome, as opposed to the β -lactamase gene, which was discovered on a plasmid. This portion of the chromosome was identified as "foreign DNA," and it was found exclusively in resistant strains. Strains that were susceptible lacked this "foreign DNA" area (Schwendener *et al.* 2017).

SCCmec is a mobile genomic element that contains the methicillin-resistance determinant *mecA*. Methicillin resistance in MRSA is caused by a gene that is not natural to *S. aureus*. Instead, this gene came from a different species and was passed on to *S. aureus* in an unknown way. For decades, the evolutionary origins of *mecA* have largely remained a mystery. Transfer and transposon mutagenesis tests done in the Tomasz laboratory confirmed the involvement of *mecA* in giving methicillin resistance (Catteau 2018).

2.4.2 Penicillin-binding proteins

The rigid envelope that encloses a bacterial cell, known as the cell-wall, dictates the composition and integrity of the cell. Peptidoglycan (PG), a heteropolymeric structure composed of saccharide backbones that are cross-linked by peptide subunits, is the major component of this prokaryotic only protective coating (García-Fernández *et al.* 2017).

The composition and structure of PG are connected to cell-wall thickness and integrity, hence affecting antibiotic sensitivity and host immunity. The biosynthesis of PG is dependent on the coordinated activity of numerous bacterial enzymes. The PBPs, which facilitate the last stages of PG assembly by polymerizing and cross-linking PG chains, are of enormous importance. PBPs are a group of proteins that are essential for the last phases of bacterial cell wall construction, namely at the septa of dividing cells (Ferrer-González *et al.* 2017). All four natural PBPs of *S. aureus* are known to relocate to the septa of dividing cells, where their catalytic transpeptidase activity is essential for cross-linking peptidoglycan strands in the newly formed cell wall (Straume *et al.* 2020).

The classification of PBPs is based on their molecular weight and conserved amino acid patterns. Based on their molecular weight, PBPs are split into two major subclasses. Low molecular mass (LMM) and high molecular mass (HMM). HMM PBPs include several domains and participate in the polymerization and cross-linking of PG chains, processes called as transglycosylation (TG) and transpeptidation (TP), respectively (Miyachiro *et al.* 2019).

HMM PBPs are further categorized based on the number of processes that each enzyme is capable of catalyzing. Unlike class A PBPs, which catalyze both TG and TP processes, class B PBPs solely catalyze TP processes (Walkowiak 2017). LMM PBPs, also known as class C, catalyze a process known as d,d-carboxypeptidation, in which the stem pentapeptide is hydrolyzed into a tetrapeptide (Sharifzadeh *et al.* 2020).

β -lactam antibiotics block the TP domain of PBPs (and carboxypeptidase activity of LMM PBPs), obstructing the cross-linking process in a manner similar to the terminal D-alanyl-D-alanine bond of the stem peptide (Maya-Martinez *et al.* 2019). The cell wall becomes mechanically weak without PG cross-linking, some cytoplasmic contents leak out, and the cell dies (Glossop *et al.* 2018).

PBPs were identified and called for their propensity to bind to β -lactam antibiotics, such as penicillin, which are the most extensively utilized class of antibiotics in clinical settings owing to their high effectiveness and the availability of several variants. Since

the discovery of penicillin G in the 1920s, various derivatives of penicillin and other types of β -lactams, including cephalosporins, monobactams, and carbapenems, have been developed (Ribeiro da Cunha *et al.* 2019). Methicillin-resistance in clinical isolates has been attributed to the presence of a methicillin-hydrolyzing β -lactamase and an altered form of PBP2 (76 KDa) with a lower penicillin-binding affinity and a faster rate of drug release compared to the normal PBP2 (Yangomudou 2019, Fisher and Mobashery 2020)

The key mechanism of MRSA is the production of a foreign PBP, PBP2a, that is resistant to the action of is situated at the membrane cell wall interface, is important for cell wall biogenesis by facilitating peptidoglycan cross-linking (Woroszyło *et al.* 2021). Synthesis of PBP2a is generally controlled and maintained at a low level, however mutations in the regulatory genes may increase the amount of synthesis (Kulanthaivel and Mishra 2021). The PBP2a encoding *mecA* gene causes resistance to methicillin and all other β -lactam antibiotics. The *mecA* gene is located on the SCCmec, which is a movable genetic element (Milheiriço *et al.* 2017).

2.4.3 *MecA* gene and staphylococcal chromosome cassette

Staphylococci have a lot of genomic variation, and over 20% of their genome is made up of "dispensable regions" that may code for virulence factors or proteins that help bacteria resist antibiotics (Argemi *et al.* 2018). Resistance to methicillin is controlled by the *mecA* gene, which encodes the PBP with low affinity, PBP2A (Fergestad *et al.* 2020). Resistance levels in MRSA are dependent on efficient PBP2' synthesis and chromosomal factors (Renzoni *et al.* 2017). The transpeptidase domain of all natural PBPs are inactivated in the presence of β -lactam antibiotics, but *mecA*-containing bacteria continue to build cell wall owing to the cooperation between the transpeptidase domain of PBP2A and the transglycosylase domain of the native staphylococcal PBP2 (Shalab *et al.* 2020). *MecA* encodes a high molecular weight class B PBP named PBP2A with two domains: the C-terminal domain, which is known to have transpeptidation activity; and an N-terminal region, the so-called non-binding domain, for which no function has been given (Tavares 2019).

The *mecA* gene is found on the SCCmec, a structurally complicated mobile genetic element (Saber *et al.* 2017). SCCmec has the ability to transform vulnerable staphylococci into virulent multidrug-resistant bacteria that have evolved to flourish in infection situations, notably in hospitals. The widespread distribution of SCCmec in nosocomial and community staphylococcal populations demonstrates the benefit for bacteria to carry this element (Rolo *et al.* 2017). SCCmec has a size range of 21 to 67 kb and is integrated into *orfX* gene, near to the replication origin. The *mec* complex, which contains *mecA* as well as intact and deleted forms of its regulators (*mecI*, *mecR1*), and the cassette chromosome recombinases (CCR) complex, which is made up of CCR involved in mobility (Moreno Grúa 2021).

Non-essential parts of SCCmec include additional metal and genes for antibiotic resistance that are delivered by transposons and plasmids, as well as genes with uncertain functions known as J regions. The J3 region is sandwiched between *orfX* and the *mec* complex, the J2 region is sandwiched between the *mec* complex and the CCR complex, and the J1 region is sandwiched between the CCR complex and the element's right extremity (Miragaia 2018). Depending on the arrangement of the CCR genes and the *mec* complex, the SCCmec may be subdivided into seven forms ranging in size from 20.9 to 66.9 kb. The Roman numerals (I-VII) are also used to name the SCC mec kinds, which are based on the order in which they were characterized (Yang *et al.* 2016). SCCmec type II and III have multi-resistance, according to the investigations, because they carry more drug resistance genes on integrated plasmids (pT181, pI258, pUB110) in addition transposon Tn 554 (Kirmusaoğlu 2017).

The few β -lactam to which *mecA* does not confer resistance include ceftobiprole and ceftaroline which are active against MRSA and penicillin G, ampicillin and amoxicillin which are active against penicillinase-negative MRSA strains (a minority, nowadays). Because of the related problems in treatment and illness outcome, the spread of SCC mec among *S. aureus* strains poses a genuine publichealth issue world wide (Asiimwe *et al.* 2017).

2.4.4 Epidemiology of MRSA

The major human pathogen *S. aureus* has virulence factors and the capacity to gain resistance to the majority of drugs (Wang *et al.* 2018). The genetic composition of *S. aureus* has gradually changed over the last 50 years, leading to the emergence of antibiotic-resistant strains that have been a source of sickness in hospital settings since the 1960s (Papadopoulos *et al.* 2018).

Methicillin resistance has been one of the most challenging parts of treating *S. aureus* infections to date. Clinical usage of methicillin has led to the emergence of MRSA, a clinical indicator of total β -lactam antibiotic resistance (Dai *et al.* 2019). This potential is further enhanced by the continual development of new clones, therefore transforming *S. aureus* into a "superbug." (Akanbi *et al.* 2017). New MRSA clones have emerged during the last few decades. In contrast to conventional MRSA, which present in hospitals, the new clones are capable of invading community settings and infecting individuals without predisposing risk factors (Berla-Kerzhner *et al.* 2017).

MRSA infections are more likely to occur in children, the elderly, athletes, military personnel, people who inject drugs, people with indigenous ancestry or who live in urban, underserved areas, people with HIV or cystic fibrosis, people who frequently interact with healthcare professionals, and people who live in institutions like prisons. Between the 1990s and the early 2000s, MRSA infection rates rose rapidly (Shrestha *et al.* 2021). Therefore, MRSA infections can be divided into two categories: those contracted by people currently in or recently discharged from a healthcare facility called HA-MRSA, and those contracted by otherwise healthy people in the community called CA-MRSA (Peng *et al.* 2018). HA-MRSA infections are infections acquired while getting medical treatment in a healthcare institution that were not present before admission. In 2011, the World Health Organization indicated that 7-15 percent of the population in industrialized and poor nations suffer with HA-MRSA, with a death rate of over 10% (Shahid *et al.* 2018). A patient's flora starts to take on the features of the surrounding bacterial pool few hours after admission. Hospital-acquired infections are those that develop clinically after 48 hours of admission. An infection that develops

after a patient leaves the hospital may be termed healthcare-associated if the patient contracted the infection while in the hospital (Tirimba 2018). Within the first 24 hours of admission to the hospital, MRSA bacteremia was caused by HA-MRSA "strains, not community-acquired strains (Jadhav *et al.* 2021). In addition to their susceptibility to various antibiotics, the community isolates can encode virulence factors like "PVL, which has been connected to necrotizing pneumonia, skin, and soft tissue infections, because they carry a novel methicillin-resistance cassette gene element not seen in hospital-acquired strains (Ferreira *et al.* 2021).

Since the 1990s, CA-MRSA colonization and infection have become more common in community settings across the globe (Gopal and Divya 2017). CA-MRSA colonizes people regardless of the risk factors listed above, and they cause a rising number of basic to severe skin and soft tissue infections, as well as dangerous invasive infections such necrotizing pneumonia and fasciitis (Sartelli *et al.* 2018). CA-MRSA strains infect a diverse group of patients, have different clinical signs, antibiotic sensitivity patterns, spread swiftly in the community, and cause infections in health care settings. The *mecA* gene, which encodes methicillin and other betalactam antibiotic resistance, is found on the SCCmec type IV that is shared by the vast majority of CA-MRSA strains (Fri *et al.* 2020). Additionally, MRSA was the predominant cause of community-associated *S. aureus* infections in hospitalized patients, and there was no clinical profile that could definitively rule out MRSA (Strommenger *et al.* 2018, Golan 2019).

2.4.5 Treatment of MRSA

In the last ten years, MRSA strains have become serious pathogens. These strains are frequently multiresistant to numerous antibiotic classes and are a leading source of significant hospital and now community-acquired infections, as well as the morbidity and mortality that accompany them. As a result of the rise in antimicrobial resistance, glycopeptides such as vancomycin are frequently employed as the first-line treatment for severe MRSA infections (Ortwine and Bhavan 2018).

Ceftobiprole's pharmacology, antimicrobial activity, pharmacokinetics, pharmacodynamics, clinical efficacy, and safety indicate that it is a novel, broad-spectrum, parenteral cephalosporin that inhibits the cell-wall synthesis of PBPs PBP2a and PBP2x, which are responsible for the resistance in staphylococci and pneumococci. In addition, it has been shown that the new cephalosporin ceftaroline is a potent treatment substitute for severe MRSA infections, including osteomyelitis (Falcó *et al.* 2018). In the recently issued guidelines on the treatment and prevention of MRSA by the British Society for Antimicrobial Chemotherapy, glycopeptides and linezolid are suggested as first-line therapy for severe MRSA infections (Horner *et al.* 2017). Quinupristin/dalfopristin has also been used successfully, however its utility is restricted by resistance rates, intolerance issues, and drug interaction profiles (Mercuro *et al.* 2018). The most often given antibiotic for *S. aureus* infections in US children's hospitals is now clindamycin, and this fact highlights the need of continued monitoring of regional *S. aureus* susceptibility developments (Medernach and Logan 2018). Linezolid and vancomycin were compared against MRSA, it was discovered that linezolid medication was just as effective as vancomycin in terms of in hospital survival and readmission for patients who had MRSA (Rupali *et al.* 2019). In a different research, the effectiveness of moxifloxacin versus vancomycin against MRSA was examined, and moxifloxacin was shown to have superior anti-biofilm action (Masadeh *et al.* 2019). On the other hand, the lipopeptide doptomycin has been effective in treating bacteremia, endocarditis, and MRSA infections in wounds that developed after heart surgery (Ye *et al.* 2019). The linezolid, a novel oxazolidinone, has shown encouraging outcomes in the treatment of nosocomial pneumonia and MRSA-caused complicated skin and soft tissue infections (Múnera *et al.* 2017). Vancomycin is often combined with rifampin or gentamicin for severe MRSA infections. Published findings from trials testing this and other vancomycin-based combinations, both in vitro and in animal infection models, are often conflicting (Cyphert *et al.* 2019). However, individuals with rifampicin-resistant *S. aureus* were shown to benefit from a combination of vancomycin and rifampicin (Rieg *et al.* 2017). In addition, vancomycin and teicoplanin are often used to treat staphylococci infections, particularly MRSA infections (Kulkarni *et al.* 2019). In Australia, the standard treatment regimen after glycopeptide therapy is

rifampicin+fusidic acid, however many individuals are intolerant (David and Daum 2017).

To reduce the immense burden produced by pathogenic staphylococci, new techniques to effectively battle staphylococcal infections have recently been urgently sought (Zheng *et al.* 2018). Among the successful nonpharmacologic treatments for MRSA, biofilm breakdown and eradication utilizing two distinct laser approaches are included (Han *et al.* 2018).

