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Department of Public health

Molecular Characterization Of *Staphylococcus aureus* Isolated From Milk and Milk products

Thesis

Submitted to the council of the College of Veterinary Medicine/University of Kerbala , as a Partial Fulfillment of the Requirement for the Degree of Master in Science of Veterinary Medicine / Public Health

By

Mohsen Yaseen Taha

Supervised by

Asst.Prof Dr. Ali Jasim Jafer **Asst.Prof Dr. Hayder . Ali Mohammed**

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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We certify this thesis entitled (Molecular Characterization Of *Staphylococcus aureus* Isolated From Milk and Milk products) was carried out by(*Mohsen yaseen Taha*) has been prepare under us supervision at the college of Veterinary Medicine/ University of Kerbala in partial fulfillment of the requirements for the Degree of Master in the Sciences of Veterinary Medicine / Veterinary Public health

Supervisor:

Asst. Prof. Dr. Ali Jasim Jafer

Co-advisor:

Asst. Prof .Dr.Hayder Ali Mohammed

The recommendation of the Department

In the view of the above recommendation, I forward this thesis for scientific discussion by the examining committee

Asst.Prof .Dr. Ihab Ghazi Mahdi

Vice Dean for Postgraduate studies and scientific Affairs

College of Veterinary Medicine

University of Kerbala

Committee Certification

We, the members of the examining committee , certify that we have read this thesis entitled :(Molecular Characterization Of *Staphylococcus aureus* Isolated From Milk and Milk Products) Presented by the (Mohsen Yaseen Taha) from the Department of (Public Health) and We have examined the student in its contents, We have found that it is adequate for award of Degree of Master in Science of the Veterinary Medicine (Public Health

Asst.Prof .

Dr.Ali Redha Abid

College of Veterinary Medicine

University of Kerbala

Chairman

Asst.Prof .
Dr.Juman Khaleel AL-Sabbagh

College of Veterinary Medicine

University of Kerbala

Member

Asst.Prof.

Dr.Hayder Ali Mohammed

College of Veterinary Medicine

University of Kerbala

(Member & Supervisor)

Asst.Prof.
Dr. Makki Kaalaf Hussein

Technical College of Al-Musib

University of AL-Furat AL-Awsat

Member

Asst.Prof.

Ali Jasim Jafer

College of Veterinary Medicine

University of Kerbala

(Member & Supervisor)

Asst.Prof.Dr.Ali Redha Abid

Head of Department of Public Health

Prof.Dr.Wefak Jbori Al-bazi

Dean of the College

Certification of Linguistic Elevator

I Certificated this thesis entitled (Molecular Characterization Of *Staphylococcus aurous* Isolated From Milk and Milk Products) from the student (Mohsen Yaseen Taha) Linguistically reviewed by me and necessary correction and has been made it Linguistically ready For examination .

Linguistic Evaluator

Instructor Dr. Hamid Gitban Jawad

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I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

Mohsen Yaseen Taha

/ / 2023

Dedication

To almighty Allah Creator of the heavens and the earth

I present fragments of my humble research as a gift to my master

To the soul of my father, may God have mercy on him

For whom is that paradise under her feet. My mother

To my wife who supported me and encouraged me in every step and to my children Nour, Areej, Muhammad and Ahmed

To my friends and colleagues in my studies and all my teachers

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I would like to thank everyone who supported me to successfully complete my researc*h*

Mohsen

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Summary

Staphylococcus aureus is a pathogenic bacterium-contaminating milk and milk products causing food poisoning primarily due to its enterotoxins Production, it possessed a repertoire of virulence factors, multiple antibiotic resistance and its capability to biofilm formation , in addition to its capacity to gain new resistance genes via integron, which could lead to difficult treatment. For these reasons, the aim of study to determine the prevalence rate ,accurant some virulence pattern and biofilm formation in *S,aureus* isolated from milk and milk product (mecA ,femA, Icaa and Icad) genes .

Study was conducted to collect a total of 300 samples divided into six groups 50 Samples for each (cow raw milk ,imported milk ,imported dairy products ,locally dairy products (factory) ,farm dairy product and buffalo raw milk),the study was beginning from October 2022 to February 2023. The samples were collected randomly from different locations in Karbala city .The samples were manipulated using bacteriological and biochemical methods for isolation and identification of *S. aureus*, then biofilm production and antimicrobial susceptibility tests were done. Molecular tools were used to study the prevalence some virulence genes (mecA,femA,Icaa and Icad) and the ability of *S.aureus* to produce β-lactamase enzyme was examined by rapid iodiometric method.

The result of our study revealed of 300 samples cultured and identified,121 (40,3%), isolates were characterized as *S. aureus*,it was distributed as cow raw milk 17 (34%),imported milk13 (26%),imported dairy products 18 (36%),locally dairy products (factory) 23 (46%),farm dairy products 30 (60%)and buffalo raw milk 20 (40%). Regarding resistance rates of *S. aureus*, the isolates were most frequently resistant to penicillin G 61 (50.77%) and oxacillin 49 (40.49%) but more susceptible to ciprofloxacin (100%) gentamycin(100%), Azythromycine 89 (73.66%), Erythromycin 77 (63.24%) ,Tetracycline 52 (42.9%)

.

Furthermore, the molecular prevalence and percentage of Methacillin resistance antibiotic depending on the detection of the mecA genes was 49 (40,49 %) and remaining 72 (59,50). the molecular prevalence of MRSA among different groups was recorded as, cow raw milk 7(41,17%),imported milk 6 (46,15%),imported dairy products 7 (38,88%),locally dairy products (factory) 9 (39,13%),farm dairy products 12 (40%) and buffalo raw milk 8 (40%) .

The rate of biofilm production in overall *S. aureus* was 100% this result was divided into three phases (strong, moderate and weak)formation the percentage of these phases among different milk and milk products was ,strong formation 74 (62,5%) moderate formation 28 (22,08%) and weak formation 19 (15,42%)

On the other hand the number and percentage of Icaa gene in all sample of *S.aureus* was 81 (67.86%) distributed among the study groups it as ; Cow raw milk 12 (70.59%), imported milk 10 (76.92%),imported dairy products.14 (77.87%) , Locally dairy products(factory) 15 (65,22%),farm dairy product 20 (66.67%)and buffalo raw milk 10 (50%) while the result was form Icad was 77 (64.24%) among the groups of milk and milk products; cow raw milk 11 (64.71%),imported milk 8 (61.54%),imported dairy products 15 (83.33%),locally dairy products (factory)14 (60.87%),farm dairy products18 (60%)and buffalo raw milk 11
(55%).

All *Staphylococcus aureus* have ability to produce the beta-lactamase enzyme by a rapid direct iodine method by useing starch-iodine complex and most of strain are strong biofilm formation.

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List of abbreviation

Abbreviation	Meaning
Aap	accumulation associated proteins

Agr	accessory gene regul|ator
AMR	antimicrobial resistance
CA-MRSA	community-associated MRSA
CLSI	Clinical and Laboratory Standards Institute
CoNS	coagulase negative staphylococci
Clf	clumping factor
CoPS	coagulase positive staphylococci
CP5	capsular polysaccharide 5
CP8	capsular polysaccharide 8
CWP	cell wall-anchored proteins
DNase	Staphylococcal Nucleases
Embp	extracellular matrix binding protein
EPS	extracellular polymeric substance
ESBL	extended spectrum-beta-lactamase
ETs	Staphylococal Exfoliative Toxin
femA	Factor essential for methicillin resistance
FnBP	fibrinogen binding protein
HA-MRSA	hospital-associated MRSA
Ica A	Polysaccharide Intracellular adhesion
mecA	Gene coding for penicillin-binding protein type (2α)
MLST	multi locus sequence typing
MRSA	methicillin-resistant Staphylococcus aureus
MSSA	Methicillin- susceptible Staphylococcus aureus
Omps	outer membrane proteins
PBP	penicillin-binding protein
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PGN	Peptidoglycan
PVL	Panton-Valentine Leukocidin
PFGE	pulsed-field gel electrophoresis
PIA	polysaccharide intercellular adhesion
Sar	staphylococcal accessory regulator
SCC mec	Staphylococcal Cassette Chromosome mec Typing

SSSS	staphylococcal scalded skin syndrome
Spa	surface binding protein A
TSST-1	Toxic shock syndrome toxin
UTI	Urinary Tract Infection

1.Introduction

Milk is a source of many essential nutrients including proteins, lipids, carbohydrates, vitamins, and minerals, It is widely consumed in various forms all over the world and represent a crucial part of the human diet

(Claeys *et al., 2014*;Alegbeleye *et al., 2018*). A high demand for milk and milk products by the increasing human population has led to a growing interest and concern for quality and safety of milk products (Suh, 2022).

Staphylococcus aureus, including Methicillin resistance *Staphylococcus aureus* , is a major pathogen responsible for severe nosocomial and community-associated infections of humans and infections of economically important livestock species (Fitzgerald, 2012). *Staphylococcus aureus* can colonize in a wide range of animals, including the domestic animals, wildlife, and food production livestock (i.e., pigs, cattle, sheep, goat, chicken, and turkey) (Cuny *et al., 2015*).

Methicillin resistance occurs due to the production of an altered penicillin-binding protein (PBP2a) with a low affinity for all penicillin classes. mecA gene encodes PBP2a(Archer and Niemeyer,1994) and its expression is regulated by associated repressor and inducer genes such as mecR, mecI, ccr, and by various other *S. aureus* genes like fem (factors essential for methicillin resistance) and aux (auxillary genes).(Ito *etal.,2003*) These genes have been reported to also have importance in the expression of methicillin resistance, in addition to mecA(Hegde *etal.,2001*).