2.4.6 Preventative actions

Despite the amount of information, health care institutions have undertaken expanded monitoring and infection control methods to combat the endemicity and frequent illness out breaks caused by MRSA in hospitals and health care facilities. This bacterium is still responsible for around 50% of hospital-acquired *S. aureus* infections globally, with rising morbidity and death (Tang *et al.* 2017). It has been shown that suggestions for infection control that integrate proactive patient care with active monitoring, such as patient isolation, decontamination, and other measures, limit transmission and subsequent infections (Fernando *et al.* 2017).

MRSA may be removed from hospitals by enforcing high cleanliness standards and maintaining a controlled atmosphere. In the Netherlands and Denmark, for example, 'search and destroy' procedures were used to reduce the spread of both HA and CA-MRSA in healthcare settings. Following that, all MRSA-positive people are separated and, if feasible, treated to get rid of the bacteria (Crespo-Piazuelo and Lawlor 2021). Similar preventative strategies have been used in the United States, such as the reduceMRSA experiment, which found a 37% decrease in MRSA carriage when all patients in the same intensive care units were decolonized using intranasal mupirocin and washing with chlorhexidine-impregnated towels (Bozzella *et al.* 2019).

In the past few decades, the incidence of HA-MRSA bacteraemia has decreased as a result of measures for the prevention and control of MRSA infections and the stoppage of its transmission in United States hospital settings. However, in the United States, since 2012, the pace of fall in HA-MRSA infections has slowed, while the prevalence of CA-MRSA infections has risen somewhat. This implies that additional preventative actions are required (Udo and Boswihi 2017). It has also been stated that the speed with which MRSA carriage is diagnosed has a vital influence, since it is a critical component of any efficient MRSA prevention approach. Quick detection procedures (often referred to as "MRSA rapid tests") employing PCR technologies and, more recently, rapid culture methods have been developed since MRSA culturing has a 2-3 day wait before the final findings are available (Morris *et al.* 2017). The use of fast testing decreases the time it takes to find MRSA carriers from 48 to 72 hours to 2 to 5 hours. MRSA may therefore be recognized with a high degree of sensitivity, according to clinical assessment data. False-positive PCR findings in mixed flora specimens, on the other hand, might reduce specificity. A culture screen must always be performed concurrently to rule out any false-positive PCR findings (Stürenburg 2009, Tacconelli *et al.* 2009).

The findings suggest that a PCR test may help minimize nosocomial MRSA transmission in high-risk patients or localities. Preventive measures should cover community-acquired infections. Clinicians working in outpatient or emergency departments may consider making practice changes in locations where CA-MRSA infections are known to be widespread to reduce clinical consequences (Yadegarynia 2021). More frequent culture and susceptibility testing of *S. aureus* isolates from clinical infections, especially in pediatric patients, might be part of these adjustments. Infections may be surgically drained if necessary. When treating a suspected staphylococcal infection, cautious selection of empirical antimicrobials is necessary (Naimi *et al.* 2003).

3. MATERIALS AND METHODS

Materials

3.1.1 Instruments and equipments

The instruments and equipments which used throughout this study are listed in Table 3.1.

Table 3.1 Instruments and equipments

INSTRUMENT	COMPANY/ORIGIN
Autoclave	Memmert (Germany)
Bacteriological loop	Himedia (India)
Centrifuge	Fisher Scientific (USA)
Cool box	-
Compound microscope	Olympus (Japan)
Electric oven	Memmert (Germany)
Microwave oven	GOSONIC (China)
Electric-sensitive balance	Mettler (Switzerland)
Electrophoresis supply and miditank	Major science
Flasks	-
Glass cylinders Schott	Duran (Germany)
Incubator	Memmert (Germany)
Micro spin centrifuge	My Fugene (China)
Micropipettes	Brand (Germany)
Plastic petri dishes	-
Plastic test tubes	Afco (Jordan)
1.5 mL, 0.5 mL and 0.2 mL tube	JET BIOFIL (Singapore)
Gel imaging system	MajorScience (Taiwan)
OWL Electrophoresis System	Thermo (USA)
Thermal Cycler	Thermo Fisher Scientific (USA)
Quantus fluorometer	Promega (USA)
Vortex	Quality Lab System (England)
Refrigerator	TEKA (Spain)
Screw-capped bottles (20 mL)	-
Sensitive balance	Sartorius (Germany)
Slides	-
Sterile bacteriological swab	Citoswab (China)
Sterile bacteriological swabs with transport media	Ataco (China)
Tips (100 µL)	Himeco (China)
Water bath	(China)
Water distillator	Gallen Kamp (England)
VITEC2 compact	BioMerieux (France)

3.1.2 Culture media

The culture mediums used in this research are detailed in Table 3.2.

Table 3.2 The culture medias

MEDIUM	MANUFACTURED COMPANY (COUNTRY OF ORIGIN)
Blood agar	Himedia (India)
Nutrient broth	
Mannitol-Salt agar	
Mueller-Hinton agar	Oxoid (England)
Brain-Heart infusion broth	
Nutrient agar	

3.1.3 Chemicals and antibiotic agents

Solutions, chemicals were used in the study are listed in the Table 3.3.

Table 3.3 Solutions, chemicals and their origins

AGENTS	AGENTS MANUFACTURED COMPANY
Agarose 2.5%	Sigma (USA)
Catalase reagent (3% hydrogen peroxide)	Fluka (Switzerland)
Ethanol 70%	ROMIL pure chemistry (UK)
Ethidium bromide solution 10 mg/mL	Sigma (USA)
Glycerol	BDH (England)
Lysostaphin solution	-----
Standard turbidity solution (McFarland tubes No. 0.5)	Biomérieux (France)
1x TBA buffer (0.44 M Tris Borate, 0.0125 M EDTA)	-----

Antibiotic discs that were used in this research, as shown in Table 3.4.

Table 3.4 Antibiotic discs and their origins

N O	ANTIBIOTIC	CODE	CONCENTRATION (MG)	MANUFACTURED COMPANY (COUNTRY OF ORIGIN)
1.	Methicillin	MET	5	Oxoid / UK
2.	Ciprofloxacin	CIP	5	
3.	Clindamycin	CD	2	
4.	Penicillin	P	10	
5.	Vancomycin	VA	30	
6.	Cefoxitin	FOX	30	
7.	Gentamycin	GN	10	
8.	Amikacin	AK	30	
9.	Novobiocin	NO	5	
10.	Cefotaxim	CTX	30	

The diagnostic kits used in this research listed in Table 3.5.

Table 3.5 Diagnostic kits and their origins

KITS	MANUFACTURED COMPANY
VITEC 2 AST-P580 REF 22233	BioMerieux, France
Presto™ Mini gDNA Kit	Geneaid, Taiwan
(10 mg/mL), Go Tag Green Master Mix, Nuclease Free Water, TAE 40 X, Quantifluor DNA System	Pro.mega, USA

All PCR primers were manufactured by (Macrogen, Korea), listed in Table 3.6.

Table 3.6 Primers used throughout the current research

PRIMER	SEQUENCES	ANNEALING TEMP. (°C)	PRODUCT SIZE (BP)
<i>mecA</i> -F	5'-TCCAGATTACAACCTCACCAGG-3'	50	162 bp
<i>mecA</i> -R	5'-CCACTTCATATCTTGTAACG-3'		
<i>16SrDNA</i> -F	5'-TATAAGACTGGGATAACTTCGGG-3'		200bp
<i>16SrDNA</i> -R	5'-CTTTGAGTTTCAACCTTGCGGTTCG-3'		

Methods

3.2.1 Patients and sample collection

The present study was carried out in Iraq-Wasit/AL-Suwira Hospital. Samples were collected from patients placed in hospital wards and incoming for the period from 1st December 2021 to 2nd April 2022. 100 samples were collected from patients residing in the hospital and incoming, whose ages ranged from 15-75, where they were collected from eyes, ears, urine, and wounds.

3.2.2 Sterilization

Glassware and other metal tools were sterilized by hot air oven at 180 °C for 2 hours. The solutions and culture media were sterilized by autoclave at 121°C at 15 lb/inch² for 15 minutes from when the temperature reached 121°C (Bidya and Suman 2014).

3.2.3 Preparation of culture media

All culture media were prepared according to the instructions shown on the packages by the manufacturer and then placed in the autoclave for sterilization at a temperature of 121 °C at 15 lb/inch² for 15 minutes and left to cool until a temperature of 45-50 °C.

3.2.4 Preparation of buffer and solutions

3.2.4.1 Standart turbidity solution

A: 1.174 grams of BaCl₂-H₂O was dissolved in 1L of distilled water to have 0.048 M solution. B: 1% of H₂SO₄ was prepared to get 0.36 M. C: 0.5 mL of solution A was mixed with 99.5 mL of solution B. The mixture was kept in closed bottles and kept in dark place. The optimal density for the turbidity standard was determined using a spectrophotometer with a 1cm light bath and a matching cuvette to measure absorbance.

For the 0.5 MacFarland standard, the absorbance at 620 nm should be between 0.80 and 0.10 (Guzmán *et al.* 2018).

3.2.4.2 Ethidium bromide solution (10 ng/mL)

100 mg of ethidium bromide (BDH-England) was dissolved in 10 mL of distilled water and stored at 4°C in dark bottles.

3.2.4.3 Preparation of catalase

To make this reagent, 3 mL of H₂O₂ at a 30 % concentration were dissolved in 97 mL of pure water, so to obtain a solution of concentration of 3% of H₂O₂ solution was used to detect the bacterial isolates producing the enzyme catalase, keeping it in dark bottle until use (Goud *et al.* 2021).

3.2.5 Culture techniques and characteristics

Cotton swab was used to transport the samples. The samples were inoculated on Blood Agar medium and incubated at 37 °C for a period ranging from 18 to 24 hours. The initial diagnosis of the isolated bacteria was made based on the colony's shape, color, size and the type of decomposition it caused on the Blood Agar plates. The pure isolates were kept in Nutrient Broth at a temperature of 4°C in order to perform phenotypic and biochemical tests (Almutawif *et al.* 2019).

5-10 µl were sub-cultured on Blood Agar and Mannitol-Salt Agar medium for traditional identification. At 37°C, the plates were incubated for 24 hours. A portion of a typical colony was taken and placed on a clean glass slide with a drop of physiological solution, mixed well, and allowed to dry at room temperature. It was fixed with flame, then dyed with gram stain, and examined under the compound light microscope using an oil lens, followed by further confirmatory tests (Farrell *et al.* 2019). The slide method was used for the detection of catalase enzyme activity. A small amount of pure growth

was transferred by a plastic loop onto a clean slide. Drops of 3% H₂O₂ were placed onto the colony on the slide and rubbed. The appearance of gas bubbles indicates a positive result.

3.2.6 Antimicrobial sensitivity test

The sensitivity to antibiotics was tested on the isolates under study using the Kirby-Bauer diffusion method. The disk diffusion technique (also known as the Kirby-Bauer method) is used by the majority of clinical microbiological labs in our country to assess the susceptibility of bacteria to antibiotics and chemotherapeutic drugs. It has the benefits of being simple, inexpensive, and replicable.

The approach used by the National Committee for Clinical Laboratory Standard (NCCLS), formerly known as Clinical and Laboratory Standard Institutes (CLSI), followed the instructions provided by (Bauer 1966). Ten types of antibiotic tablets were used on Muller Hinton medium.

Four to five pure isolates were taken by sterile wire ring to prepare the bacterial suspension. From Blood Agar medium were picked up and introduced into a test tube containing 5 mL distilled water and mix it well. Its turbidity was compared to that of MacFarland 0.5 barium sulfate standard.

A sterile swab was placed into the bacterial solution, and surplus fluid was removed by spinning the swab against the tube's interior above the fluid level. To achieve a uniform dispersion of inoculums, the surface of the Mueller-Hinton plate was streaked in three distinct plane using a cotton swab. With a sterile forceps, the selected disks were placed on the inoculated plates and pressed firmly to ensure complete contact with the agar. Disks were distributed evenly so that no closer than 24 mm from center to center then the plates were inverted and incubated at 37°C for 18-24 hours.

Utilizing reflected light and a ruler, the diameters of the whole zone of inhibition were observed and measured after incubation. The region displaying no discernible growth was chosen as the end point, which was measured to the closest mm. By using an interpretive CLSI/NCCLS chart, the width of the inhibition zone for each antimicrobial agent was converted into sensitive and resistant groups.

3.2.7 Identification and antibiotic susceptibility test

VITEC 2 is an automated microbiology system using technologies based on microbial growth. VITEC 2 compact which represents an advanced colorimetric technology for bacterial identification and antibiotic susceptibility. The kit contains Gram-positive card (ID-GPB) used for identification of Gram-positive bacteria. Gram-positive susceptibility card: it contains 20 microwells, contained benzylpenicillin, oxacillin, gentamicin, methicillin, tobramycin, clindamycin, amikacin, erythromycin, linezolid, levofloxacin, cefotaxime, nitrofurantoin, tigecycline, rifampicin, teicoplanin, tetracycline, fusidic acid, trimethoprim-sulfamethoxazole and vancomycin. All the following steps were done according to the manufacture's instruction. 3 mL of normal saline was placed in plan tube and inoculated with loopful of isolated colony. The test tube was standardization of colony with McFarland solution to obtain cell density 1.5×10^8 cfu/mL. The standard inoculum was inserted in the cassette, and the sample identification number was input via barcode into the computer program. The barcode on the VITEC 2 card was scanned by a scanner to identify the antibiotic susceptibility test.

3.2.8 Molecular techniques

3.2.8.1 DNA extraction

Following the Gene-aid extraction technique, genomic DNA was extracted from bacterial growth as follows:

Suspension and protein digestion: 1 mL of the overnight culture was centrifuged for 3 minutes at 12000 rpm to isolate pellet cells. The supernatant was discarded thereafter. 100 µl of nuclease-free water and 100 µl of lysozyme solution were added to the pellet and then vortex. 20 µl of proteinase K were added to the solution for protein digestion. The whole mixture was incubated for at least 10 minutes at 60 °C.

Cell lysis: 200 µl of GB Buffer was added to the sample and vortexed for 10 seconds for cell lysis. To ensure that the sample lysate is clear, the mixture was incubated at 70 °C for 10 minutes. Following incubation, samples were centrifuged for 10 seconds to eliminate air bubbles.