Bacteria on biofilm structures are protected from environmental conditions, antimicrobial agents, and host immune responses, and they also exhibit up to 1000-fold increased antibiotic resistance to a wide range of antimicrobial agents, thus leading to persistence of infection (Chen *et al., 2018*) . Treatment of biofilm-related infections has become an important part of antimicrobial chemotherapy because biofilms are not affected by therapeutic concentrations of antibiotics (Fabres-Klein *et al.,2015).*

Multiple genes are responsible for biofilm formation in *S. aureus*. The genes present in ica(intracellular adhesion) locus (icaA and icaD) play a significant role in biofilm formation (Aslantaş Ö, and Demir C., 2016) .

β-lactamase is the predominant extracellular enzyme synthesized after exposure of *S. aureus* to β-lactam antibiotics (Cies *et al.,2018)*. The enzyme is encoded in the plasmid or chromosome and its expression can either be constitutive or inductive. It deactivates the drug by cleaving the β-lactam ring. The hydrolytic ability of β-lactamase in conferring resistance in *S. aureus* largely depends on its location, kinetics, quantity Physiochemical conditions and interplay of determinants (Livermore, 1995).

Objective of this study.

1.The study was determine the prevalence and the contamination rate of *staphylococcus aureus* in raw milk , milk products and differentiation of *Staphylococcus aureus* MRSA and MSSA in Karbala province.

2. The aim of study isolation of bacteria by the molecular technique via conventional PCR by study the some virulence genes (mec A, fem A ,Icca and Icad).

3.Determination the bacteria resistance for different antibiotic and beta-lactamase production enzyme via idometric assay.

2. Review of Related Literatures

2.1 General characteristics of bacteria

The family *Staphylococcaceae* contains 98 properly disseminated species within nine genera comprising *Abyssicoccus, Aliicoccus, Aureococcus, Corticoccus, Jeotgalicoccus, Macrococcus, Nosocomiicoccus, Salinicoccus, and Staphylococcus Some* Members of this family are Gram-positive, (Madhaiyan *et al., 2020*) non-sporous, spherical or spherical cells with sizes ranging from 0.5 to 2.5 µm, nonmotile, occurring single pairs or tetrapods(Tania *et al., 2021*), strictly aerobic to anaerobic, catalase-positive (typically)(Bitew *et al., 2021*), Among this family, the most populous species is Staphylococcus with 55 properly propagated species and 23 subspecies(Madhaiyan *et al., 2020*).

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2.1.1The *Staphylococcus* genus

Staphylococcus genus belongs to the *Staphylococcaceae* family (Silva *et al., 2021*). According to the 16S rRNA analysis, the Staphylococcus genus is divided into 62 species and 30 subspecies (Kayili *et al*., 2012). composed of non-motile facultative anaerobic Gram positive cocci that appear as clusters under microscopic examination and are, with some exceptions, catalase positive(Naureen *et al., 2022*).

Staphylococci have two groups using the coagulase test. It is assumed that coagulase positive *staphylococci* (CoPS) are usually pathogenic, even when in some cases they can cause asymptomatic colonization in healthy individuals, and coagulase negative *staphylococci* (CoNS) are saprophytic or cause opportunistic infections (Martín *et al., 2020).*

2.1.2 *Staphylococcus aureus*

Staphylococcus. aureus belongs to the genus *Staphylococcus*, is positive for Gram stain, ~0.8 μm in diameter, arranged in a "string of grapes" under a microscope(Naureen *et al., 2022*), an aerobic or anaerobic; and grows optimally at 37°C, and at pH7.4 . The colonies on blood agar plate are thick, shiny, and round with a diameter of 1~2 mm(Bulock *et al., 2021)* .

Most of them are hemolytic, forming a transparent hemolytic ring around the colonies on blood agar plates due to production of four types of haemolysins (alpha, beta, gamma and delta)(Bulock *et al., 2021*) . Moreover, *S. aureus* does not form spores or flagella, but possesses a capsule and can produce golden yellow pigment, and decompose mannitol that can grow in up to 10% salt (Guo *et al., 2020*).

The yellow or golden colour of the colonies is imparted by carotenoids produced by the organism typical biochemical identification tests include catalase positive (all pathogenic *Staphylococcus* species)(Durrani, 2021), coagulase positive (to distinguish *S. aureus* from other *Staphylococcus* species), novobiocin sensitive (to distinguish from *Staphylococcus* saprophyticus), and mannitol fermentation positive to distinguish from

Staphylococcus epidermidis (Taylor and Unakal, 2017)

The successful colonization of *S. aureus* is due to the large number of virulence factors that include the production of a large number of enzymes and toxin enhance the virulence of this microorganism (Adame *et al., 2020)*

2.1.3 History and Classification of *Staphylococcus aureus*

In 1880, the Scottish surgeon Sir Alexander Ogston first described staphylococcus in pus from a surgical abscess in a knee joint(Algammal *et al*., 2020) and he involvement in wound infections was established in the 1881 report , who described the formation of new abscesses in guinea pigs and mice injected with pus taken from his patients.

He called the clusters of bacteria he observed in the abscesses “staphyle” come From the Greek staphyle (bunch of grapes) and kokkos (berry) because the arrangement of the bacterial cells resembled a cluster of grapes (Adhikari, 2021) .In 1884, the German physician Friedrich Julius Rosenbach differentiated the *staphylococci* by the color of their colonies*: S. aureus* (from the Latin aurum, gold) and *S. albus* (Latin for white*). S. albus* was later renamed *S. epidermidis* because of its ubiquity on human skin (Adhikari, 2021).

Taxonomic opener for the genus *Staphylococcus* as reported(Milner, 2015)(Berman, 2019):

Domain: *Bacteria*

Phylum*: Firmicutes*

Class: *Bacilli*

Order*: Lactobacillales*

Family: *Staphylococcaceae*

Genus: *Staphylococcus*

Species*: Staphylococcus aureus*

2.2. Pathophysiology

Staphylococcus. aureus are one the most common bacterial infections in humans, including infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), septic arthritis, gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections(Taylor and Unakal, 2021) .

It is one is a leading causative agent in pulmonary infections (e.g., pneumonia and empyema) and other respiratory tract infections, surgical site, prosthetic joint, and cardiovascular infections (Cheung *et al.*, *2021*).Osteomyelitis is an infection of bone that can result from contiguous spread from surrounding tissue, direct bone trauma due to surgery or injury, or haematogenous spread from systemic bacteraemia or haematogenous spread from systemic bacteraemia it remains a significant health-care burden with a prevalence of ~22 cases per 100,000 person-years , and its incidence has been rising over time, especially in the elderly (Masters *et al.*, *2022)*

Staphylococcus aureus bacteremia remains a distinct entity in the realm of infectious disease, singular in its ability to adhere to vascular structures,

cause deep-seated infections, disseminate, and result in a high mortality of those cases (Suarez *et al., 2021)* . .

Staphylococuss. aureus is one of the most common bacteria isolated from burn infections. *Methicillin Resistance Staphylococcus aureus* including hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) have been reported as important bacterial causes of burn wound infections(Tajik *et al., 2019*).Urinary Tract Infection (UTI) is the invasion and subsequent multiplication of microorganisms anywhere in the urinary tract infection is one of the most common infectious diseases causing over 150 million cases per year (Belete and Saravanan, 2020)

Staphylococcus. aureus also can make use in an opportunistic method of primary harm done by other pathogens or predisposing conditions. This occurs, for example, in lung infections that have been initiated by a viral infection such as the flu, in which *S. aureus* secondary infection is often the ultimate cause for death(Cheung *et al*., 2021)

2.3. Methicillin-resistant Staphylococcus aureus (MRSA)

Fleming discovered penicillin in the 1940s and pioneered the era of antibiotics for infection treatment (Wenzel, 2020). At the time, the infectious diseases caused by *S. aureus* were well-controlled, but with the widespread use of penicillin in the 1950s, penicillin-resistant *S. aureus* appeared in the clinic(Guo *et al., 2020*).

Penicillin-resistant *S. aureus* can produce penicillinase, which can hydrolyze the penicillin βlactam ring, leading to resistance to penicillin. Later, scientists developed a new penicillinase-resistant semisynthetic penicillin named methicillin, which is resistant to the hydrolysis of β-

lactamase (Tyagi *et al., 2021*). After being applied to the clinic in 1959, methicillin effectively controlled the infection of penicillin-resistant *S. aureus* (Sharma *et al., 2021*).

However, only 2 years after methicillin was applied, in 1961, British scientist Jevons reported the isolation of an MRSA strain; this resistance was produced by a gene encoding the penicillin-binding protein 2a or 2′ (PBP2a or PBP2′) (mecA) which was integrated into the chromosomal element (SCCmec) of methicillin-sensitive *S. aureus*(Dinescu *et al., 2021)* .

Moreover, MRSA has rapidly become the most frequently occurring resistant pathogen identified in many parts of the world, including Europe, the United States, North Africa, the Middle East and East Asia (Romero and de , 2021). According to its original source, MRSA is classified into hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) . In China, the proportion of hospital-acquired MRSA has reached 50.4% (Gupta *et al., 2021).*

2.4. Incidence of MRSA in milk and milk products

There are a high number of scientific works focused on the study of *S. aureus,* including MRSA, in milk and milk products in many countries. However, in most of them, the main objective was to detect the presence of methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA without any molecular characterization of the isolated strains. In Italy, various studies were conducted to assess the presence of MRSA in milk (cow, sheep, goat, and buffalo milk) and milk products Six MRSA strains were isolated from milk and cheese samples out of 1634 foods of animal origin (Normano *et al., 2007*).

Prevalences ranging between 0.34% and 8.3% were observed in other studies, demonstrating the probable role of milk and milk products in the transmission of MRSA strains through the food chain (Normano *et al*., 2020).

Studies from Greece revealed prevalences of 10%, 11.1%, and 8.3% in bovine bulk tank milk (Angelidis *et al.,(2019*) The same authors revealed an MRSA prevalence of 14.3% in ovine bulk tank milk (Papadopoulos, *et al., 2019).* However, (Pexara *et al*.,(2016) isolated only one-MRSA strain in ovine milk out of 175 samples of ovine and caprine raw bulk tank milk samples in the region of Thessaly, central Greece.

In Germany, a survey conducted by(Kreausukon *et al.,(2012*) reported an MRSA prevalence of 4.4% in bulk tank milk of dairy herds.(Ariza-Miguel *et al.,(2014)* identified both mecA-MRSA and mecC-MRSA in dairy sheep farms in Spain, with values of 1.31% and 0.44%, respectively. No MRSA strains were detected both in cow and goat milk in Poland (Rola, Korpysa-Dzirba, *et al*., 2015).

In Great Britain, (Cui *et al*.,(2021) isolated both mecA and mecC MRSA from bulk tank milk, with values of 0.29% and 0.57%, respectively. (Tegegne *et al*.,(2018) announced a high MRSA contamination level (28.6%) in milk produced in Republic Czech. Recently, in Portugal,Oliveira *et al.,(2022)* reported a prevalence of 8.1% in bulk tank milk -

In the Asian continent, only few studies identified MRSA strains in raw milk and milk products. In China, (Song *et al*.,(2015) observed an MRSA prevalence of 12.1% out of 248 milk samples. In another study was conducted in the northern part of China, a prevalence of 8.2% was reported

(Liu *et al*.,(2017). Recently, a low prevalence of 0.7% was observed in Shandong dairy farms (Zhao *et al*., 2021) and (Cai *et al*.,2021) reported a prevalence of 11.3% in Kazak cheese in Xinjiang. (Mahanti *et al*.,2020) observed an MRSA contamination of 9.6% in raw milk produced in India.

A higher incidence of MRSA in milk and milk products was found in Iran, with a value of 16.2% (Jamali *et al*., 2015). In Turkey,(Keyvan *et al*.,2020) reported a high prevalence of 75.4%.(Elal Mus *et al*.,2019) identified a value of 22.8% out of 650 samples of milk (cow milk and sheep milk) and dairy products (cheeses, yoghurt, butter, and ice cream). However, a low MRSA contamination (9%) was observed in study conducted by (Saka and Terzi Gulel ,2018).

Recently,(Taban *et al*.,(2021) reported the presence of both MRSA-mecA and MRSA-mecC among isolates from raw milk and traditional artisanal dairy foods. In Saudi Arabia, (Yehia *et al*.,2020) isolated MRSA strains from pasteurized camel milk. A high incidence of MRSA (72.8%) was reported by (Alghizzi and Shami ,2021) among strains isolated from milk and dairy products in Riyadh, Saudi Arabia

In America, a study was conducted in USA in a Minnesota dairy farm found two MRSA-positive samples (4%) out of 150 pooled bulk tank milk samples (Haran *et al., 2012*).(Gonzalez *et al.,2017*) isolated seven MRSA strains (3.3%) from one menas frescal cheese sold at city of Niteroi (Brazil). The same finding was reported by (Herrera *et al.,2016*), who reported the detection of MRSA strains in raw milk fresh cheeses in Colombia. In Mexico, an incidence of 14% was observed in dairy products (Avila-Novoa *et al., 2021*)

In the African continent, a high number of papers describing the presence of MRSA in foods (including milk and milk products) made from healthy animals were pub-lished. A study from Tunisia revealed the presence of MRSA in 0.8% of milk samples (Khemiri *et al.,2018*). In Algeria, (Chaalal *et al.,2018*) isolated MRSA strains from20.3% of raw milk and pasteurized milk samples.

Low prevalence were observed in studies conducted in other areas of Algeria, with rates of 4.1% (Titouche *et al*.,2019)and 6% (Titouche *et al.,2020*). In Egypt, (Kamal *et al*.,2013) reported a low prevalence of MRSA (5.3%) in raw milk and milk products, and similar results were obtained by (Ahmed *et al*.,2019). However, high prevalence were reported in other studies conducted in Egypt(Sadat *et al*.,2022).

Low prevalence were reported in Nigeria (Aliyu *et al.,2021*)and Mozambique (Nhatsave *et al.,2021*), with values of 5% and3%, respectively. However, a high rate of 38.5% was found by (Lemma *et al*.,2021) in Addis Ababa, Ethiopia .The presence of MRSA in milk and milk products has been frequently reported worldwide over the last two decades. Overall, there is a clear variation in the MRSA prevalence in milk and milk products, according to the type of products and countries.

The maximum values were observed in Saudi Arabia (Alghizzi and Shami,2021)and Egypt (Zaydaetal.,2020), with a values of 72.8% and60%, respectively. However, the minimum values were assigned to bulk tank milk in Great Britain (0.29%)(Cui*et al.,2021*) and sheep milk in Italy (0.57%)(Carfora *et al.,2016*).

It is important to notice that the frequencies of MRSA isolation in milk and milk products differed between studies conducted in different areas among different countries or even regions within the same country which might reflect the heterogeneity of the methods used and factors such as the type of tested samples (geographical origin, manufacturing technology, use of pasteurized or raw milk, sample storage, and handling), sensitivity of the MRSA screening methods, and the sample size(Al-Ashmawy *et al.,2016;* Gharsa *et al.,2019*)

2.5. Virulence Factors of *Staphylococcus Aureus*

2.5.1.Capsular Polysaccharides

Staphylococcus aureus has developed many mechanisms to escape from human immune responses. The first shielding mechanism is represented by the formation of a capsule, a polysaccharide structure surrounding the bacterial cell wall(Huitema *et al., 2021*) . The two main serotypes was produced by clinical *S. aureus* strains are the serotype consisting of capsular polysaccharide 5 (CP5) and capsular polysaccharide 8 (CP8) are produced by a 75 % to 80% of *S. aureus* isolates from humans and play a significant role in the pathogenesis of staphylococcal infections(Suligoy *et al., 2020*).

2.5.2 Cell Wall Components

The cluster cell wall is characterised by a complex structure of polysaccharides that give rigidity and strength, The outer structure of the cell, which helps in the process of adhesion(Dinescu *et al., 2021*), consists of a layer with a thickness of 20-30 nanometers of peptidoglycan (PGN). Apart

from being a protective barrier for bacteria, PGN has other functions such as being a scaffold, where it can cleave surface proteins essential for bacterial virulence.

Because of the critical role it plays in maintaining the structure and growth of bacteria(Jin *et al., 2021*). Furthermore, the cell wall controls the tactile response of bacteria, influencing a wide range of behaviours such as cell adhesion, environmental sensing, or host defence evasion (Kumar *et al., 2022*).

2.5.3. Surface Associated Protein

2.5.3.1- Staphylococcal Protein-A

Protein A (spa A) is a component of the cell wall of *Staphylococcus aureus* strains and is a surface protein It is located within the adhesion molecules (MSCRAMMs) which is encoded by the spa gene (Vlaeminck *et al., 2020*). It is a cell wall-mounted protein containing immunoglobulin-binding domains that binds the Fcγ portion of human IgG antibodies and Fab to certain IgM subtypes, Protein A binding to the Fcγ portion of IgG limits antibody-mediated phagocytosis. Antibodies are essential for the immune response against bacteria. To catalyse the killing of bacteria, the antibodies must bind to the bacterial cell and catalyse the complement reaction. Interestingly, pathogenic bacteria produce IgG-binding molecules that specifically bind to the Fc region required for the hexamerase process (Ford *et al., 2020*).

2.5.3.2- Clumping Factor

There are two types of clumping factor in *S. aureus*, clumping factor A and B, which are encoded by the specific genes. The bacterial Clf A and Clf B accumulate in blood plasma, increase adhesion to fibrinogencoated surfaces, and cause endocarditis. The Clf A gene is produced during bacterial growth which enables the bacteria to attach to surfaces that have fibrinogen in its composition, while the Clf B gene is only produced during the logarithmic phase which enables the bacteria to colonize only the nose(Algammal *et al., 2020).*

2.5.4 .Extracellular Toxins

2.5.4.1.*Staphylococcal* Hemolysins

Hemolysins are important virulence factors for *S. aureus* that contribute to bacterial invasion and escape from the host's immune response and cause tissue damage and facilitate spreading and nutrients uptake. Witch hemolysins α, β, γ, δ are unique in that they drill pores in the membrane, leading to the efflux of vital molecules and metabolites (*Pérez et al., 2020).*α-Hemolysin, also known as α-toxin, is the most prominent cytotoxin which damages a wide range of host cells including epithelial cells, endothelial cells, erythrocytes, monocytes, keratinocytes and it damages cell membrane and induces apoptosis (Bennett and Thomsen, 2020).