Binding: To bind the DNA, 200 µl of absolute ethanol was placed in the sample lysate, and the mixture was mixed immediately by vigorous shaking. Next, the mixture, (including any solids that would not dissolve), was put into the GD column and spun at 12000 rpm for a minute. The GD collection tube with the flow-through was discarded, and a fresh GD collection tube was inserted with the GD column.

Washing: The GD column was washed with 400 µl of W1 Buffer, and the mixture was centrifuged at 12000 rpm for 30 seconds before discarding the flow-through. The GD column put back in the 2 mL collection tube, and 600 µl of wash buffer (ethanol was supplied) was added to the GD column. 3 minutes of 12000 rpm centrifugation. The flow-through was then discarded and the GD column was reinserted into the 2 mL collection tube. The column matrix was dried by centrifuging it at 12000 rpm for 3 minutes.

Elution: A fresh 1.5 mL micro centrifuge tube was used to house the dried GD column. In the center of the column matrix, 100 µl of pre-warmed elution buffer¹ was poured. After at least 3 minutes (to allow elution buffer to be completely absorbed). To get the purified DNA out, the tube was spun at 9000 rpm for 3 minutes.

3.2.8.2 Quantitation of DNA

The concentration of extracted DNA was measured using a Quentus fluorameter to determine the sample quality for further uses. 199 μl of diluted Quantifluor dye were combined with 1 μl of DNA. DNA concentration readings were found during a 5 minute incubation period at room temperature.

3.2.8.3 Primer preparation

Macrogen company supplied these primers in a lyophilized form. As a stock solution, primers were dissolved in nuclease-free water to give final concentration of 100 pmol/ μl . The working solution of these primers was made by combining 10 μl of primer stock solution (stored at $-20\text{ }^{\circ}\text{C}$) with 90 μl of nuclease-free water to yield a 10 pmol/ μl working primer solution.

3.2.8.4 Amplification reaction

The PCR technique was used for detection 16S rDNA and *mecA* gene to confirm the identification of 20 selected species. PCR master was mixed and primers solutions were stirred by vortex, at room temperature. These solutions were added as described in Table 3.7, the amplification program is shown in Table 3.8. The components of tubes were mixed by vortex and then placed in PCR apparatus.

Table 3.7 The component of PCR reaction

MASTER MIX COMPONENTS	STOCK	UNIT	FINAL	UNIT	VOLUME
Master Mix	2	X	1	X	10
Forward primer	10	μM	1	μM	1
Reverse primer	10	μM	1	μM	1
Nuclease Free					6
DNA	10	ng/ μL	10	ng/ μL	2
Total volume					20
Aliquot per single rxn	18 μl of master mix per tube and add 2 μL of template				

Table 3.8 PCR program

STEPS	°C	M:S	CYCLE
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	50	00:30	
Extension	72	01:00	
Final extension	72	07:00	1
Hold	10	10:00	

3.2.8.5 Agarose gel electrophoresis

Agarose gel electrophoresis was used to verify the existence of amplification after PCR amplification. PCR was entirely reliant on the extracted DNA criteria.

3.2.8.6 Preparing agarose

Agarose was prepared. A flask containing 100 mL of 1XTAE was used. Agarose 1.5 g (for 1.5 %) was added to the buffer. Using a microwave, the solution was heated to boiling in order to dissolve all of the gel particles. To the agarose, 1 µl of ethidium bromide (10 mg/mL) was added. To combine the agarose and prevent bubbles, it was swirled. At 50-60 °C, the solution was allowed to cool.

3.2.8.7 Pour the agarose gel into horizontal mold

The edges of the gel box were sealed with reinforced adhesive tape, the agarose solution was poured inside, and the agarose was allowed to firm for 30 minutes at room temperature. The gel was put in the gel tray after the comb had been gently removed.

The pits are filled with 1XTAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel.

3.2.8.8 DNA loading

Each 5 µl DNA sample was loaded with 2 µl of loading dye, and samples were carefully added to the individual wells. PCR products were immediately loaded. 5 µl of PCR product was put straight into each well. 100 v/mA of electricity was switched on for 60 minutes. Using the gel imaging equipment, the ethidium bromide-stained bands in gel were seen (Mishra *et al.* 2010).

3.2.8.9 Sequence analysis of 16s rDNA and *mecA* gene

The PCR product from the amplification of 16s rDNA and *mecA* gene for both forward and reverse strand of 10 species were sent for Sanger sequencing using ABI3730 XL automated DNA sequence, by Macrogen. The results were analyzed and edit by use genious software. The edited sequence was compared with database by use BLAST (Basic Local Alignment Search Tool) to detect the close relation with submitted sequences.

4. RESULTS AND DISCUSSIONS

4.1 Collection and Identification of Isolates

There is no doubt that the importance of the current research stems from a number of factors, the most important of which is the increasing prevalence of MRSA around the world, as well as its tendency to cause hospital and community outbreaks. To achieve this goal, 100 samples were collected from different sites which initially were diagnosed in hospital. Specimens obtained from inpatients were classified according to the site of anatomical origin as well as by species. Samples were taken from wound (25), ears (25), urine (25), and eyes (25). Swabs were cultured primarily on Blood Agar. Of the 100 samples, only 37 (37%) were positive, while 44 (44%) samples showed different types of bacteria like *Staphylococcus epidermidis*, *Pseudomonas* spp. and *Escherichia coli*. 19 samples (19%) did not show any bacterial growth, which may be attributed to the administration of antibiotics. Another cause may be infection with another causative germ, which may need special technology. Among the 37 samples, the highest level of infection in wound patients was 14 (56%), eyes 10 (40%), ears 7 (28%), and the lowest level in urine was 6 (24%). The bacterial isolates preliminary were cultured on Blood Agar, as shown in Table 4.1.

Isolates of the genus *Staphylococcus* were initially diagnosed based on their phenotypic characteristics when grown on Blood Agar in aerobic conditions at 37 °C for 24 hours. Medium to large *Staphylococcus* colonies appeared, ranging in size from 1-3 mm, with regular edges, smooth, convex, glossy, and opaque, of a buttery texture (Figure 4.1), and many of them were found to show beta hemolytic activity on Blood Agar due to the production of hemolysin by isolates, forming a clear zone surrounding the colonies. After that the suspected cultures with growth of *Staphylococcus* species was affirmed by making biochemical tests catalase to differentiate between *Staphylococcus* and *Streptococcus*. Where *Staphylococcus* species are positive for catalase and negative for oxidase. All isolates of *S. aureus* produce catalase enzyme, which catalyzes the breakdown of hydrogen peroxide to water and oxygen. The positive result appeared in the form of gas bubbles (Figure 4.3) (Azizan et al. 2018). Then, to differentiate between

different species of Staphylococcus species, Mannitol Salt Agar was performed. All the isolates were cultured on Mannitol Salt Agar and after incubation at 37°C for 24 hours. All those 37 isolates appeared as golden colonies in color surrounded by a wide yellow zones and turned the medium color from purple to yellow due to mannitol fermentation (Figure 4.2), which is a unique feature of *S. aureus* (Duza 2021). This result is the same result obtained from (Garoy et al. 2019). MRSA is a globally recognized public health concern. There is non statistically significant effect ($P < 0.05$) of the type of surgical location on the infection rate of *S. aureus*.

Table 4.1 *S. aureus* according to the source of samples

SOURCE OF SAMPLE	TOTAL NO	POSITIVE SAMPLES	%
Wound	25	14	56
Ear	25	6	24
Eye	25	10	40
Urine	25	7	28
Total	100	37	37
X ²	6.65		
P value	0.084		

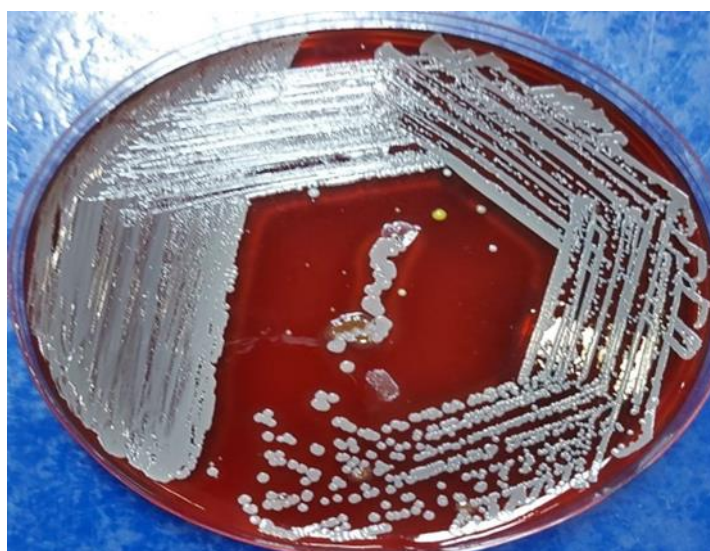


Figure 4.1 *S. aureus* colonies on Blood Agar and the appearance of clear areas indicate hemolysis due to the production of hemolysin by the isolates

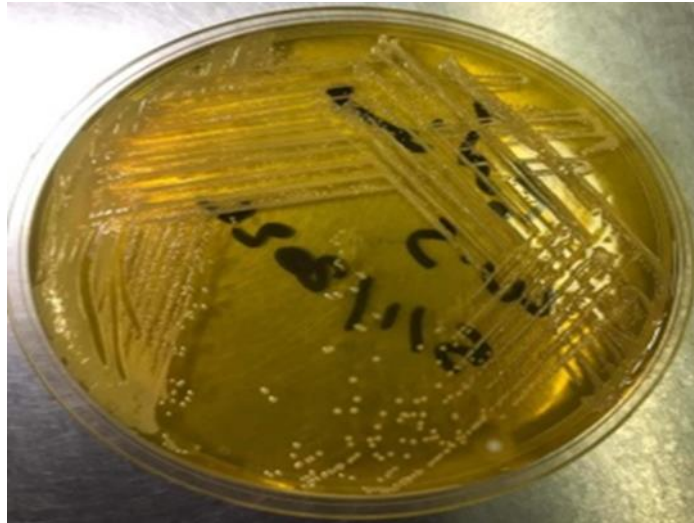


Figure 4.2 *S. aureus* appears in golden color on Mannitol Agar



Figure 4.3 Catalase test

4.2 Microscopic Characterization

Microscopic examination of the slices stained with gram stain showed, the plates after they were incubated for 24 hours at 37°C. The shape of the cells, their regularity, the manner of their assembly, and their interaction with the gram stained cells were observed. In colonies suspected to be *S. aureus*, colonies appeared yellow in color, shiny, convex with a diameter of about 2-3 mm, with spherical cells that were clustered (grape-like) and positive for gram staining (Figure 4.4). This corresponds to the characteristics of the genus *S. aureus* (Van Nassau 2018).

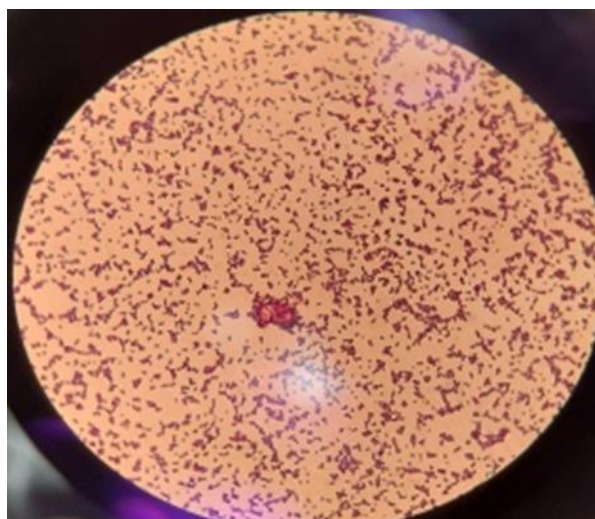


Figure 4.4 *S. aureus* under a light microscope

4.3 Antibiotic Susceptibility

In accordance with CLSI standards, a standardized Kirby-Bauer disk diffusion technique using the Muller-Hinton Agar plate technique was carried out (Teklu *et al.* 2019). For inoculation, a bacterial suspension corresponding to the 0.5 McFarland turbidity standard was produced. Antimicrobial disks have been selected from several drug classes. Methicillin (5g), cefoxitin (30g), cefotaxime (30g), penicillin (10g), vancomycin (30g), amikacin (30g), gentamycin (10g), clindamycin (2g), ciprofloxacin (5g), and novobiocin (5g).

At 37°C the plates were incubated for 18-24 hours in Mueller Hinton Agar. Then the area of inhibition for each antagonist was measured and interpreted as sensitive (S), intermediate (I), and resistant (R). These features were described in Figure 4.5. All the antibiotics used in this study were UK products. Inhibition zones were interpreted according to CLSI 2021 guidelines.

S. aureus isolates showed disparate level of resistance almost for all β -lactam antibiotic classes under study as showing in Table 4.2, which included; most *Staphylococcus* isolates were resistance to methicillin 35 (94.59%), cefoxitin 35 (94.59%), penicillin 37

(100%). cefotaxime 26 (70.27%), gentamycin 5 (13.51%), clindamycin 4 (10.81%), ciprofloxacin 4 (10.81%), novobiocin 0 (0%).

An exception for *Staphylococcus* isolates which being susceptible to vancomycin 0 (0%), amikacin 1 (2.7%). *S. aureus* showed resistant to cefoxitin and methicillin (approximately 95%), these results is the same results obtained by recent study done by (Parvin *et al.* 2021). In comparison to other antibiotics, vancomycin significantly showed $p < 0.05$ higher level of inhibition of *S. aureus*. This result is the same result obtained by researcher (Khanal *et al.* 2018).

Vancomycin is an antibacterial cationic glycopeptide (Salwan and Sharma 2020). Vancomycin kills bacteria by forming a persistent, non-covalent compound with the C-terminal D-alanyl-D-alanine residues of the peptidoglycan precursor lipid II in the cytoplasmic membrane. This prevents the precursor from being used for cell wall construction. The steric hindrance transported by vancomycin may further inhibit the glycosyltransferase and transpeptidase activities of PBPs. Vancomycin is used clinically to treat infections caused by *S. aureus*, *Streptococcus pneumoniae*, *Clostridium difficile*, *Enterococcus spp.* etc. Since the 1980s, the rising prevalence of MRSA and other Gram-positive bacterial infections in hospitals has led to an increase in the global usage of vancomycin. Since the 1980s, vancomycin has been one of the only medicines to which the majority of MRSA and other multidrug-resistant Gram-positive bacteria were still dependably sensitive (Hu *et al.* 2016).

Table 4.2 Sensitivity rate of *S. aureus* isolates different antimicrobials

TYPE OF ANTIBIOTIC	RESISTANT	INTERMEDIATE	SENSITIVE
Met (Methicillin)	35 (94.59)	0 (0)	2 (5.40)
CTX (Cefotaxim)	26 (70.27)	7 (18.91)	4 (10.81)
VA (Vancomycin)	0 (0)	0 (0)	37 (100)
AK (Amikacin)	1 (2.7)	1 (2.7)	35 (94.59)
GN (Gentamycin)	5 (13.51)	0 (0)	32 (86.48)
CD (Clindamycin)	4 (10.81)	2 (5.40)	31 (83.78)
CIP	4 (10.81)	1 (2.7)	32 (86.48)
NO (Novobiocin)	0 (0)	0 (0)	37 (100)
P (Pencillin)	37 (100)	0 (0)	0 (0)
FOX (Cifoxitin)	35 (94.59)	0 (0)	2 (5.40)
X ²	319.7		
P value	0 (Significant difference at P<0.05)		



Figure 4.5 Antibiotic susceptibility test of *S. aureus*

4.4 Antibiotic Susceptibility Test of *S. aureus* Using VITEC 2 System

Furthermore, to confirm the antibiotic sensitivity pattern, automated susceptibility testing was performed with the Gram-positive susceptibility card (AST P580, REF 22233) using the VITEC 2 compact system according to the manufacturer's

recommendation. The interpretation for oxacillin followed the breakpoint recommendation (resistant, ≥ 4 $\mu\text{g/mL}$; susceptible, ≤ 0.25 $\mu\text{g/mL}$).

20 isolates of *S. aureus* that obtained from different sources revealed a various resistance level towards 20 antimicrobial agents by VITEC 2 compact systems. *S. aureus* isolates demonstrated varying degrees of antibiotic resistance, as follows: benzylpenicillin 20/20 (100%), methicillin 20/20 (100%), oxacillin 20/20 (95.8%), gentamicin 6/20 (30%), clindamycin 6/20 (30%), amikacin 4(20%) while all isolates were sensitive to vancomycin, as showing in Table 4.3.

Table 4.3 Antibiotic susceptibility test of *S. aureus* using VITEC 2

ANTIMICROBIAL	RESISTANCE	%
Methicillin	20	100
Amikacin	4	20
Clindamycin	6	30
Gentamycin	6	30
Oxacillin	20	100
Cefotaxime	20	100
Vancomycin	0.0	0.0

4.5 Effect of Gender on the Infection Rate with *S. aureus*

The data regarding infection rate with positive samples with staph in both sex are presented in the Table 4.4. *S. aureus* isolation rate from the 100 samples was found to be 37 (37%) divided in to 23 samples from male (62%), while 14 samples from female (38%). No statistically significant correlation was discovered between these dates and sex ($p= 0.068$). This result is matched to a recent result from research done by (Upreti *et al.* 2018).

Table 4.4 MRSA distribution according to gender

SOURCE OF SAMPLE	TOTAL NO.	POSITIVE SAMPLES	%
Male	45	23	51.11
Female	55	14	25.45
Total	100	37	37
X ²		6.98	
P value		0.068 (Non significant difference at P>0.05)	

4.6 Effect of Age on the Infection Rate of *S. aureus*

In current study the data related to the age of the participants that enrolled in this study was shown in Table 4.5. The age groups were divided in to five groups 12-24, 25-37, 38-50, 51-63, and 64-75. The number of positive samples with *S. aureus* was higher in age group 12-24 and the lower number of positive samples was in age group 64-75. Regarding infection rate of status, the higher percentage was in age group 51-63, which equals to approximately 77%. The lower percentage of infection rate was found in age group 38-50, which equals to 25%. Overall, infection rate was higher in age more than 51 years compared to the other age groups. There is significant difference ($p=0.034$) among age groups especially in groups older than 51 year. This is relevant no senescence is present in various degrees in all older persons, and there is a correlation between frailty and immunocompetency (Elias *et al.* 2018).

Age-related declines in the immune system's ability to fight off infections, malignant cells, and autoreactive cells lead to a weakened defense against these threats and an increase in the risk of infection, cancer, autoimmune illnesses, and poor wound healing. (Akha 2018). Immunosenescence, along with age-related organ changes, comorbidities, geriatric syndromes, frailty, malnutrition, functional dysfunction, and polypharmacy, impair the prognosis of geriatric patients with infectious illnesses, causing immunosuppression in many older persons (Esme *et al.* 2019).

Table 4.5 Prevalence of MRSA by age

SOURCE OF SAMPLE	TOTAL NO	POSITIVE SAMPLES	%
12-24	44	15	34.09
25-37	31	10	32.25
38-50	12	3	25
51-63	9	7	77.77
64-75	4	2	50
X ²		7.91	
P value		0.034 (Significant difference at P<0.05)	

4.7 Identification of Bacterial Species by 16s rDNA

Recently, numerous PCR-based molecular identification techniques have been presented as an alternate strategy. The diagnostic gene, *16srDNA* gene was used in this study to denote isolates belonging to *S. aureus* (Rana *et al.* 2018).

Sequencing DNA was extracted from bacteria *S. aureus*, conventional PCR was carried out using these DNA samples for the amplification of specific *16S rDNA* primer. PCR analysis was applied in all study *S. aureus* isolates, previously phenotypic diagnosed, (Figure 4.7). After that gel electrophoresis showed that all of these isolates 20/20 (100%) which were identified by conventional methods have *16SrDNA* gene of DNA fragment when compared with ladder, then, the isolates were recorded in NCBI-gene sequencing as first described in our local study (Figure 4.6).

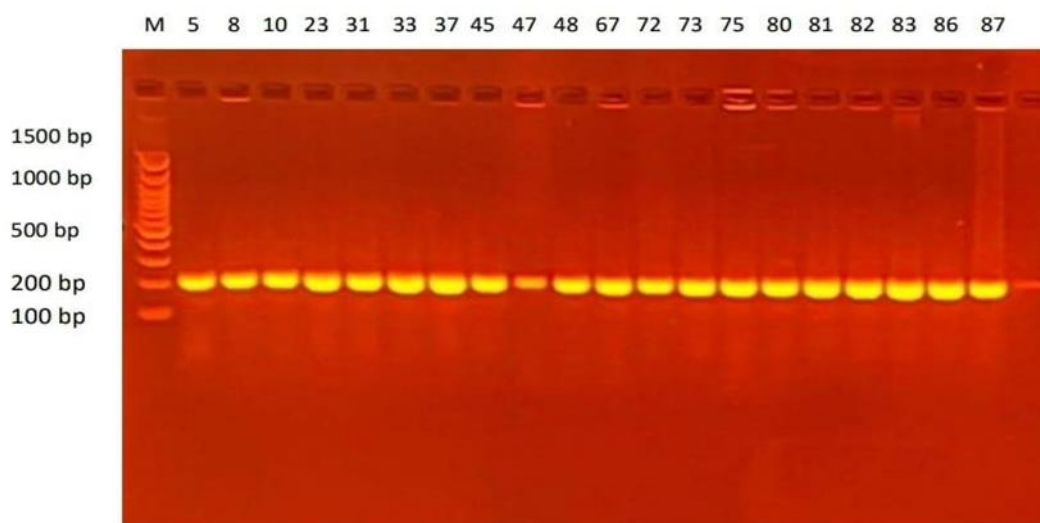


Figure 4.6 Diagnosis of staphylococci by using PCR. The appearance of bundles defining the *16srDNA* gene across an agarose gel was documented using a UV



Figure 4.7 Amplification of the *mecA* gene for *S. aureus* on agarose-stained gel

4.8 Detection of the *mecA* gene in *S. aureus* using PCR technology

The standard for identifying MRSA in *S. aureus* is the detection of the *mecA* gene, which is responsible for the making of the protein PBP2a responsible for classic β -lactam resistance (Siddiqui *et al.* 2018).

After identification of *S. aureus* isolates by cultivation, biochemical tests, susceptibility tests, lethal inhibitory concentration by VITEC 2, and 16s rDNA gene identification, all isolates were confirmed as *S. aureus*, which included 35 resistant isolates and 2 sensitive isolates. These 35 isolates were identified by PCR technique to detect the presence of the *mecA* gene. Among 35 samples, 1 sample was negative for the *mecA* gene, whereas 34 were positive. This results is similar to the study (Al-Saadi and Abd Al-Mayahi 2021). The primary proof of the discovery of an MRSA isolate is the *mecA* gene. Numerous researchers from throughout the globe concurred with this assessment. in Egypt (Hafez *et al.* 2009), in Sudan (Elhassan *et al.* 2015), in Iraq (Al-Zu'bi *et al.* 2004), in Turkey (Cikman *et al.* 2019) and in USA (Murakami *et al.* 1991).

In the absence of the *mecA* gene, it may be possible to explore for additional intrinsic factors that may compete with the *mecA* gene in causing the phenomena of resistance in MRSA-endemic regions. On the other hand, the absence of resistant gene among *S. aureus* was also reported around the globe (Bignardi *et al.* 1996, Hawraa *et al.* 2014). In addition, prior research in Nigeria found the complete lack of 5 main SCCmec types and *mecA* genes, in addition to the gene product of PBP2a, in isolates that had the MRSA phenotype, indicating a likelihood of hyperproduction of β -lactamase as the origin of the occurrence (Olayinka *et al.* 2009). Additionally, Ba and coworkers have pointed out how little alterations to amino acids in the protein binding protein cascade (PBPs1, 2, and 3) may be the root cause of resistance. These results are strong proof that MRSA's resistance to β -lactam antibiotics is caused by something other than the *mecA* gene (Ba *et al.* 2014)..

4.9 Sequence Analysis of 16s rDNA and *mecA* Gene

The results were analyzed and edited by Macrogen Corporation Korea, using special and accurate programs. The edited sequence was checked with the databases by using BLAST to detect the close relationship with submitted sequences. as shown in the Table 4.6 and 4.7.

Table 4.6 Comparison between 16sDNA sequences of *S. aureus* strains with gene bank by using BLAST software

SAMPLE CODE	ACCESSION NUMBER	COVER (%)	SIMILARITY %
s10-sf	<u>KX373891.1</u>	98%	98.89
s33-sf	<u>KP318059.1</u>	100%	98.33
s45-sf	<u>ON526674.1</u>	98%	98.88
s48-sf	<u>KX373891.1</u>	98%	99.44
s67-sf	<u>KX373891.1</u>	95%	99.42
s75-sf	<u>HM285203.1</u>	96%	98.84
s80-sf	<u>KP318059.1</u>	98%	98.32
s83-sf	<u>JF194816.1</u>	97%	99.43
s86-sf	<u>MT379497.1</u>	97%	100
s87-sf	<u>JF197580.1</u>	98%	98.89

Table 4.7 Comparison between *mecA* sequences of *S. aureus* strains with gene bank by using BLAST software

SAMPLE CODE	ACCESSION NUMBER	COVER (%)	SIMILARITY %
M10-MF	<u>MN635692.1</u>	92	89.72
M33-MF	<u>LC632937.1</u>	89	88.68
M45-MF	<u>LC632937.1</u>	94	93.37
M48-MF	<u>CP092565.1</u>	95	96.76
M67-MF	<u>LC414617.1</u>	93	88.15
M80-MF	<u>LC632937.1</u>	96	86.90
M86-MF	<u>KM235679.1</u>	92	86.74
M87-MF	<u>CP092561.1</u>	95	91.46

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

MRSA makes up roughly one-third of the total bacterial isolates found in various pathological samples. Moreover, the detection rate of MRSA was higher among wound patients compared to other sites that were detected. The molecular methodology was determined to be the most sensitive way for identifying *S. aureus* at the genus and species levels, with 100 % accuracy in detecting MRSA. Cefoxitin as antibiotic disc is a more specific and sensitive test for accurate phenotypic detection of MRSA. Since vancomycin is seldom prescribed by clinicians to treat staphylococcal infections, no cases of vancomycin-resistant *S. aureus* among patients at Al-Swuaire Hospital have been isolated.

5.2 Recommendations

Use of cefoxitin (30g) during the susceptibility test method for staphylococci, which provides an easier-to-read test and results in an equivalent detection (sensitivity and specificity) of methicillin-resistance in *S.aureus*. Further research should be conducted to determine resistance in the absence of the gene *mecA*. Using more advanced and accurate methods for the diagnosis of MRSA in Iraqi hospitals, especially the PCR technique to differentiate between antibiotic sensitive and resistant strains that are independent of physical and chemical conditions. MRSA carriers in the population are successfully treated and followed up on, especially if they work in fields related to public health.