2.5.4.2. *Staphylococcal* Enterotoxins

Staphylococcus aureus produce several exoproteins including *staphylococcal* enterotoxins , exfoliative toxins , leukocidin Toxic shock syndrome toxin (TSST-1) and staphylococcal enterotoxins are a subset of the superantigen family, collectively termed as pyrogenic toxin

superantigens , The staphylococcal enterotoxins are superantigens which trigger T-cell activation and proliferation; their mode of action probably includes activation of cytokine release and cell death via apoptosis and potentially lethal toxic shock syndrome(Shettigar and Murali, 2020).

Enterotoxins are resistant to heat, digestive enzymes, denaturing agents, and a wide range of pH. Hence, it does not degrade in the digestive tract, can pass through the stomach and attack the intestinal cells Among these enterotoxins, the five classical types (A, B, C, D, and E) are the most important and are responsible for 95% of staphylococcal food poisoning and resistant to the action of intestinal enzymes and are a cause of food poisoning plays a role in vomiting and diarrhea (Mahfoozi *et al., 2019).* Enterotoxin genes there is a toxin element on the chromosome element that reacts with the accessory genetic elements (Bae *et al., 2021*).

2.5.4.3. Panton-Valentine Leukocidin

It is one of the most important virulence factors in bacteria, as PVL is a potent cytotoxin . The active toxin causes the decomposition of neutrophils by forming pores on their membranes, this toxin causes channels open to Calcium, necrosis, and leukocyte programmed death(Divyakolu *et al*., 2019).

It is associated with skin necrosis, chronic mucosal dermatitis, recurrent mucocutaneous infections, and necrotizing pneumonia. Moreover, the breeds that It contains pvl enzyme, which has a high virulence and is often accompanied by the occurrence of boils, skin abscesses and severe infections And the wound aggravated (Shettigar and Murali, 2020).

2.5.4.4. Staphylococal Exfoliative Toxin (ETs)

The cutaneous toxins *in S. aureus* contain two distinct proteins of the same weight molecular, exfoliative toxin A is encoded by the *eta* gene located on the chromosome and is heat stable (resisting boiling for 20 minutes) and exfoliative toxin B is encoded on a plasmid and is not stable in temperature (Bennett and Thomsen, 2020).

These epidermal toxins consist of secreted serine proteins that attack the skin by cleaving cadherins and destroying cell-cell adhesions and epidermal junctions. cause coccal burn skin syndrome aureus staphylococcal scalded skin syndrome(SSSS) By lysis of the epidermal layer and bullous impetigo, toxins are considered mucopolysaccharide matrix superantigens (Singh and Phukan, 2019).

2.5.4.5.Toxic Shock Syndrome Toxin

Most strains of *S. aureus are* isolated from toxic shock patients produce a toxin called 1-toxic shock syndrom, which is encoded by the tst gene Interestingly, the gene encoding this toxin is carried by only a limited number of strains. by *S. aureus* these toxins bind Major Histocompatibility complex(MHC) class II molecules with T-cell receptors, thus activation of T cells leads to a cytokine storm the lethality of, such as IL-8 and MIP-3α, IL-2, and TNF, activation of immune cells will enhance inflammation and cause mucosal cell barrier disruption, allowing further interaction of the toxin with T-cells and macrophages, leading towards toxic shock syndrome disease known as toxic shock syndrome, Symptoms include high fever, rash and hypotension(Dinescu *et al., 2021)*

2.5.5. Extracellular Enzymes

2.5.5.1. Staphylococcal Nucleases (DNase)

In 1956 *Staphylococcal* nuclease (DNase) was firstly recognized as culture filtrates of *S. aureus*. Staphylococcal nuclease is an extracellular enzyme, produced by various microorganisms that are heat-stable and Ca2+ dependent, two types are found: endo- and exonuclease that destroy the DNA and RNA substrates(Samani *et al., 2021*).

The total genome sequencing revealed two various types of staphylococcal nuclease genes: *nuc* and *nuc2* the main difference between Nuc and Nuc2 is the cell-localization: nuc is an extracellular enzyme with two various isoforms, *NucB* and *NucA,* while *nuc2* is surface-bound(Algammal *et al., 2020).*

2.5.5.2 Staphylococcal Coagulase

S. aureus bacteria produce coagulase that causes plasma coagulation in the host. It causes conversion of fibrinogen to fibrin and fibrin production may protect staphylococci from phagocytosis(Javid *et al*., 2018)(Singh and Phukan, 2019).Coagulase a protein-like enzyme become enzymatically active by binding to prothrombin and begin the polymerization of fibrin, and this coagulation results in the deposition of fibrin on the surfaces of bacteria cells, thus preventing The process of being ingested by phagocytes .This enzyme is encoded by the Coa gene it is a major gene that distinguishes *Staphylococcus aureus* from other cocci The Coagulase includes two types, the bound coagulase, which detects It is detected by the glass slide method

and the free coagulant enzyme, which is detected by the tube method(GonzálezMartín *et al., 2020)* .

2.5.5.3. Catalase

Catalase is an antioxidant that is a defense mechanism that contributes to the protection of *Staphylococcus aureus* from phagocytosis within neutrophils(Buvelot *et al*., 2017), that separates hydrogen peroxide (H_2O_2) into molecular oxygen (O2) and water (H2O) and thus remains undigested Staphylococcus inside neutrophils Catalase has a molecular weight It is equal to 250 kDa and consists of four protein groups (Hadwan, 2018) .

2.5.5.4. Staphylokinase

Bacterial staphylokinase is an extracellular enzyme that stimulates the plasminogen for the lyses of fibrin clot with subsequent bacterial propagation is an extra cellular protein composed of 136 amino acids synthesized through the late exponential growth phase of lysogenic *S.aureus*(Aziz and Noori, 2020) .

It has ideal fibrin specific plasminogen activator and clot specificity converting a precursor plasminogen to plasmin which results in dissolving a blood clot along with destroying the natural components of the blood clotting system, leading to life-threatening as well as death consequence. After activation, plasmin cleaves variable substrates such as fibrin and extracellular matrix proteins and activates proteases and other growth factors

then fibrinolysis including staphylokinase and plasmin are implicated in a variety of physiological and pathophysiological procedures for example wound curing, irritation, cell migration, embryogenesis, cancer development, metastasis, angiogenesis and atherosclerosis(Faujdar *et al., 2019*).

2.5.5.5. Staphylococcal Proteases

S. aureus secretes three types of staphylococcal proteases is serine, proteases, metalloproteases, and 3-cysteine proteases. They play a significant role in host-defense evasiveness and bacterial dissemination that they interact with neutrophils, plasma proteins and antimicrobial peptides to weaken host immunity Expression and synthesis of these proteases is mainly modulated by two global regulatory elements, one is sarA (staphylococcal accessory regulator) and other one is agr (accessory gene regul|ator). agr expression is directly or indirectly regulated by sarA which in turn modulates virulence determinant synthesis(Algammal *et al., 2020).*

2.6. Antibiotic Resistance

Emergence of resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans worldwide. Multidrug-resistant organisms have not only emerged in the hospital environment but are now often identified in community settings, suggesting that reservoirs of antibiotic-resistant bacteria are present outside the hospital (Munita and Arias, 2016) .

The bacterial response to the antibiotic “attack” is the prime example of bacterial adaptation and the pinnacle of evolution. “Survival of the fittest” is

a consequence of an immense genetic plasticity of bacterial pathogens that trigger specific responses that result in mutational adaptations, acquisition of genetic material, or alteration of gene expression producing resistance to virtually all antibiotics currently available in clinical practice. Therefore, understanding the biochemical and genetic basis of resistance is of paramount importance to design strategies to curtail the emergence and spread of resistance and to devise innovative therapeutic approaches against multidrug-resistant organisms(Majumder *et al., 2020*).

2.6.1. Resistance of *S. aureus* to Antibiotics

Discovery of antibiotics has been one of the greatest medical achievements of the twentieth century. Regrettably, their excessive, unreasonable, and inappropriate use has led to the selection and expansion of resistant bacterial strains and dramatically increased treatment failure ratio. Bacteria have developed many different mechanisms of resistance(Foster, 2017).

The ability of bacteria to resist antibiotics began with its resistance to penicillin, which was used as a treatment in the early 1940s, when strains resistant to it quickly appeared in 1946, and to avoid this problem and overcome the spread of those strains, cephalosporins were used as effective antibiotics against these bacteria in 1960(Masumi *et al., 2022*).

And soon, new strains that were resistant to cephalosporins also appeared, and then other antibiotics followed, such as erythromycin, tetracycline, streptomycin, gentamicin and chloramphenicol, with the emergence of strains resistant to the antibiotic phenylphenyl 59 and the semiillin, which was introduced in the year 19 To reduce the spread of penicillin-resistant

cocci and after two years Its use appeared resistant strains(Miranda *et al., 2021).*

Staphylococcus aureus can exemplify better than any other human pathogen the adaptive evolution of bacteria in the antibiotic era, as it has demonstrated a unique ability to quickly respond to each new antibiotic with the development of a resistance mechanism, starting with penicillin and methicillin. Resistance mechanisms include enzymatic inactivation of the antibiotic (penicillinase and aminoglycoside-modification enzymes), alteration of the target with decreased affinity for the antibiotic (notable examples being penicillin-binding protein 2a of methicillin-resistant *S. aureus*), trapping of the antibiotic (for vancomycin and possibly daptomycin) and efflux pumps fluoroquinol and tetracycline(Nadeem *et al., 2020).*

Nowadays, in all countries *staphylococci* are one of the major public health problems. Errors in the anti-staphylococcal treatment strategies resulted in the selection and spread of drug resistant strains. multi-drug resistant staphylococci are one of the most common cause of nosocomial infections, particularly for hospitalised and immunocompromised patients(Stewart-Johnson *et al., 2019*)

2.7. Mechanisms of Antimicrobial Resistance

2.7.1. Intrinsic Antibiotic Resistance

The endogenous resistance mechanism mainly includes three aspects(Guo *et al., 2020)* .