REFERENCES

- Akanbi, O. E., Njom, H. A., Fri, J., Otigbu, A. C., and Clarke, A. M. 2017. Antimicrobial susceptibility of *Staphylococcus Aureus* isolated from recreational waters and beach sand in eastern cape province of south africa. *International Journal of Environmental Research And Public Health*, 14(9): 1001.
- Akha, A. A. S. 2018. Aging and the immune system: An overview. *Journal of Immunological Methods*, 463: 21-26.
- Alam, M. M., Islam, M. N., Hawlader, M. D. H., Ahmed, S., Wahab, A., Islam, M., and Hossain, A. 2021. Prevalence of multidrug resistance bacterial isolates from Infected wound patients in Dhaka, Bangladesh: a cross-sectional study. *International Journal of Surgery Open*, 28: 56-62.
- Alani, H. A., Hassawi, D. S., and Flayih, M. T. 2017. Patterns of Antibiotic resistance in *Staphylococcus Aureus* isolates and detection the heteroresistance to vancomycin by population analysis method. *JUAPS*, 11(3): 26-33.
- Almutawif, Y., Hartmann, B., Lloyd, M., Lai, C. T., Rea, A., and Geddes, D. 2019. *Staphylococcus Aureus* enterotoxin production in raw and pasteurized milk: The effect of selected different storage durations and temperatures. *Breastfeeding Medicine*, 14(4): 256-261.
- Al-Zu'bi, E., Bdour, S., and Shehabi, A. A. 2004. Antibiotic resistance patterns of *mecA*-positive *Staphylococcus Aureus* isolates from Clinical specimens and nasal carriage. *Microbial Drug Resistance*, 10(4): 321-324.
- Argemi, X., Matelska, D., Ginalski, K., Riegel, P., Hansmann, Y., Bloom, J., and Prévost, G. 2018. Comparative genomic analysis of *Staphylococcus lugdunensis* shows a closed pan-genome and multiple barriers to horizontal gene transfer. *BMC Genomics*, 19(1): 1-16.
- Armin, S., Fallah, F., Navidinia, M., and Vosoghian, S. 2017. Prevalence of *blaOXA-1* and *blaDHA-1* AmpC β -lactamase-producing and methicillin-resistant *Staphylococcus Aureus* in Iran. *Archives of Pediatric Infectious Diseases*, 5(4).
- Asante, J., Amoako, D. G., Abia, A. L., Somboro, A. M., Govinden, U., Bester, L. A., and Essack, S. Y. 2020. Review of Clinically and epidemiologically relevant

- coagulase-negative Staphylococci in Africa. *Microbial Drug Resistance*, 26(8): 951-970.
- Asiimwe, B. B., Baldan, R., Trovato, A., and Cirillo, D. M. 2017. Prevalence and molecular characteristics of *Staphylococcus Aureus*, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. *BMC Infectious Diseases*., 17(1): 1-8.
- Askarian, F., Ajayi, C., Hanssen, A. M., Van Sorge, N. M., Pettersen, I., Diep, D. B., and Johannessen, M. 2016. The interaction between *Staphylococcus Aureus* SdrD and desmoglein 1 is important for adhesion to host cells. *Scientific Reports*, 6(1): 1-11.
- Azizan, N. H., Kisim, N. F. M., Saedudin, R. R., Abidin, Z. A. Z., Ashaari, M. M., and Kasim, S. 2018. Isolation and Characterization of Hydrocarbon Tolerant Microorganisms from Marine Environment. *International Journal of Integrated Engineering*, 10(6).
- Ba, X., Harrison, E. M., Edwards, G. F., Holden, M. T., Larsen, A. R., Petersen, A., and Holmes, M. A. 2014. Novel mutations in penicillin-binding protein genes in Clinical *Staphylococcus Aureus* isolates that are methicillin resistant on susceptibility testing, but lack the *mec* gene. *Journal of Antimicrobial Chemotherapy*, 69(3): 594-597.
- Bauer, A. W. 1966. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 45: 149-158.
- Bencardino, D., Amagliani, G., and Brandi, G. 2021. Carriage of *Staphylococcus Aureus* among food handlers: An ongoing challenge in public health. *Food Control*, 130: 108362.
- Berla-Kerzhner, E., Biber, A., Parizade, M., Taran, D., Rahav, G., Regev-Yochay, G., and Glikman, D. 2017. Clinical outcomes and treatment approach for community-associated methicillin-resistant *Staphylococcus Aureus* (CA-MRSA) Infections in Israel. *European Journal of Clinical Microbiology and Infectious Diseases*, 36(1): 153-162.
- Bidya, S., and Suman, R. S. 2014. Comparative study of three β lactamase test methods in *Staphylococcus Aureus* isolated from two Nepalese hospitals. *Open Journal of Clinical Diagnostics*, 2014.

- Bignardi, G. E., Woodford, N., Chapman, A., Johnson, A. P., and Speller, D. C. E. 1996. Detection of the *mec-A* gene and phenotypic detection of resistance in *Staphylococcus Aureus* isolates with borderline or low-level methicillin resistance. *Journal of Antimicrobial Chemotherapy*, 37(1): 53-63.
- Boswihi, S. S., and Udo, E. E. 2018. Methicillin-resistant *Staphylococcus Aureus*: An update on the Epidemiology, treatment options and infection control. *Current Medicine Research and Practice*, 8(1): 18-24.
- Bozzella, M. J., Soghier, L., Harris, T., Zell, L., Short, B. L., and Song, X. 2019. Impact of decolonization on methicillin-resistant *Staphylococcus Aureus* transmission and Infection in a neonatal intensive care unit. *Infection Control and Hospital Epidemiology*, 40(10): 1123-1127.
- Bush, K. 2018. Past and present perspectives on β -lactamases. *Antimicrobial Agents and Chemotherapy*, 62(10): e01076-18.
- Bush, K., and Bradford, P. A. 2020. Epidemiology of β -lactamase-producing pathogens. *Clinical Microbiology Reviews*, 33(2): e00047-19.
- Chalmers, S. J., and Wylam, M. E. 2020. Methicillin-Resistant *Staphylococcus Aureus* Infection and treatment options. *Methicillin-Resistant Staphylococcus Aureus (MRSA) Protocols.*, 229-251.
- Chatterjee, A., Rai, S., Guddattu, V., Mukhopadhyay, C., and Saravu, K. 2018. Is methicillin-resistant *Staphylococcus Aureus* Infection associated with higher mortality and morbidity in hospitalized patients? A cohort study of 551 patients from South Western India. *Risk Management and Healthcare Policy*, 11: 243.
- Cheung, G. Y., Bae, J. S., and Otto, M. 2021. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, 12(1): 547-569.
- Cikman, A., Aydin, M., Gulhan, B., Karakeçili, F., Kurtoglu, M. G., Yuksekkaya, S., and Ozekinci, T. 2019. Absence of the *mecC* gene in methicillin-resistant *Staphylococcus Aureus* isolated from various Clinical samples: The first multi-centered study in Turkey. *Journal of Infection and Public Health*, 12(4): 528-533.
- Crespo-Piazuelo, D., and Lawlor, P. G. 2021. Livestock-associated methicillin-resistant *Staphylococcus Aureus* (LA-MRSA) prevalence in humans in close contact with

- animals and measures to reduce on-farm colonisation. *Irish Veterinary Journal*, 74(1): 1-12.
- Cyphert, E. L., Lu, C. Y., Marques, D. W., Learn, G. D., and von Recum, H. A. 2019. Combination antibiotic delivery in PMMA provides sustained broad-spectrum Antimicrobial activity and allows for postimplantation refilling. *Biomacromolecules*, 21(2): 854-866.
- Dahlen, G., Basic, A., and Bylund, J. 2019. Importance of virulence factors for the persistence of oral bacteria in the inflamed gingival crevice and in the pathogenesis of periodontal disease. *Journal of Clinical Medicine*, 8(9): 1339.
- Dai, Y., Liu, J., Guo, W., Meng, H., Huang, Q., He, L., and Li, M. 2019. Decreasing methicillin-resistant *Staphylococcus Aureus* (MRSA) Infections is attributable to the disappearance of predominant MRSA ST239 clones, Shanghai, 2008–2017. *Emerging Microbes and Infections*, 8(1): 471-478.
- David, M. Z., and Daum, R. S. 2017. Treatment of *Staphylococcus Aureus* Infections. *Staphylococcus Aureus*, 325-383.
- de Vor, L., Rooijackers, S. H., and van Strijp, J. A. 2020. *Staphylococci* evade the innate immune response by disarming neutrophils and forming biofilms. *FEBS Letters*, 594(16): 2556-2569.
- Doulgeraki, A. I., Di Ciccio, P., Ianieri, A., and Nychas, G. J. E. 2017. Methicillin-resistant food-related *Staphylococcus Aureus*: A review of current knowledge and biofilm formation for future studies and applications. *Research in Microbiology*, 168(1): 1-15.
- Dweba, C. C., Zishiri, O. T., El Zowalaty, and M. E. 2018. Methicillin-resistant *Staphylococcus aureus*: livestock-associated, Antimicrobial, and heavy metal resistance. *Infection and Drug Resistance*, 11: 2497.
- Ersoy, S. C., Abdelhady, W., Li, L., Chambers, H. F., Xiong, Y. Q., and Bayer, A. S. 2019. Bicarbonate resensitization of methicillin-resistant *Staphylococcus Aureus* to β -lactam antibiotics. *Antimicrobial Agents and Chemotherapy*, 63(7): e00496-19.
- Esme, M., Topeli, A., Yavuz, B. B., and Akova, M. 2019. Infections in the elderly critically-ill patients. *Frontiers in Medicine*, 6: 118.

- Falcó, V., Burgos, J., and Almirante, B. 2018. Ceftobiprole medocaril for the treatment of community-acquired pneumonia. *Expert Opinion on Pharmacotherapy*, 19(13): 1503-1509.
- Farrell, D. J., Mendes, R. E., and Bensaci, M. 2019. In vitro activity of tedizolid against Clinical isolates of *Staphylococcus lugdunensis* and *Staphylococcus Haemolyticus* from Europe and the United States. *Diagnostic Microbiology and Infectious Disease*, 93(1): 85-88.
- Fathi, H. A., Abdelkader, A., AbdelKarim, M. S., Abdelaziz, A. A., El-Mokhtar, M. A., Allam, A., and Elsabahy, M. 2020. Electrospun vancomycin-loaded nanofibers for management of methicillin-resistant *Staphylococcus Aureus*-induced skin Infections. *International Journal of Pharmaceutics*, 586: 119620.
- Fergestad, M. E., Stamsås, G. A., Morales Angeles, D., Salehian, Z., Wasteson, Y., and Kjos, M. 2020. Penicillin-binding protein PBP2a provides variable levels of protection toward different β -lactams in *Staphylococcus Aureus* RN4220. *Microbiologyopen*, 9(8): e1057.
- Fernando, S. A., Gray, T. J., and Gottlieb, T. 2017. Healthcare-acquired Infections: prevention strategies. *Internal Medicine Journal*, 47(12): 1341-1351.
- Ferreira, C., Costa, S. S., Serrano, M., Oliveira, K., Trigueiro, G., Pomba, C., and Couto, I. 2021. Clonal lineages, Antimicrobial resistance, and PVL carriage of *Staphylococcus Aureus* associated to skin and soft-tissue Infections from ambulatory patients in Portugal. *Antibiotics*, 10(4): 345.
- Ferrer-González, E., Kaul, M., Parhi, A. K., LaVoie, E. J., and Pilch, D. S. 2017. β -Lactam antibiotics with a high affinity for PBP2 act synergistically with the FtsZ-targeting agent TXA707 against methicillin-resistant *Staphylococcus Aureus*. *Antimicrobial Agents and Chemotherapy*, 61(9): e00863-17.
- Fisher, J. F., and Mobashery, S. 2020. β -Lactams against the Fortress of the Gram-Positive *Staphylococcus Aureus* Bacterium. *Chemical Reviews*, 121(6): 3412-3463.
- Gajdács, M. 2019. The continuing threat of methicillin-resistant *Staphylococcus Aureus*. *Antibiotics*, 8(2): 52.

- García-Fernández, E., Koch, G., Wagner, R. M., Fekete, A., Stengel, S. T., Schneider, J., and Lopez, D. 2017. Membrane microdomain disassembly inhibits MRSA Antibiotic resistance. *Cell*, 171(6): 1354-1367.
- Geetha, S. H., Kalghatgi, A. T., and Rama, N. K. 2015. Characterization of methicillin resistant *Staphylococcus Aureus* strains from Clinical isolates in a tertiary care hospital of south India. *Int. J. Med. Res. Rev.*, 3(9): 1077-1083.
- Gillet, Y., Henry, T., and Vandenesch, F. 2018. Fulminant Staphylococcal Infections. *Microbiology Spectrum*, 6(5): 6-5.
- Glossop, H. D., Pearl, E., De Zoysa, G. H., and Sarojini, V. 2018. Linear Analogues of the Lipopeptide Battacin with Potent In Vitro Activity Against *S. Aureus*. *Advances in Protein Chemistry and Structural Biology*, 112: 385-394.
- González-Martín, M., Corbera, J. A., Suárez-Bonnet, A., and Tejedor-Junco, M. T. 2020. Virulence factors in coagulase-positive *Staphylococci* of veterinary interest other than *Staphylococcus Aureus*. *Veterinary Quarterly*, 40(1): 118-131.
- Gopal, S., and Divya, K. C. 2017. Can methicillin-resistant *Staphylococcus Aureus* prevalence from dairy cows in India act as potential risk for community-associated Infections?: a review. *Veterinary World*, 10(3): 311.
- Goud, R., Gupta, S., Neogi, U., Agarwal, D., Naidu, K., Chalannavar, R., and Subhaschandra, G. 2011. Community prevalence of methicillin and vancomycin resistant *Staphylococcus Aureus* in and around Bangalore, southern India. *Revista da Sociedade Brasileira de Medicina Tropical*, 44: 309-312.
- Gruttadauria, M., Pollard, J., Kim, S., and Lian, X. 2021. Streptococcal toxic shock syndrome in the setting of recent gynecologic surgery: A case report. *Case Reports in Women's Health*, 32: e00352.
- Guo, Y., Song, G., Sun, M., Wang, J., and Wang, Y. 2020. Prevalence and therapies of Antibiotic-resistance in *Staphylococcus Aureus*. *Frontiers in Cellular and Infection Microbiology*, 10: 107.
- Guzmán, L., Ramirez, B. S., Maribel, C. F., Pescador, M. G. N., and Cruz, F. J. M. 2018. Low accuracy of the McFarland method for estimation of Bacterial populations. *African Journal of Microbiology Research*, 12(31): 736-740.