2.7.1.1. Outer Membrane Permeability

When the cell membrane permeability is lowered, the energy metabolism of the bacteria is affected, and therefore, drug absorption is reduced, leading to drug resistance (Anuj *et al*., 2019). For example, the resistance of *S. aureus* to aminoglycosides is caused by a decrease in membrane permeability and finally results in a decrease in drug intake(Zhang *et al., 2022).*

2.7.1.2. Efflux Systems

The active efflux system of bacteria was discovered in 1980 by Ball and McMurry when studying the resistance of *Escherichia coli* to tetracycline(Ahirrao *et al., 2022*) . Afterwards, the scholars conducted many experiments on the active efflux system, which confirmed that the active efflux system is a normal physiological structure of bacteria, and exists in sensitive strains (Dos Santos Barbosa *et al., 2021*).

When induced by substrates in the environment for a long time, efflux systemencoding genes are activated and expressed, and the ability to efflux drugs is greatly enhanced, thus leading to drug resistance (Guo *et al., 2020).* Active drug efflux systems play a role in resistance to multiple drugs (Zgurskaya, 2021).

There are three types of multidrug-pumping proteins present on the *S.aureus* cell membrane: *QacA*, *NorA*, and Smr (DashtbaniRoozbehani, 2021) considering QacA to be an important factor in MRSA . Multidrug pumping proteins are all proton kinesins (Swanson, 2022). That is, instead of

relying on ATP hydrolysis to release energy, material exchange is performed by an electrochemical gradient formed by H+ on both sides of the cell membrane (Srinivasan *et al., 2021*).

Usually, it is a reversible process, that is, H+ moves from extracellular to intracellular, while intracellular harmful substances such as dyes and antibacterial drugs flow from the inside of the cell to the outside . . also demonstrated the role of active efflux systems in MRSA resistance (Ahirrao *et al., 2022).*

2.7.1.3. Excessive Production of β-Lactamase

β-lactamase is an enzyme that catalyzes the hydrolysis of various β-lactam antibiotics (including carbapenem), is encoded by bacterial chromosomal genes, and is transferable (*Kumar et al., 2022*). β-lactam antibiotics have a lethal effect on bacteria mainly through two mechanisms: first, by binding to penicillin-binding protein (PBPs, i.e., cell wall mucin synthase), which represses cell wall mucin synthesis, disrupts the cell wall, and leads to bacterial expansion and lysis; second, by triggering the autolytic enzyme activity of the bacteria, which resulted in autolysis and death . Excessive secretion of β-lactamase by MRSA mainly reduces the effect of antibiotics through two mechanisms, which lead to MRSA resistance (Guo *et al., 2020).*

The first is the hydrolysis mechanism that is β-lactamase hydrolyze and inactivates β-lactam antibiotics the second is the mechanism of pinching, that is, a large amount of β-lactamase binds quickly and firmly to extracellular antibiotics, preventing the antibiotics from reaching the intracellular space and therefore the antibiotics are not able to reach the

target site, ultimately leading to MRSA resistance to antibiotics (Hochvaldová *et al., 2022*).

2.7.2. Acquired Antibiotic Resistance

2.7.2.1. Resistance by Mutations

Staphylococcus aureus can become drug-resistant by genetic mutations (*Hussain et al., 2021)*that alter the target DNA gyrase or reduce outer membrane proteins, thereby reducing drug accumulation (Kime *et al., 2019*). For example, the principle of resistance to clindamycin and erythromycin is caused by a modification in ribosomal RNA methylase(Roemhild *et al., 2022)* .

2.7.2.2.Acquisition of Resistance Genes

Acquired resistance is a type of plasmid-mediated resistance(Dureja *et al*., 2022) . Through plasmid-mediated transduction, transformation, and insertion of drug-resistant genes, excessive β-lactamase can be produced, leading to bacteria resistance(Hussain *et al., 2021*). The mechanism of MRSA resistance is mainly because plasmids, or drug-resistant gene transmission mediated by plasmids, which can expand the genome and resistance genes can be transferred between *S. aureus* and other bacteria (Vestergaard *et al., 2019*). For example, MRSA can obtain drug-resistant plasmids from Enterococcus, further expanding and enhancing its resistance (Dinescu *et al., 2021*).

2.8. β-lactam antibiotics

β-lactams are a large group of antibiotics, all containing the βlactam ring. There are four major groups, penicillins, cephalosporins, carbapenems, and monobactams, which differ from one another in the nature of the additional ring attached to the β-lactam ring. In penicillins, there is a fivemembered thiazolidine ring, in cephalosporins a six-membered cephem ring, adouble ring in carbapenems whereas in monobactams only the β-lactam ring is present. The various types of βlactams within each group differ in the side chains attached to the core rings (Samaha-Kfoury and Araj, 2003).

Monobactams are active against Gram-negative rods but not against Gram-positive bacteria or anaerobes. The first such drug to become available was azetreonam (Rupp and Fey, 2003). Imipenem the first drug of the carbapenems has good activity against many Gram-negative rods, Grampositive organisms and anaerobes. It is very stable in the presence of bacterial β-lactamase (Wilson *et al., 2015*).

Clavulanic acid, a naturally occurring βlactam, was the first inhibitor, which was produced by Streptomyces clavuligerus in 1977 (Paradkar, 2013). Subsequently, a few more inhibitors sulbactam, a penicillanic acid sulphone, and tazobactam, etc., were founded (Shahid *et al*., 2009). Amoxicillin-clavulanate is a β-lactam- β-lactamase inhibitor combination that has antimicrobial activity against Gram-positive, Gram-negative, and anaerobic organisms (Bush and Bradford, 2016).

The β-lactamases confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four-membered β -lactam ring (Gupta, 2007). Over the last decades, many new β-lactams have been developed that were specifically designed to be resistant to hydrolytic

actions of βlactamase (Bush and Bradford, 2016). Development of the "third-generation" cephalosporins in the early 1980s based heavily on the ability of these agents to escape hydrolysis by all the common β-lactamases in both Gram-positive and Gram-negative bacteria (Rawat and Nair, 2010).

2.8.1. Mechanisms of β-lactams action

β-lactam antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems target transpeptidase enzymes that synthesize the bacterial cell wall and act cytostatically on bacteria by inactivating peptidoglycan transpeptidases irreversibly. The transpeptidases are members of the family of penicillin-binding proteins (PBPs) from which β-lactamases are likely to have evolved (Öztürk *et al., 2015)*.

The desirable attributes of this class of antibiotic arise from the facts that these enzymes are localized to the outer leaflet of the bacterial cytoplasmic membrane (i.e. are relatively accessible) and that they are specific to bacteria (with no functional or structural counterpart in the human host) (Walther-Rasmussen and Høiby, 2006).

The transpeptidases catalyze the cross-linking of the peptidoglycan polymers in the bacterial cell wall (Sauvage and Terrak, 2016). In the presence of the antibiotic, the transpeptidases form a lethal covalent penicilloyl enzyme complex that served to block the normal transpeptidation reaction and inhibition of the polymerization process. This resulted in weakly crosslinked peptidoglycan, which makes the growing bacteria highly susceptible to cell lysis and death (Wilke *et al., 2005).*

2.8.2. Mechanisms of β-Lactams Resistance

There are several mechanisms of resistance to βlactam antibiotics and they are generally due to point mutations on the chromosome or to the acquisition of mobile elements such as plasmids or transposons (Munita and Arias, 2016). The resistance to β-lactam antibiotics can be due to the expression of a single mechanism of resistance or to the additive effect of several mechanisms, resistance to β-lactam antibiotics in bacteria could be due to four mechanisms (Peterson and Kaur, 2018).

I. Resistance by Increased Efflux Pump Genes encoding efflux pumps may be on the chromosome or transmissible elements, such as plasmids, are present in antibiotic-susceptible and antibiotic-resistant bacteria, efflux systems are now recognized as an important contributor to antimicrobial resistance, and are more commonly found in Gram-negative bacteria, with resistance mediated by increased expression of the efflux pump protein or a mutation in the protein increases the efficiency of export(Piddock,2006) 11.Resistance by Decreased Antibiotics Uptake Before a β-lactams reaches bacterial PBP targets which are on the outer surface of the cell membrane it must diffuse across the outer membrane of the cell, using the pores that are formed by porins, and then cross the periplasm. The porins, which represent one family of outer membrane proteins (Omps), form channels to permit diffusion of small hydrophilic solutes through the outer membrane (Yildirim *et al*., 2005). Usually, K. pneumoniae strains express OmpK35 and OmpK36, while the ESBL producing strains commonly express only one of these, normally OmpK36, or no porin at all (Martínez-Martínez, 2008). In some instances, porin loss in ESBL-producing isolates increases resistance

to fourth-generation cephalosporins and /or carbapenems (Pfeifer *et al*., 2010).