- Hafez, E. E., Al-Sohaimy, S. A., and El-Saadani, M. A. 2009. The effect of the *mecA* gene and its mutant form on the response of *S. Aureus* to the most common Antibiotics. *International Journal of Immunological Studies*, 1(1): 106-122.
- Han, J., Ma, Z., Gao, P., Lu, Z., Liu, H., Gao, L., and Bie, X. 2018. The Antibacterial activity of LI-F type peptide against methicillin-resistant *Staphylococcus Aureus* (MRSA) in vitro and inhibition of Infections in murine scalded epidermis. *Applied Microbiology and Biotechnology*, 102(5): 2301-2311.
- Harkins, C. P., Pichon, B., Doumith, M., Parkhill, J., Westh, H., Tomasz, A., and Holden, M. T. 2017. Methicillin-resistant *Staphylococcus Aureus* emerged long before the introduction of methicillin into Clinical practice. *Genome Biology*, 18(1): 1-11.
- Harrison, E. M., Ba, X., Coll, F., Blane, B., Restif, O., Carvell, H., and Holmes, M. A. 2019. Genomic identification of cryptic susceptibility to penicillins and β -lactamase inhibitors in methicillin-resistant *Staphylococcus Aureus*. *Nature Microbiology*, 4(10): 1680-1691.
- Hawraa, W. A., Al-Dulaimi, T., and Al-Marzoqi, A. H. 2014. Phenotypic detection of resistance in *Staphylococcus Aureus* isolates: detection of (*mec A* and *fem A*) gene in methicillin resistant *Staphylococcus Aureus* (MRSA) by polymerase chain reaction. *Journal of Natural Sciences Research*, 4(1): 112-118.
- Hiko, A. 2019. Isolation, DNase-cross-Coagulase test and Antimicrobial resistance test on *Staphylococcus* along beef abattoir line in Addis Ababa Ethiopia. *Ethiopian Veterinary Journal*, 23(1): 90-110.
- Horner, C., Utsi, L., Coole, L., and Denton, M. 2017. Epidemiology and Microbiological characterization of Clinical isolates of *Staphylococcus Aureus* in a single healthcare region of the UK, 2015. *Epidemiology and Infection*, 145(2): 386-396.
- Hu, Q., Peng, H., and Rao, X. 2016. Molecular events for promotion of vancomycin resistance in vancomycin intermediate *Staphylococcus Aureus*. *Frontiers in Microbiology*, 7, 1601.
- Huang, G., Huang, Q., Xie, L., Xiang, G., Wang, L., Xu, H., and Zhang, L. 2017. A rapid, low-cost, and microfluidic chip-based system for parallel identification of multiple pathogens related to Clinical pneumonia. *Scientific reports*, 7(1): 1-10.

- Jadhav, S. V., Desai, D. S., Mirza, S. B., Apte-Deshpande, A. D., and Deshpande, S. 2021. Molecular Characterization of Staphylococcal Cassette Chromosome *mecA* and Concomitant Pantone-valentine Leukocidine in Clinical Isolates of Community acquired Methicillin-resistant Staphylococcus Aureus. Journal of Krishna Institute of Medical Sciences (JKIMSU), 10(2).
- Jajere, S. M. 2019. A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and Antimicrobial resistance including multidrug resistance. Veterinary World, 12(4): 504.
- Jamilatun, M. 2019. Uji Resistensi Antibiotik Staphylococcus Aureus Isolat Kolam Renang. Biomedika, 12(1): 1-8.
- Karauzum, H., Venkatasubramaniam, A., Adhikari, R. P., Kort, T., Holtsberg, F. W., Mukherjee, I., and Aman, M. J. 2021. IBT-V02: a multicomponent toxoid vaccine protects against primary and secondary skin Infections caused by Staphylococcus Aureus. Frontiers in Immunology, 12: 624310.
- Khanal, L. K., Adhikari, R. P., and Guragain, A. 2018. Prevalence of methicillin resistant Staphylococcus Aureus and Antibiotic susceptibility pattern in a tertiary hospital in Nepal. Journal of Nepal Health Research Council, 16(2): 172-174.
- Kırmusaoğlu, S. 2019. Sensitizing of β -lactam resistance by tannic acid in methicillin-resistant S. Aureus. World Journal of Microbiology and Biotechnology, 35(4): 1-9.
- Kulanthaivel, S., and Mishra, P. 2021. Shape-and Size-Dependent Antibacterial Activity of Nanomaterials. Microbial Interactions at Nanobiotechnology Interfaces: Molecular Mechanisms and Applications, 1-52.
- Lakhundi, S., and Zhang, K. 2018. Methicillin-resistant Staphylococcus Aureus: molecular characterization, evolution, and Epidemiology. Clinical Microbiology Reviews, 31(4): e00020-18.
- Lamut, A., Peterlin Mašič, L., Kikelj, D., and Tomašič, T. 2019. Efflux pump inhibitors of Clinically relevant multidrug resistant bacteria. Medicinal Research Reviews, 39(6): 2460-2504.
- Lee, A. S., De Lencastre, H., Garau, J., Kluytmans, J., Malhotra-Kumar, S., Peschel, A., and Harbarth, S. 2018. Methicillin-resistant Staphylococcus Aureus. Nature Reviews Disease Primers, 4(1): 1-23.

- Luzum, M., Sebolt, J., and Chopra, V. 2020. Catheter-associated urinary tract Infection, *Clostridioides difficile* colitis, central line-associated bloodstream Infection, and methicillin-resistant *Staphylococcus Aureus*. *Medical Clinics*, 104(4): 663-679.
- Ma, G. C., Worthing, K. A., Ward, M. P., and Norris, J. M. 2020. Commensal *Staphylococci* including methicillin-resistant *Staphylococcus Aureus* from dogs and cats in remote New South Wales, Australia. *Microbial Ecology*, 79(1): 164-174.
- MacIntyre, C. R., and Bui, C. M. 2017. Pandemics, public health emergencies and Antimicrobial resistance-putting the threat in an epidemiologic and risk analysis context. *Archives of Public Health*, 75(1): 1-6.
- Makinde, T. M., Ako-Nai, K. A., and Shittu, A. 2019. A study on the Antibiotic susceptibility of *Staphylococcus Aureus* from nasal samples of female students at the Obafemi Awolowo university campus. *Journal of Microbiology and Experimentation*, 7(2): 78-83.
- Martens, E., and Demain, A. L. 2017. The Antibiotic resistance crisis, with a focus on the United States. *The Journal of Antibiotics*, 70(5): 520-526.
- Masadeh, M. M., Alzoubi, K. H., Ahmed, W. S., and Magaji, A. S. 2019. In vitro comparison of Antibacterial and Antibiofilm activities of selected fluoroquinolones against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. *Pathogens*, 8(1): 12.
- Maya-Martinez, R., Alexander, J. A. N., Otten, C. F., Ayala, I., Vollmer, D., Gray, J., and Simorre, J. P. 2019. Recognition of peptidoglycan fragments by the transpeptidase PBP4 from *Staphylococcus Aureus*. *Frontiers in Microbiology*, 9: 3223.
- Meade, E., Slattery, M. A., and Garvey, M. 2020. Bacteriocins, potent Antimicrobial peptides and the fight against multi drug resistant species: resistance is futile?. *Antibiotics*, 9(1): 32.
- Medernach, R. L., and Logan, L. K. 2018. The growing threat of antibiotic resistance in children. *Infectious Disease Clinics*, 32(1): 1-17.
- Mercuro, N. J., Davis, S. L., Zervos, M. J., and Herc, E. S. 2018. Combatting resistant enterococcal Infections: a pharmacotherapy review. *Expert Opinion on Pharmacotherapy*, 19(9): 979-992.

- Meşeli, T., Doğan, Ş. D., Gündüz, M. G., Kökbudak, Z., Bogojevic, S. S., Noonan, T., and Nikodinovic-Runic, J. 2021. Design, synthesis, Antibacterial activity evaluation and molecular modeling studies of new sulfonamides containing a sulfathiazole moiety. *New Journal of Chemistry*, 45(18): 8166-8177.
- Mikłasińska-Majdanik, M., Kępa, M., Wojtyczka, R. D., Idzik, D., and Wąsik, T. J. 2018. Phenolic compounds diminish Antibiotic resistance of *Staphylococcus Aureus* Alinical strains. *International Journal of Environmental Research and Public Health*, 15(10): 2321.
- Milheiriço, C., de Lencastre, H., and Tomasz, A. 2017. Full-genome sequencing identifies in the genetic background several determinants that modulate the resistance phenotype in methicillin-resistant *Staphylococcus Aureus* strains carrying the novel *mecC* gene. *Antimicrobial Agents and Chemotherapy*, 61(3): e02500-16.
- Miragaia, M. 2018. Factors contributing to the evolution of Meca-mediated β -lactam resistance in *Staphylococci*: update and new insights from whole genome sequencing (WGS). *Frontiers in Microbiology*, 9, 2723.
- Mishra, V., Nag, V. L., Tandon, R., and Awasthi, S. 2010. Response surface Methodology-based optimisation of agarose gel electrophoresis for screening and electropherotyping of rotavirus. *Applied Biochemistry and Biotechnology*, 160(8): 2322-2331.
- Miyachiro, M. M., Contreras-Martel, C., and Dessen, A. 2019. Penicillin-binding proteins (PBPs) and Bacterial cell wall elongation complexes. *Macromolecular Protein Complexes II: Structure and Function*, 273-289.
- Mohammadzadeh, R., Mohammadi-Gollou, A., and Salehabadi, Y. 2019. The Prevalence of CTX-M Beta-Lactamase Gene in Urinary Tract Infections in Patients Referring to Bonab City Hospitals. *Iranian Journal of Medical Microbiology*, 13(03).
- Moreno Grúa, E. 2021. Characterization of methicillin-resistant " *Staphylococcus Aureus*" in commercial and wild rabbits (" *Oryctolagus cuniculus*") and immunological evaluation of a paternal line of commercial rabbits: study of the pathogen-host interaction.

- Morris, D. O., Loeffler, A., Davis, M. F., Guardabassi, L., and Weese, J. S. 2017. Recommendations for approaches to methicillin-resistant Staphylococcal Infections of small animals: diagnosis, therapeutic considerations and preventative measures. *Clinical Consensus Guidelines of the World Association for Veterinary Dermatology*. *Veterinary Dermatology*, 28(3): 304-e69.
- Müller, A., Seinige, D., Jansen, W., Klein, G., Ehrlich, R., Monecke, S., and Kehrenberg, C. 2016. Variety of Antimicrobial resistances and virulence factors in *Staphylococcus Aureus* isolates from meat products legally and illegally introduced to Germany. *PLoS One*, 11(12): e0167864.
- Múnera, J. M. V., Ríos, A. M. O., Urrego, D. M., and Jiménez Quiceno, J. N. 2017. In vitro susceptibility of methicillin-resistant *Staphylococcus Aureus* isolates from skin and soft tissue Infections to vancomycin, daptomycin, linezolid and tedizolid. *Brazilian Journal of Infectious Diseases*, 21: 493-499.
- Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H., and Watanabe, S. 1991. Identification of methicillin-resistant strains of Staphylococci by polymerase chain reaction. *Journal of Clinical Microbiology*, 29(10): 2240-2244.
- Naimi, T. S., LeDell, K. H., Como-Sabetti, K., Borchardt, S. M., Boxrud, D. J., Etienne, J., and Lynfield, R. 2003. Comparison of community-and health care-associated methicillin-resistant *Staphylococcus Aureus* infection. *Jama*, 290(22): 2976-2984.
- Nasser, A., Moradi, M., Jazireian, P., Safari, H., Alizadeh-Sani, M., Pourmand, M. R., and Azimi, T. 2019. *Staphylococcus Aureus* versus neutrophil: Scrutiny of ancient combat. *Microbial Pathogenesis*, 131: 259-269.
- Nasser, A., Dallal, M., Jahanbakhshi, S., Azimi, T., and Nikouei, L. 2022. *Staphylococcus Aureus*: Biofilm Formation and Strategies against it. *Current Pharmaceutical Biotechnology*, 23(5): 664-678.
- Negm, S. H., El-Hamid, A., Abd Allh, M., Gado, M. A., and El-Gendy, H. S. 2019. Selective uranium adsorption using modified acrylamide resins. *Journal of Radioanalytical and Nuclear Chemistry*, 319(1): 327-337.
- Olayinka, B. O., Olayinka, A. T., Obajuluwa, A. F., Onaolapo, J. A., and Olurinola, P. F. 2009. Absence of *mecA* gene in methicillin-resistant *Staphylococcus Aureus* isolates. *African Journal of Infectious Diseases*, 3(2).

- Ortwine, J. K., and Bhavan, K. 2018. Morbidity, mortality, and management of methicillin-resistant *S. Aureus* Bacteremia in the USA: update on Antibacterial choices and understanding. *Hospital Practice*, 46(2): 64-72.
- Painter, K. L., Hall, A., Ha, K. P., and Edwards, A. M. 2017. The electron transport chain sensitizes *Staphylococcus Aureus* and *Enterococcus faecalis* to the oxidative burst. *Infection and Immunity*, 85(12): e00659-17.
- Panchal, V. 2018. The molecular basis of high-level methicillin resistance in *Staphylococcus Aureus* (Doctoral dissertation, University of Sheffield).
- Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A., Papa, A., and Sergelidis, D. 2018. Prevalence of *Staphylococcus Aureus* and of methicillin-resistant *S. Aureus* (MRSA) along the production chain of dairy products in north-western Greece. *Food Microbiology*, 69: 43-50.
- Parvin, M. S., Ali, M. Y., Talukder, S., Nahar, A., Chowdhury, E. H., Rahman, M. T., and Islam, M. T. 2021. Prevalence and multidrug resistance pattern of methicillin resistant *S. Aureus* isolated from frozen chicken meat in Bangladesh. *Microorganisms*, 9(3): 636.
- Paterson, G. K. 2020. Genomic Epidemiology of methicillin-resistant *Staphylococcus sciuri* carrying a SCCmec-mecC hybrid element. *Infection, Genetics and Evolution*, 79: 104148.
- Peker, N., Couto, N., Sinha, B., and Rossen, J. W. 2018. Diagnosis of bloodstream Infections from positive blood cultures and directly from blood samples: recent developments in molecular approaches. *Clinical Microbiology and Infection*, 24(9): 944-955.
- Peng, H., Liu, D., Ma, Y., and Gao, W. 2018. Comparison of community-and healthcare-associated methicillin-resistant *Staphylococcus Aureus* isolates at a Chinese tertiary hospital, 2012–2017. *Scientific Reports*, 8(1): 1-8.
- Pérez, V. K. C., da Costa, G. M., Guimarães, A. S., Heinemann, M. B., Lage, A. P., and Dorneles, E. M. S. 2020. Relationship between virulence factors and Antimicrobial resistance in *Staphylococcus Aureus* from bovine mastitis. *Journal of Global Antimicrobial Resistance*, 22: 792-802.