111.Resistance by Alteration of the Target Site Resistance caused by alterations in PBPs can occur by the acquisition of an increased target PBP number andreduced affinity of this target. PBPs with reduced affinity are an important mechanism of resistance to β-lactams especially in Gram-positive when βlactamases are absent (Rice, 2012)

IV. Resistance by Enzymatic Inactivation Antibiotic-inactivation enzymes, like β-lactamses, are the most important single cause of resistance to β-lactams. Over 700 unique enzymes have been identified and they were either chromosomally or plasmid-mediated. These enzymes made biologically inactive products of the antibiotic by efficient hydrolysis of the amide bond in the β-lactam ring (Grace, 2012). β-lactamases were initially noted in *Staphylococcus aureus* and were common in Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, and *Proteus mirabilis*, but have also been found in other Enterobacteriaceae (Bonomo, 2017).

2.8.Methicillin Resistance *and* sensitivity *S.aureus*.

The possible differences in pathogenicity and virulence among strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) form an as yet unresolved problem. What factors contribute to virulence? On one side is the patient's ability to respond to infecting bacteria; on the other are the virulence factors produced by the bacteria, e.g., adhesins, toxins and various enzymes Methicillin resistance is encoded by the *mecA* gene, located on a genomic island termed the staphylococcal cassette chromosome *mec* (SCC*mec*) element, which

itself has five distinct types. SCC*mec* is a mobile genetic element that is capable of exchange between different staphylococcal species (Katayama *et al*.,2000).

Clinical data concerning length of hospitalisation, mortality rate and hospital costs related to MSSA and MRSA infections suggest a greater burden for MRSA infections. Patients for whom appropriate therapy is delayed have a significantly increased risk of developing MRSA bacteraemia, and their clinical response is slower than that of patients with MSSA bacteraemia (Lodise and McKinnon ,2005)

However, the sample size, the various patient populations with different underlying diseases, and differences in antibiotic use can all be postulated as confounding factors in such situations. Adjustment for disease severity and co-morbid conditions is crucial in order to obtain a more objective understanding concerning the clinical significance of methicillin resistance (Cosgrove *et al*.,2005).

According to Cosgrove *et al (2003),*the enhanced virulence of MRSA and the decreased effectiveness of vancomycin, which is invariably used to treat MRSA infections, coupled with the delay in selection of a microbiologically appropriate antibiotic regimen, are the potential reasons why MRSA infections may be associated with higher mortality (Cosgrove *et al* .,2003).

Another aspect relates to whether the *S. aureus* infection is epidemic or sporadic. A lower mortality rate was found for MRSA in an outbreak situation when compared with a non-outbreak situation. This observation can perhaps be explained by a greater suspicion of MRSA infection and earlier use of adequate empirical therapy in the outbreak situation , In addition, the immunological status and the risk-factors of the patients in whom manifest, life-threatening MRSA infections occur must be considered.

Most such patients have been immunologically compromised and have underlying diseases. So why is MRSA more virulent than MSSA? (Cosgrove *et al* .,2003).

Comparative in-vitro studies of heteroresistant MRSA and non-heteroresistant MSSA clinical isolates have investigated protein A-, fibrinogen-, fibronectin-, collagen- and vitronectin-binding proteins. The results were not consistent with respect to the frequency and content of these adhesins, either in the MRSA or in the MSSA strains(Salgado *et al*.,2004) Similarly, the magnitude of binding to epithelial cells and plastic surfaces did not differ significantly between MRSA and MSSA strains (Duckworth & Jordens 1990)

Results concerning the incidence and amount of enterotoxins produced are also conflicting, although it was thought originally that production of enterotoxin B was associated with methicillin resistance. The situation concerning the production of cytotoxins is similar (Coia *et al.,1995*).

The presence of Panton–Valentine leukocidin genes has also been investigated, but the results for different MSSA and MRSA strains were also conflicting. Panton–Valentine leukocidin is a bicomponent pore-forming cytotoxin that is used as a marker of community-acquired MRSA infections. It is now widely accepted that MRSA is not just a nosocomial pathogen, and MRSA strains involved in community-associated infections have a worldwide distribution (Robert *et al*.,2005).

Phagocytosis assays have also failed to yield consistent results, in that MRSA and MSSA strains were equally susceptible to phagocytes (Salgado *et al.,2004).*

All of these conflicting results can be attributed to the varying heterogeneity of the individual strains studied so far. It is also important to

note that the above-mentioned clinical and in-vitro studies focused on phenotypic characteristics and did not always detect the genes encoding these virulence factors. Most MRSA strains actually consist of a heterogeneous population of cells, composed of methicillin-sensitive, borderline-resistant and methicillin-resistant (MR) sub-populations. In the MR sub-population, the cocci have an enhanced level of resistance to methicillin. Such cells occur at a frequency of only 1 in 10^4–10^5. Data derived from experiments with such heterogeneous isolates mostly concern the susceptible majority of the population rather than the MR sub-population. Thus, when comparing MSSA and MRSA strains, congenic MSSA and MRSA strain pairs derived from the same isolate should be examined to obtain meaningful results(Cosgrove *et al* .,2003).

To date, few studies have compared congenic MRSA and MSSA sub-populations derived from the same strain. However, the results available show fundamental differences between MRSA and MSSA cells, with MRSA cells possessing significantly higher quantities of lipids of all classes than do MSSA cells. Electron-microscopic examinations have shown that the separation of MRSA daughter cells along the cross-walls is delayed, but that the formation of new cross-walls occurs more quickly, resulting in multi-septated cocci. In contrast, the division of MSSA occurs regularly in two planes, while the separation of MRSA has three planes ,MSSA cocci have a shorter generation time, resulting in a higher cell count within an hour, than that of MRSA , The log phase of MRSA has been found to be *c.* 5 h longer than that of MSSA with the same inocula (Rozgonyi *et al.,1982*)..

In-vivo animal models may also reveal characteristic differences between congenic MRSA and MRSA cells. A higher number of MRSA cells was required to cause the same rate of death in Balb/c mice as that caused by

congenic MSSA cells (Rozgonyi *et al.,1984).* In contrast, the persistence of MRSA cells in the organs of surviving mice was twice that of the congenic MSSA cells (*Majoros et al.,1996).* These results indicate that both bacterial populations are virulent for mice, but that the mechanism(s) of pathogenesis of MRSA and MSSA infections may be different.

The main objective difference between MRSA and MSSA cells remains the antibiotic susceptibility pattern. MRSA cells are resistant to all β-lactam antibiotics, and can acquire resistance to other antibiotics easily, leading to the development of multiresistant strains. Multiple drug resistance might play a role as an indirect virulence factor by providing a selective advantage for MRSA cells (Vriens *et al.,2002*)

In conclusion, the data available in the literature do not, as yet, unequivocally support the hypothesis that MRSA strains are more virulent than MSSA strains. The most important reason for the conflicting results is probably the heterogeneic nature of the resistant population. Further investigations with congenic MRSA and MSSA strains are required to correlate the genetic background with the phenotypic expression of virulence. Such investigations would better mimic many clinical situations, since manifest infections are, of necessity, treated empirically with standard antibiotics to which the MR sub-population is usually resistant. As a consequence, an MRSA sub-population is selected and may become predominant, which then determines the subsequent clinical response(Cosgrove *et al* .,2003).

2.10. Molecular Typing Methods for MRSA

Molecular-typing methods, are an effective and important way to quickly identify and monitor pathogenic strains that are prevalent and to control their

diffusion method to control them and thus control the diseases they cause it.(Rezai, *et al., 2020*).

MRSA can be distinguished by antibiotic susceptibility patterns and by molecular typing methods, which include; DNA fragment restriction profile, pulsed-field gel electrophoresis(PFGE), protein A (spa-typing) , multi locus sequence typing (MLST) and the accessory gene regulator (agr), determination of direct repeat unit the Mec associated hyper-variable region (dru), dru locus is placed in a highly variable region of MecA gene (SCCmec), Mec gene complexes in SCC element has been standardized internationally.(Ho, *et al., 2015*) .

Most appropriate typing method is chosen according to the reason for taking the test; typically, outbreaks of the disease need distinctive and highly accurate methods. An excellent molecular typing method should have an adequate discriminatory power, be highly reproducible, easy performance and interpretation, generate un-changeable data, inexpensive and not time-consuming (Nazareth, *et al., 2012).*

2.11.Mec A and Fem A

MecA, a structural gene located on the chromosome of *Staphylococcus aureus,* characterizes methicillin-resistant *S. aureus* (MRSA),and femB(fem) genes encode proteins which influence the level of methicillin resistance of *S. aureus*. In order to examine effectiveness of detecting mecA and fern genes in identification of MRSA,

Methicillin-resistant Staphylococcus aureus (MRSA) is an important cause of nosocomial bacterial infection in many countries. Coagulase-

negative staphylococci (CNS) derived from normal skin flora have also been recognized as nosocomial pathogens, and the emergence of multiply drug-resistant strains, which may mostly be ascribed to the acquisition of extrachromosomal DNA, is a matter of recent concern (Thore *et al.,1990*).

It has been established that the production of an additional penicillin-binding protein PBP-2' (PBP-2a), with low-affinity for betalactam antibiotics, is mainly involved in the mechanism of methicillin resistance of *S. aureus* (Utsui *et al*.,1985) . While the PBP-2', which is encoded by a chromosomal structural gene designated as mecA, is usually induced by beta-lactam antibiotics, it is known to be constitutively produced in some MRSA (Ubukata *et al*.,1990).

Further epidemiological studies revealed that mecA genes are also distributed widely among CNS, and are associated with methicillin-resistance (Ryffel *et al* .,1990). Recently two chromosomal mec regulator genes mecRl and mecl have been identified (Hiramatsu *et al*.,1992) . Surveys of the distribution of mec regulator genes among clinical isolates of methicillin-resistant staphylococci indicated that mecl encodes the repressor protein of the mecA gene and it is deleted or mutated in methicillinresistant strains (Suzuki *et al*.,1993).

Although the mechanism of regulation of the mecA gene has not been completely elucidated, the presence of the mecA gene in staphylococci has been considered recently as a molecular basis for the identification of MRSA or methicillin-resistant CNS. even though the strain appears methicillin-sensitive by the measurement of minimum inhibitory concentration (MIC)

(Hiramatsu *et al* .,1992) . On the basis of these findings, attempts have been made to identify MRSA by polvmerase chain reaction (PCR) amplification of mecA gene fragments derived not only from isolated strains but also from clinical specimens directly (Higashiyama *et al*.,1993) .

However, it has also been recognized that detection of a certain marker which is specific for *S. aureus* is needed to distinguish MRSA from methicillin-resistant CXS. in addition to demonstrating the mecA gene by PCR. Besides the mec regulator genes, femA and femB genes on the chromosome have been shown to encode proteins which considerably affect the level of methicillin resistance of *S. aureus* , Although fern genes were suggested to be specific for *S. aureus* (TOKUE *et al*.,1991).

2.12.*Staphylococcus aureus* Biofilm

Staphylococcus aureus secretes an extracellular polymeric substance (EPS), known as biofilm, that helps the microbe to resist and minimise the effect of antibacterial drugs (Kaplan *et al.,2018*). Similar to any other bacterial biofilm, a *Staphylococcus aureus* biofilm also has two distinct components, i.e., water (about 97%) and the organic matter which includes EPS and micro colonies (Nazir *et al.,2019*).

The EPS constitutes about 50 to 90% of the total organic matter of a biofilm and is a complex of different polymeric substances, such as extracellular DNA (eDNA), proteins and polysaccharides (Donlan,2002) The remaining portion, 10–25%, consists of microcolonies ,In *Staphylococcus aureus* biofilm, the major component of EPS is the polysaccharide intercellular adhesin (PIA) (Reffuveille *et al.,2017*).

The polysaccharide component of EPS has been given the name PIA due its function, i.e., intercellular adhesion of bacterial cells, and poly-β(1-6)-Nacetylglucosamine (PNAG), due to its chemical composition. PIA are cationic in natur and play a significant role in colonisation, biofilm formation and biofilm-related infections, immune evasion, resistance to antimicrobials and phagocytosis (Nguyen *et al.,2020*).

Staphylococcus aureus EPS also contains a range of proteins including accumulation associated proteins (Aap), surface binding protein A (Spa), fibrinogen binding protein (FnBP) A and B, extracellular matrix binding protein (Embp), amyloid fibres and *S. aureus* surface binding protein (SasG) (Dutta*et al.,2016).*

Other *S. aureus* proteins that are found covalently attached to cell wall peptidoglycan (PG) by trans peptidases (sortases) are known as cell wall-anchored proteins (CWP) (Lacey *et al.,2017*). There are as many as 25 different CWPs, categorised as microbial surface component recognising adhesive matrix molecule (MSCRAMM), near iron transporter (NEAT), three-helical bundle and G5-E repeat proteins (Foster *et al*.,2014). These *S. aureus* proteins perform different functions. For example, accumulation associated protein Aap interacts with PIA and plays a role in biofilm maturation (Reffuveille *et al.,2017*).

SasG protein and surface binding protein A are responsible for surface attachment and causing infections (Corrigan *et al*.,2007). CWA proteins facilitate adhesion to EPS, to host surface, and their interaction with CWA proteins on adjacent cells contributes to the accumulation of biofilm , Similarly, amyloid fibres act as a scaffold that *keeps S. aureus* cells

anchored to the biofilm matrix and thus maintain the stability of the biofilm(Taglialegna*etal.,2016).*

Alongside PIA and EPS proteins, the third important component *of S. aureus* biofilm EPS is eDNA. eDNA has been reported to be involved in irreversible attachment, horizontal gene transfer, maintaining biofilm integrity, antimicrobial resistance and host immune system evasion (Miao *et al.,2016*).The extra polymeric substance of a biofilm also contains charged (both positive and negative) groups and hydrophobic groups.

The negatively charged groups found in EPS include carboxyl groups, phosphates, sulphates, glutamic acid and aspartic acid, while positively charged ones include amino sugars (Neu *et al.,2010)* . Despite of the presence of both positively and negatively charged species, the overall charge on the EPS surface is negative and thus can serve as a better target for positively charged moieties (Algburi *et al.,2017*).

2.12.1. Biofilm Formation

The formation of biofilm proceeds through four different stages (Landini *et al.,2010*) , which are

1. Attachment of planktonic cells to the surface (either a biotic host or any abiotic surface)
2. Colonization and biofilm formation
3. Biofilm maturation
4. Biofilm dispersal

Biofilm formation in *S. aureus* is initiated when free floating, planktonic cells attach to the available surface and start colonising (Petrova & Sauer.,2012).. *S. aureus* adherence to a surface is influenced by hydrophobic

and hydrophilic interactions between the *S. aureus* cell surface and any biotic or abiotic surface (Maikranz *et al.,2020*). It has been found that the *S. aureus* cell surface adheres to hydrophobic surfaces by the help of many weakly binding macromolecules, while its adherence to hydrophilic surfaces involves fewer but stronger binding macromolecules (Otto,2018).

The formation of micro colonies is followed by the formation of an extracellular polymeric substance (EPS) that develops into a fully matured biofilm (Landini *et al., 2010*). Once the biofilm is fully matured, the bacterial cells residing inside it release certain chemicals, i.e., D-amino acids and EPS-degrading enzymes such as alginate lyase, to break and disperse the biofilm (Kostakioti, *et al.,2013*) . These planktonic cells are ready to either recolonise the same site or attach to a different site and repeat the process to form a new biofilm (Donla, 2002). Figure (2.1) depicts different stages involved in the formation of a bacterial biofilm.

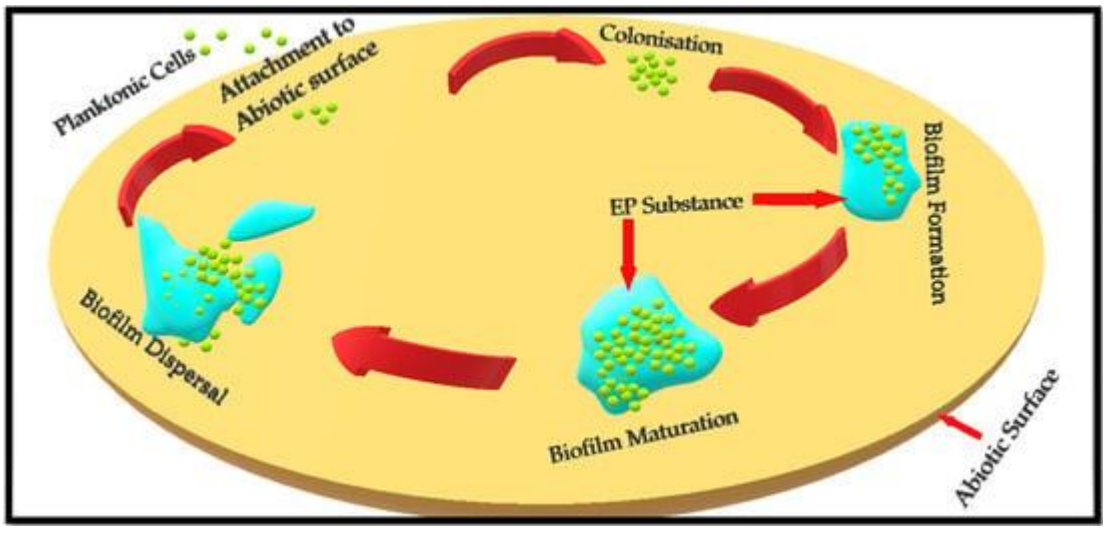


Figure (2-1). Depiction of *Staphylococcus aureus* biofilm formation on an abiotic surface. Basic concept has been adopted from Idrees *et al.* (2020) and Paharik (2016) [Paharik & Horswill.,2016).

Staphylococcus aureus cells that are encased and protected by biofilms show different phenotypic characters compared to cells in their planktonic form. Biofilm-associated *Staphylococcus aureus* cells are more resistant to antibiotics and exhibit differences in cell size and growth, gene expression and protein production, compared to their free living counterparts (Otto,2018).

Biofilm-associated *S. aureus* cells have been reported to have four different metabolic states, i.e., they can either be growing aerobically, can be fermentative, can be dormant, or can even be dead (Archer *et al.,2011).* Besides the extracellular polymeric matrix that shelters the cells against antibiotics, the dormant and metabolically slow growing cells have also been reported to add to antimicrobial resistance (Lister & Horswill,,2014). Moormeier (Moormeier& Bayles.,2017) reported that *S. aureus* cells encased in a biofilm grow at different rates, i.e., some cells grow at a faster rate as compared to other cells within the same biofilm. These cells are smaller in size and attain their normal size once released upon the dispersal of the biofilm.