- Pietrocola, G., Nobile, G., Rindi, S., and Speziale, P. 2017. *Staphylococcus Aureus* manipulates innate immunity through own and host-expressed proteases. *Frontiers in Cellular and Infection Microbiology*, 7: 166.
- Rahman, M. M., Amin, K. B., Rahman, S. M. M., Khair, A., Rahman, M., Hossain, A., and Alam, M. M. 2018. Investigation of methicillin-resistant *Staphylococcus Aureus* among Clinical isolates from humans and animals by culture methods and multiplex PCR. *BMC Veterinary Research*., 14(1): 1-6.
- Rajan, V., Sivaraman, G. K., Vijayan, A., Elangovan, R., Prendiville, A., and Bachmann, T. T. 2022. Genotypes and phenotypes of methicillin-resistant *Staphylococci* isolated from shrimp aquaculture farms. *Environmental Microbiology Reports*, 14(3): 391-399.
- Rana, E., Rania, A. K., Hamdallah, Z., Alaa, E. D. S. H., and Tarek, H. E. 2018. Study on prevalence and genetic discrimination of methicillin-resistant *Staphylococcus Aureus* (MRSA) in Egyptian hospitals. *African Journal of Microbiology Research*, 12(27): 629-646.
- Reddy, P. N., Srirama, K., and Dirisala, V. R. 2017. An update on Clinical burden, diagnostic tools, and therapeutic options of *Staphylococcus Aureus*. *Infectious Diseases: Research and Treatment*, 10: 1179916117703999.
- Regecová, I., Výrostková, J., Zigo, F., Gregová, G., Pipová, M., Jevinová, P., and Becová, J. (2022). Detection of Resistant and Enterotoxigenic Strains of *Staphylococcus Warneri* Isolated from Food of Animal Origin. *Foods*, 11(10): 1496.
- Renzoni, A., Kelley, W. L., Rosato, R. R., Martinez, M. P., Roch, M., Fatouraei, M., and Rosato, A. E. 2017. Molecular bases determining daptomycin resistance-mediated resensitization to β -lactams (seesaw effect) in methicillin-resistant *Staphylococcus Aureus*. *Antimicrobial Agents and Chemotherapy*, 61(1): e01634-16.
- Ribeiro da Cunha, B., Fonseca, L. P., and Calado, C. R. 2019. Antibiotic discovery: where have we come from, where do we go?. *Antibiotics*, 8(2): 45.
- Rieg, S., Joost, I., Weiß, V., Peyerl-Hoffmann, G., Schneider, C., Hellmich, M., and Kaasch, A. 2017. Combination Antimicrobial therapy in patients with

- Staphylococcus Aureus bacteraemia-a post hoc analysis in 964 prospectively evaluated patients. *Clinical Microbiology and Infection*, 23(6): 406-e1.
- Rolo, J., Worning, P., Nielsen, J. B., Bowden, R., Bouchami, O., Damborg, P., and Miragaia, M. 2017. Evolutionary origin of the Staphylococcal cassette chromosome mec (SCC mec). *Antimicrobial Agents and Chemotherapy*, 61(6): e02302-16.
- Rupali, P., Palanikumar, P., Shanthamurthy, D., Peter, J. V., Kandasamy, S., Zacchaeus, N. G. P., and Zervos, M. J. 2019. Impact of an Antimicrobial stewardship intervention in India: evaluation of post-prescription review and feedback as a method of promoting optimal Antimicrobial use in the intensive care units of a tertiary-care hospital. *Infection Control and Hospital Epidemiology*, 40(5): 512-519.
- Saber, H., Jasni, A. S., Jamaluddin, T. Z. M. T., and Ibrahim, R. 2017. A review of Staphylococcal cassette chromosome mec (SCCmec) types in coagulase-negative Staphylococci (CoNS) species. *The Malaysian Journal of Medical Sciences: MJMS*, 24(5): 7.
- Salwan, R., and Sharma, V. 2020. Molecular and Biotechnological aspects of secondary metabolites in actinobacteria. *Microbiological Research*, 231: 126374.
- Sartelli, M., Guirao, X., Hardcastle, T. C., Kluger, Y., Boermeester, M., Raşa, K., and Catena, F. 2018. 2018 WSES/SIS-E consensus conference: recommendations for the management of skin and soft-tissue Infections. *World Journal of Emergency Surgery*, 13(1): 1-24.
- Schmelcher, M., and Loessner, M. J. (2016). Bacteriophage endolysins: applications for food safety. *Current opinion in Biotechnology*, 37: 76-87.
- Schwendener, S., Cotting, K., and Perreten, V. 2017. Novel methicillin resistance gene mecD in Clinical *Staphylococcus caseolyticus* strains from bovine and canine sources. *Scientific Reports*, 7(1): 1-11.
- Scudiero, O., Brancaccio, M., Mennitti, C., Laneri, S., Lombardo, B., De Biasi, M. G., and Pero, R. 2020. Human defensins: A novel approach in the fight against skin colonizing *Staphylococcus Aureus*. *Antibiotics*, 9(4): 198.
- Seni, J., Bwanga, F., Najjuka, C. F., Makobore, P., Okee, M., Mshana, S. E., and Kateete, D. P. (2013). Molecular characterization of *Staphylococcus Aureus*

- from patients with surgical site Infections at Mulago Hospital in Kampala, Uganda. *PloS One.*, 8(6): e66153.
- Shahid, U., Sharif, H., Farooqi, J., Jamil, B., and Khan, E. 2018. Microbiological and Clinical profile of infective endocarditis patients: an observational study experience from tertiary care center Karachi Pakistan. *Journal of Cardiothoracic Surgery*, 13(1): 1-9.
- Shalaby, M. A. W., Dokla, E. M., Serya, R. A., and Abouzid, K. A. 2020. Penicillin binding protein 2a: An overview and a Medicinal chemistry perspective. *European Journal of Medicinal Chemistry*, 199: 112312.
- Sharifzadeh, S., Dempwolff, F., Kearns, D. B., and Carlson, E. E. 2020. Harnessing β -lactam antibiotics for illumination of the activity of penicillin-binding proteins in *Bacillus subtilis*. *ACS Chemical Biology*, 15(5): 1242-1251.
- Shrestha, L. B., Syangtan, G., Basnet, A., Acharya, K. P., Chand, A. B., and Pokhrel, K. 2021. Methicillin-resistant *Staphylococcus Aureus* in Nepal. *JNMA: Journal of the Nepal Medical Association*, 59(237): 518.
- Siddiqui, T., Khan, M. N., Fatima, S., Alam, N., Masood, R., Saeed, R., and Naqvi, T. 2018. Prevalence of *mecA*: Genotyping screening of community acquired-MRSA isolates in Karachi, Pakistan. *Pakistan Journal of Pharmaceutical Sciences*, 31(5): 2091-2094.
- Straume, D., Piechowiak, K. W., Olsen, S., Stamsås, G. A., Berg, K. H., Kjos, M., and Håvarstein, L. S. 2020. Class A PBPs have a distinct and unique role in the construction of the pneumococcal cell wall. *Proceedings of the National Academy of Sciences*, 117(11): 6129-6138.
- Tacconelli, E., De Angelis, G., De Waure, C., Cataldo, M. A., La Torre, G., and Cauda, R. 2009. Rapid screening tests for methicillin-resistant *Staphylococcus Aureus* at hospital admission: systematic review and meta-analysis. *The Lancet Infectious Diseases*, 9(9): 546-554.
- Tang, P., Croxen, M. A., Hasan, M. R., Hsiao, W. W., and Hoang, L. M. 2017. Infection control in the new age of genomic Epidemiology. *American Journal of Infection Control*, 45(2): 170-179.
- Teklu, D. S., Negeri, A. A., Legese, M. H., Bedada, T. L., Woldemariam, H. K., and Tullu, K. D. 2019. Extended-spectrum beta-lactamase production and multi-drug

- resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. *Antimicrobial Resistance and Infection Control*, 8(1): 1-12.
- Teng, F., Liu, X., Guo, S. B., Li, Z., Ji, W. Q., Zhang, F., and Zhu, X. M. 2019. Community-acquired bacterial co-infection predicts severity and mortality in influenza-associated pneumonia admitted patients. *Journal of Infection and Chemotherapy*, 25(2): 129-136.
- Tillotson, G. S., and van Hise, N. 2021. Screening for Methicillin resistant *Staphylococcus Aureus* (MRSA)-a valuable Antimicrobial stewardship tool?. *Expert Review of Anti-infective Therapy*, 19(8): 957-959.
- Tirimba, N. J. 2018. Determination of iplex massarray as a method for genetic typing and characterization of *Staphylococcus Aureus* isolates in Kenya, doctoral dissertation, Kenyatta University.
- Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H., Takebayashi, Y., and Spencer, J. 2019. β -Lactamases and β -Lactamase Inhibitors in the 21st Century. *Journal of Molecular Biology*, 431(18): 3472-3500.
- Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., and Fowler, V. G. 2019. Methicillin-resistant *Staphylococcus Aureus*: an overview of basic and Clinical research. *Nature Reviews Microbiology*, 17(4): 203-218.
- Udo, E. E., and Boswihi, S. S. 2017. Antibiotic resistance trends in methicillin-resistant *Staphylococcus Aureus* isolated in Kuwait hospitals: 2011-2015. *Medical Principles and Practice*, 26(5): 485-490.
- Ukpanah, M. A., and Upla, P. U. 2017. Why is *Staphylococcus Aureus* Such a Successful Pathogen?. *Microbiology Research Journal International*, 19, 1-22.
- Upreti, N., Rayamajhee, B., Sherchan, S. P., Choudhari, M. K., and Banjara, M. R. 2018. Prevalence of methicillin resistant *Staphylococcus Aureus*, multidrug resistant and extended spectrum β -lactamase producing gram negative bacilli causing wound Infections at a tertiary care hospital of Nepal. *Antimicrobial Resistance and Infection Control*, 7(1): 1-10.
- Wang, X., Liu, Q., Zhang, H., Li, X., Huang, W., Fu, Q., and Li, M. 2018. Molecular characteristics of community-associated *Staphylococcus Aureus* isolates from

- pediatric patients with bloodstream Infections between 2012 and 2017 in Shanghai, China. *Frontiers in Microbiology*, 9: 1211.
- Yadegarynia, D., Tehrani, S., and Nasiri, M. (2021). Community-and Health Care-Associated Methicillin-Resistant *Staphylococcus Aureus* Infection in Tehran, Iran: Comparison of Drug Resistance and Virulence Determinants. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)*, 21(4): 553-557.
- Yang, X., Zhang, J., Yu, S., Wu, Q., Guo, W., Huang, J., and Cai, S. 2016. Prevalence of *Staphylococcus Aureus* and methicillin-resistant *Staphylococcus Aureus* in retail ready-to-eat foods in China. *Frontiers in Microbiology*, 7, 816.
- Zhan, X. Y., and Zhu, Q. Y. 2018. Evolution of methicillin-resistant *Staphylococcus Aureus*: Evidence of positive selection in a penicillin-binding protein (PBP) 2a coding gene *mecA*. *Infection, Genetics and Evolution*, 59: 16-22.
- Zheng, Y., He, L., Asiamah, T. K., and Otto, M. 2018. Colonization of Medical devices by *Staphylococci*. *Environmental Microbiology*, 20(9): 3141-3153.
- Zhou, L. L., and Yang, C. G. 2019. Chemical intervention on *Staphylococcus Aureus* virulence. *Chinese Journal of Chemistry*, 37(2): 183-193.

APPENDICES

APPENDIX 1. MIC test of VITEK 2 system for *S. aureus*

APPENDIX 2. Sequence 16s DNA for *S. aureus*

APPENDIX 3. Sequence *mecA* for *S. aureus*

APPENDIX 4. *S. aureus* resistant for antibiotics



APPENDIX 1. MIC test of VITEK 2 system for *S. aureus*

bioMérieux Customer:

Microbiology Chart Report

Printed February 15, 2022 8:24:40 AM CS*

Patient Name:

Patient ID

Location:

Physician

Lab ID: *87

Isolate Number:

Organism Quantity:

Selected Organism : *Staphylococcus aureus*

Source:

Collected:

Comments:	

Susceptibility Information	Analysis Time: 8.08 hours	Status: Final
----------------------------	---------------------------	---------------

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Inducible Clindamycin Resistance	NEG	-
Benzylpenicillin	>= 0.5	R	Erythromycin	0.5	S
+Amoxicillin		R	Clindamycin	<= 0.25	S
+Ampicillin		R	Linezolid	2	S
+Amoxicillin/Clavulanic Acid		R	Teicoplanin	<= 0.5	S
+Methicillin		R	Vancomycin	1	S
Oxacillin	>= 4	R	Tetracycline	<= 1	S
+Cefotaxime		R	Tigecycline	<= 0.12	S
+Imipenem		R	Fosfomycin		
+Meropenem		R	Nitrofurantoin	<= 16	S
+Amikacin			Fusidic Acid	<= 0.5	S
Gentamicin	<= 0.5	S	Mupirocin		
Tobramycin	<= 1	S	Rifampicin	<= 0.5	S
Levofloxacin	0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S
Moxifloxacin	<= 0.25	S			

Phenotypes

Antibiotic Family	Detected Phenotypes
BETA-LACTAMS	MODIFICATION OF PBP (mecA)
AMINOGLYCOSIDES	RESISTANT KAN (APH(3')-III),WILD
QUINOLONES	WILD
MACROLIDES/LINCOSAMIDES/ STREPTOGRAMINS	RESISTANT TO STREPTOGRAMINS (SGA-SGB),WILD
OXAZOLIDINONE	WILD
GLYCOPEPTIDES	WILD
TETRACYCLINES	WILD
FURANES	WILD
FUSIDIC ACID	WILD
RIFAMYCINES	RESISTANT (LOW LEVEL),WILD
TRIMETHOPRIM/SULFONAMIDES	TRIMETHOPRIM RESISTANT,WILD

Therapeutic Interpretations

None

MIC/Test Differences

None

Antibiotic Deductions

Deduced Antibiotic(s)	Tested Antibiotic	Reason (rule or phenotype)
Amoxicillin		MODIFICATION OF PBP (mecA)
Ampicillin		MODIFICATION OF PBP (mecA)
Amoxicillin/Clavulanic Acid		MODIFICATION OF PBP (mecA)
Methicillin		MODIFICATION OF PBP (mecA)
Cefotaxime		MODIFICATION OF PBP (mecA)
Imipenem		MODIFICATION OF PBP (mecA)
Meropenem		MODIFICATION OF PBP (mecA)
Amikacin		RESISTANT KAN (APH(3')-III),WILD

Installed VITEK 2 Systems version: 9.02

Page 1 of 1

AES Parameter Set: Global CLSI-based+Phenotypic 2019
Last Modified: Apr 15, 2021 12:27 CDT
Basis: Global CLSI-based+Phenotypic

MIC Interpretation Guideline: Global CLSI-based 2019
Basis: Global CLSI-based
Therapeutic Interpretation Guideline: PHENOTYPIC 2019
Basis: PHENOTYPIC

APPENDIX 2. Sequence 16s DNA for *S. aureus*

s10-sf

CTGGACTCCTTTGACACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAA
AGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGT
TGGGCACTCTAAGTTGACTGCCGGTGACATA

s33-sf

CTGACTCCTTTGACACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAG
TGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTTG
GGCACTCTAAGTTGCTGCCGGTGACAA

s45-sf

CGACTCCTTTGACACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAG
TGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTTG
GGCACTCTAAGTTGACTGCCGGTGACAT

s48-sf

CTGACTCCTTTGACACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAA
GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTT
GGGCACTCTAAGTTGACTGCCGGTGACATA

s67-sf

CGACTCCTTTGACACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTAAGCTTAATTGCCATCATTAAAGTTGG
GCACTCTAAGTTGACTGCCGGTGACATAA

s75-sf

TGACTCCTTTGACACTCTAGAGATAGAGCTTTCCCCTTCGGGGGACAAAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTTGG
GCACTCTAAGTTGCTGCCGGTGACATA

s80-sf

CTGACTCTTTGACACTCTAGAGATAGAGCTTTCCCCTTCGGGGGACAAAG
TGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
AAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTTG
GGCACTCTAAGTTGACTGCCGGTGACATAA

s83-sf

CGACTCTCTGACACTCTAGAGATAGAGTTTTCCCCTTCGGGGGACAAAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
AGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTTG
GCACTCTAAGTTGACTGCCGGTGACAAA

s86-sf

CGACTCTCTGACCCTCTAGAGATAGAGTTTTCCCCTTCGGGGGACAGAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
AGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTTG
GCACTCTAAGTTGACTGCCGGTGACAAA

s87-sf

CTGACTCCTTTGACACTCTAGAGATAGAGCTTTCCCCTTCGGGGGACAAA
GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAAGTT
GGGCACTCTAAGTTGACTGCCGGTGACATA

APPENDIX 3. Sequence *mecA* for *S. aureus*

M10-mf

AAATTCTGGTCGTGCTCACGTAACAAATATGAAGTGGTAAATGGTAATAT
CGACTTAAAACAAACACTACAATCATCAAATTTTTTTTTCTTTGCTAGAG
TACCATTCTGAATTAGGCAGTAAGAAATTTGAAAAAGGCATGAAAGTACTT
GGTGTTGATGAAGATATACCTAGTGATTTTCCTTTTTATAATGCTCAAAT
TTCAAACAAAAATTTAAATAATGAATTATTATTACCTGATTCGGGTACG
GACAAGGTGAAATACTGATTAACCCAATACTTATCCTTTCAATCTATAGC
GCAATAAAAAATAATGGCAATATCCCCCCCCCTCACTTATTAAAAAACAC
AAAAAATGAAGTTAGAAAAAAATTATTATTTCCAAAAAAATATCTATC
TATTAGCTGATGGTATGCAACAAGTCATAAATAAAACACATAAAAAAAAT
ATTTATAGATCTTATGCAATCTTAATTGCCAAACCCCCTACATAAGAATT
AA

M33-mf

GAATCCCTTTGCCCTCTAACTCGTGAGATTCACCTTCAAATATTACTGTT
CAATGGTCTTTTCGACTTAAACAAGCACTACCATCATCTTTTTTTTTTTCT
TTGCTAGAGTAACACTCGAATTAAGCAATAAGAAATTTGAAAAAGGCATG
AAAAAACTAGGTGTTGATGAAGATATACCTAGTGATTTTCTTTTTTATAA
TGCTCTAATTTCAAATTAATAATTTAAATAATGAATTATTATTACCTGATT
CAGGTTACGGACAAGGAGAAATACTGATTAACCCAATACTTATCCTTTCA
ATCTATAGCGCAATAAAAAATAATGGCAATATTACCCCTCCTCACTTATT
AAAAAACACAAAAAACGAAGTTTGGAAAAAAATTATTATTTCCAAAAAA
ATATCAATCTATTAGCTGATGGTATGCAACAAGTCGTAAATAAAACACAT
AAAGATAATATTTATAGATCTTATGCAATCTTAATTGGCGCATCCGGTAC
TTAAAAAAATCAAAAAA

M45-mf

AAGTCTGGAAGGTGGTTACACGTAACAGATATGAAGTGGTAAATGGTAAT
ATCGACTTAAAACAAGCAATACAATCATCAAATTTTTTTTTCTTTGCTAG
AGTACCACTCGAATTAGGCAGTAAGAAATTTGAAAAAGGCATGAAAAAAC
TAGGTGTTGATGAAAATATACCAAATGATTATCCATTTTATAATGCTCAA
ATTTCAAACAAAAATTTAAATAATGAATATTATTAGCTGATTCAGGTTA
CGGACAAGGTGAAATACTGATTAACCCAGTACTTATCCTTTCAATCTATA

GCGCAATAAAAAATAATGGCAATATTACCCCCCTCACTTATTAAAAAAC
ACAAAAACGAAGTTAGGAAAAAAATTATTATTTCCAAAAAAATATCAA
TCTATTAGCTGATGGTATGCAACAAGTCATAAATAAAACACATAAAAAAA
ATATTTATAGATCTTATGCAAACCTTAATTGGCAAACCCGGCCCTAAAAAC
TAAAAAAAGA

M48-mf

TAAAAAATCTGGGGGGGTGGTTAAACGTTACAGATATGAAGTGGTAAATG
GTAATATCGACTTAAAACAAGCAATAGAATCATCAGATATCTTTTTCTTT
GCTAGAGTACCACTCGAATTAGGCAGTAAGAAATTTGAAAAAGGCATGAA
AAAAGTAGGTGTTGGTGAAGATATACCAAGTGATTATCCATTTTATAATG
CTCAAATTTCAAACAAAAATTTAAATAATGAAATATTATTAGCTGATTCA
GGTTACGGACAAGGTGAAATACTGATTAACCCAGTACAGATCCTTTCAAT
CTATAGCGCATTAAAAAATAATGGCAATATTACCCCCCTCACTTATTAA
AAGACACAAAAAACAAAGTTTGGAAAAAAATTATTATTTCCAAAGAAAAT
ATCAATCTATTAGCTGATGGTATGCAACAAGTCGTAAATAAAACACATAA
AGAATATATTTATAGATCTTATGCAAACCTTAATTGGCAAATCCGGTACTG
AAGAAACTAATTAA

M67-mf

AATTCCTGTGTCGATGTTACACGTAACAGATAGGAAGTGGTAAATGGTAC
TATCGACTTAAAACAAGCACTACCATCATCAAATTCCTTTTTCTTTGCTA
CAGTAGCCTTCGAATTAGGCAGTAAGAAATTTGAAAAAGGCATGAAAAAG
CTGGGTGTTGGTGAATAATACCTAGTGATTTTCCTTTTTTATAATGCTCC
AATTTCAAACAAAAATTTAAATAATGAATTATTATTTGCTGATTCGGGT
ACGGACAAGGTGAAATACTGATTAACCCAATACTTATCCTTTCAATCTAT
AACACAATAAAAAATAATGGCAATATCCCCCTCCTCACTTATAAAAAAA
CACAAAAACAAAGTTAGAAAAAATATTATTATTTCCAAAAAAATATCT
ATCTATTAGCTGATGGTATGCCCAAGTCAAAAATAAAACACATAAAAAA
AATATTTATAGATCTTATGCAATCTTAATTGGCAAACCCGGAATTAAAAA
ATAATAAATG

M80-mf

ATATTCTGTGGACATGCTTACACATTACAAATAGGAAGTGGTAAATGGTA
CTTTGACTTAAATCCAGCACTACCATCATCTTTTCTTTTTTCTTTGCTA

CAGTACCACTCGAATTAAGAAGTTGAAATTTGAAAAAGGCATGAAAAAAT
TGGGTGTTGATGACATATACCTAGTGATTTTCCATTTTATAATGCTCAA
TTTCAAACAAAAATTTAAATAATGAATTATTATTTGCTGATTCAGGTAC
GGACAAGGTGAAATACTGATTAACCCAATACTTATCCTTTCAATCTATAA
CGCAATAAAAAATAATGGCAATATCCCCCCCCTCACTTATTAAAAAACA
CAAAAAACGAAGTTTGGAAAAAAATTATTATTTCCAAAAAAATATCAAT
CTATTAGCTGAGGGTATGCCCCAAGTCATAAATAAAACACATAAAAAAAA
TATTTATAGATCTTCTGCCAACTTAATTGGCACATCCCCTACAGAAAAAA
ATAAAAAAGA

M86-mf

AAATTCTTTGTCATGCTTACACGTAACAGATAGGAAGTGGTAAATGGTAC
TATCGACTTAAAACAAACACTACCATCATCTTTTTTTTTTTCTTTGCAAC
AGTACCATTTCGAATTAAGCAATAAGAAATTTGAAAAAGGCATGAAAGTGC
TGGGTGTTGATGAAGATATACCTAGTGATTTTCCATTTTATAATGCTCAA
ATTTCAAACAAAAATTTAAATAATGAATTATTATTACCTGATTCGGGTTA
CGGACAAGGTGAAATACTGATTAACCCAATCCTTATCCTTTCAATCTATA
ACACAAAAAAAATAATGGCAATATCCCCCCTCCTCACTTAATAAAAAAC
ACAAAAAACGAAGTTAGGAAAAAAATTATTATTTCCAAAAAAATATCAA
TCTATTAGCTGAGGGTATGCCACAAGTCATAAATAAAACACATAAAAAAA
ATATTTATAGATCTTATGCAATCTTAATTGCCAAACCCCCAAATAAAAAA
ATAAAAAAAA

M87-mf

CACTTTCTTGCGGGTGCTTCACGTTACATAATAGGAAGTGGTAAATGGTA
ATATCGACTTAAAACAAACAATACCATCATCAAATTTCTTTTTCTTTGCT
ATAGTACCACTCGAATTAGGCAGTAAGAAATTTGAAAAAGGCATGAAAAA
ACTAGGTGTTGGTGAAAATATACCTAGTGATTATCCATTTTATAATGCTC
AAATTTCAAACAAAAATTTAAATAATGAAATATTATTAGCTGATTCAGGT
TACGGACAAGGTGAAATACTGATTAACCCAATACTTATCCTTTCAATCTA
TAGCGCAATAAAAAATAATGGCAATATCCCCCCCCTCACTTATTAAAAA
ACACAAAAAACAAAGTTAGGAAAAAAATTATTATTTCCAAAAAAATATC
AATCTATTAGCTGATGGTATGCACCAAGTCATAAATAAAACACATAAAAA
AAATATTTATACATCTTATGCAAACCTTAATTGGCACACCCGGTACATAAA

APPENDIX 4. *S. aureus* resistant for antibiotics

FOX	P	NO	CIP	CD	GN	AK	VA	CTX	Met	No
R..	R..	S.	R..	S.	R	R	S.	R..	R..	10
R..	R..	S.	R..	S.	R	I	S.	R..	R..	33
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	75
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	80
R..	R..	S.	I	S.	R	S.	S.	R..	R..	83
R..	R..	S.	R	S.	R	S.	S.	R..	R..	86
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	87
R..	R..	S.	S.	I	S.	S.	S.	R..	R..	45
R..	R..	S.	S.	I	S.	S.	S.	R..	R..	67
R..	R..	S.	S.	R	S.	S.	S.	R..	R..	48
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	73
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	82
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	81
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	5
R..	R..	S.	S.	S.	S.	S.	S.	I	R..	8
R..	R..	S.	S.	R	S.	S.	S.	R..	R..	23
R..	R..	S.	S.	S.	R	S.	S.	R..	R..	31
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	37
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	47
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	72
R..	R..	S.	S.	R	S.	S.	S.	I	R..	2
R..	R..	S.	S.	S.	S.	S.	S.	I	R..	44
R..	R..	S.	S.	S.	S.	S.	S.	I	R..	66
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	68
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	69
R..	R..	S.	S.	S.	S.	S.	S.	R..	R..	71
R..	R..	S.	S.	S.	S.	S.	S.	I	R..	76
R..	R..	S.	S.	S.	S.	S.	S.	R.	R..	77
R..	R..	S.	S.	S.	S.	S.	S.	R.	R..	43
R..	R..	S.	S.	S.	S.	S.	S.	R.	R..	74
R..	R..	S.	S.	S.	S.	S.	S.	I	R..	4
R..	R..	S.	S.	S.	S.	S.	S.	R	R..	70
S	R..	S.	S.	S.	S.	S.	S.	S.	S.	9
S	R..	S.	S.	S.	S.	S.	S.	S.	S.	26
R	R	S	S	S	S	S	S	S	R	93

R	R	S	S	S	S	S	S	I	R	1
R	R	S	R	R	S	S	S	S	R	3



CURRICULUM VITAE

Personal Information

Name and Surname : Mohammed Hameed Ahamed AL-SABBAGH

Education

MSc Çankırı Karatekin University
Graduate School of Natural and Applied Sciences 2019-Present
Department of Biology

Undergraduate Baghdad University
Faculty of Natural and Applied Sciences 2003-2006
Department of Biology