Biofilm associated *S. aureus* cells exhibit altered gene expressions, i.e., up- and down regulation of genes has been witnessed in the cells residing inside a *S. aureus* biofilm. The differential gene expression accounts for the variation in cell sizes within a biofilm, their growth rates and protein production (Donlan,2002).

2.12.2.Molecular typing of biofilm

Bioflms can provide protection in a number of different ways. The exo poly saccharide present in the biofilm can act as a physical barrier inhibiting

the entry of antimicrobial agents and antibodies into the biofilms (Sritharadol *et al.,2018*). There are two major ways in which biofilm forms, one relies on the *ica* operon and poly-N-acetyl-β-(1–6)-glucosamine (PNAG) production, while the second is *ica* independent, The *IccaDBC* encodes four genes including *Icca, icaB, icaC,* and *Iccd* . *Icca* and *Iccd*, which collectively produce PIA, facilitate the cells binding together and forming into biofilms,the majority *of S.aureus* strains contain the *IccaDBC* operon which is up regulated under in vivo conditions (McCarthy *et al*.,2015).

The process of *S. aureus* biofilm formation is controlled by quorum sensing which is a system used by bacteria for cell–cell communication to regulate gene expression in response to the cell density. The staphylococcal accessory gene regulator (agr) system plays an important role in QS (Dehbashi *et al.,2018)*

by activating some PIA (polysaccharide intercellular adhesion) dependent surface factors, the system can increase the pathogenicity of *S. aureus,* in several studies, this system plays the role of down regulation in bacterial colonization and up regulation in host disease (McCarthy *et al*.,2015).

The down regulation and up regulation of the genes involved in the described processes promote the establishment and development of MRSA infections. In addition, these genes play an important role in MRSA biofilm formation which, in turn, leads to a more aggressive infection giving the patient a poor prognosis (da Fonseca *et al* 2016).

2.13.Beta-lactamases enzyme

Beta-lactamases are bacterial enzymes that inactivate beta lactam antibiotics (Etok *et al.,2012*).The beta lactamases inactivating all the penicillins and cephalosporins including the extended spectrum cephalosporins are called as Extended Spectrum Beta Lactamases (ESBLs). There are almost 500 different ESBLs described, that are mutations of the classical broad-spectrum beta lactamase enzymes, initially named TEM and SHV (TEM-1, TEM-2, SHV-1).

Treatment is complicated by the presence of ESBLproducing Enterobacteriaceae, because they are often multidrug resistant. Thus, infections caused by ESBLproducing Enterobacteriaceae are of serious concerns. Many ESBLs are frequently expressed in gram-negative bacteria. They confer resistance to ampicillin, amoxicillin, and other penicillin derivatives, as well as to early but not later-generation cephalosporins (Etok *etal.,2012*).

Advances in control of infections have not eliminated the risk of post-operative wound infections due to the emergence and spread of resistant microbes. The condition is particularly serious in developing countries where irrational prescribing of antimicrobial agents is common. Measures including new antimicrobial production, better infection control program and rational use of existing antimicrobial agents have been suggested to reduce the problem (Hart and Kariuki,1998).

3.Methodology

3.1.Materials

3.1.1.Equipment and Instruments

Table (3-1): equipment and instruments used in this study

Type of Equipment	Manufacturer, origin
1.5 microcenterfuge tube	Ultra-Cruz
Autoclave	Hirayama, Japan
Bunsen burner	Labgard, USA
Centrifuge	Hettich, Germany
Class II biological safety cabinet	Labgard USA
Conventional PCR thermal cycler	Bio base, China
Cryogenic tubes	Ultra-Cruz, Germany
Deep freezer	Samsung, Korea
Drying oven	Bio base, China
Electronic balance	Bio base, China
Electrophoresis Unit	Bio base, China
ELISA-Reader	Biotek USA
Flat Bottom glass Tube with a Screw cap	Germany
High-speed refrigerated centrifuge	Bio base, China
Incubator	Bio base, China
Inoculation loop	Lab-tech, Italy
Magnetic stirrer with a hot plate	Wise stir, Belgium

Micro centrifuge	Gusto, China
Micropipette sets from 0.5µl to 1000µl	CYAN
Micropipette tips (different sizes)	Citotest, China
Microwave oven	Samsung, Korea
Petri plates (Disposable plastic)	Sunvian, China
Plain tube	Sunvian
Plastic rack	Sunvian
Refrigerator	Kelon, Japan
Steel rack	Citotest, China
Sterile swap	Sunvian
UV trans-illuminator	Bio base, China
Vortex mixer	Bio base, China
Water bath	FALC BI, Italy
Water distiller	K&K, Korea

3.1.2. The Biological and Chemical Substances

Table (3-2): Chemical and Biological Materials

Substances	Manufacturer, origin
10xTBE buffer	Promega, USA
Absolute Ethanol	Bio world, USA
Agarose	Intron, Korea
Barium chloride dehydrate	BDH, England

Crystal violet	Fluka, England
Dextrose	Difco, USA
Di sodium hydrogen phosphate (Na_2HPO_4),	BDH, UK
Ethidium bromide	BDH
Glycerol	Bio world, USA
Hydrogen peroxide (H2O2)	BDH
Lugol's Iodine	BDH
Lysozyme enzyme	Intron, Korea
Phosphate buffer	BDH,UK
Plasma-coagulase EDTA (Rabbit plasma)	MAST, USA
Ribonuclease (A) enzyme	Intron, Korea
Safranine	Fluka, England
Slouble starch	Difco Laboratories, USA
Sodium chloride (NaCl)	GCC, England
Sodium di hydrogen phosphate (NaH_2PO_4),	BDH, UK
Sulfuric acid (H2So4)	BDH, UK
tetra-methyl-p-phenylenediamine dihydrochloride	BDH
Tris-borate-EDTA (TBE) buffer stock	Intron, Korea

3.1.3. The culture media

Table (3-3): The culture medium used during the study

Culture media	Company and origin
Blood agar base	Himedia, India
Brain heart infusion agar	Himedia, India
Brain heart infusion broth	Himedia, India
Mannitol salt agar	Oxoid, England
Muller Hinton agar	Himedia, India
Nutrient agar	Himedia, India
Nutrient broth	Himedia, India

3.1.4. The antibiotics discs

Table (3-4): Antibiotic discs (MAST/USA)

Antibiotic class	Antibiotic name and content	code	Inhibition zone diameter (mm) (CLSI 2020)		
			S	I	R
β-lactams	Penicillin G (10U)	PG	≥29	-	≤28
	Oxacillin (1 µg)	Ox	≥13	11-12	≤10
Tetracyclines	Tetracycline (10 mg)	TE-10	≥19	15-18	≤14
Aminoglycoside	Gentamicin (10 µg)	CN-10	≥15	13-14	≤12
Fluoroquinolone	Ciprofloxacin (5 µg)	CIP-5	≥21	16-20	≤15
Macrolides	Erythromycin (10 µg)	E-10	≥23	12-22	≤13
	Azithromycine 15mcg	AZM-15	≥18	14-17	≤13

Lincosamides	Lincmycin (10 mcg)	L-10	≥21	15-20	≤14

3.1.5. DNA amplification materials

3.1.5.1. DNA polymerase and molecular weight marker kits

Table (3-5): DNA amplification materials

DNA amplification materials and Content		
1. DNA Extraction Kit (G-spinTM Genomic DNA) / Intron, Korea(Appendix 1)		
G-buffer	Pre buffer	Washing buffer A
Washing buffer B	Binding buffer	Elution buffer
Lysozyme powder	Ribonuclease A powder	Proteinase K powder
2. GoTaq® G2 Green Master Mix / Promega, USA		
Taq DNA polymerase	MgCl2	dNTPs
Reaction buffer	Yellow and blue loading dyes	
3.DNA Marker /intron, korea		

Marker DNA leader consists of 12 DNA fragment (double-stranded) with a size of 100 bp-1000 bp and 1500 bp, and 3000 bp, it was used to determine the size of double-stranded DNA product from 100bp to 1500bp

3.1.5.2 The primers used for antibiotic resistance determinants detection

Table (3-6): The primers used in the identification of MRSA determinants, Macrogen , Korea

Primer		Sequence 5'-3'	Amplicon size	Reference
mecA	F	GTAGAAATGACTGAACGTCCGATAA	310bp	(McClure et al.,2006)
	R	CCAATTCCACATTGTTTCGGTCTAA		
femA	F	AAAAAAGCACATAACAAGCG	134 bp	(Mehrotra et al.,2000)
	R	GATAAAGAAGAAACCAGCAG		

3.1.5.3 Primers for virulence factors detection

Table (3-7): Primers for the detection of virulence factors, Macrogen , Korea

Target Gene		Primer sequence	Size (bp)	Reference
Icaa	F	GAGGTAAAGCCAACGCACTC	151 bp	(Atshan et al.,2013)
	R	CCTGTAACCGCACCAAGTTT		
Icad	F	ACCCAACGCTAAAATCATCG	211 bp	
	R	GCGAAAATGCCCATAGTTTC		

3.2.The Methods

3.2.1. Ethical approval

This study did not include the use of genetically changed organisms or biological materials and was carried out under the supervision and recommendations of the Faculty of Veterinary Medicine, University of Kerbala , according to the controls approved by it. All samples that were worked on in this study were collected according to the research protocols for each type, without additional material or manipulation .

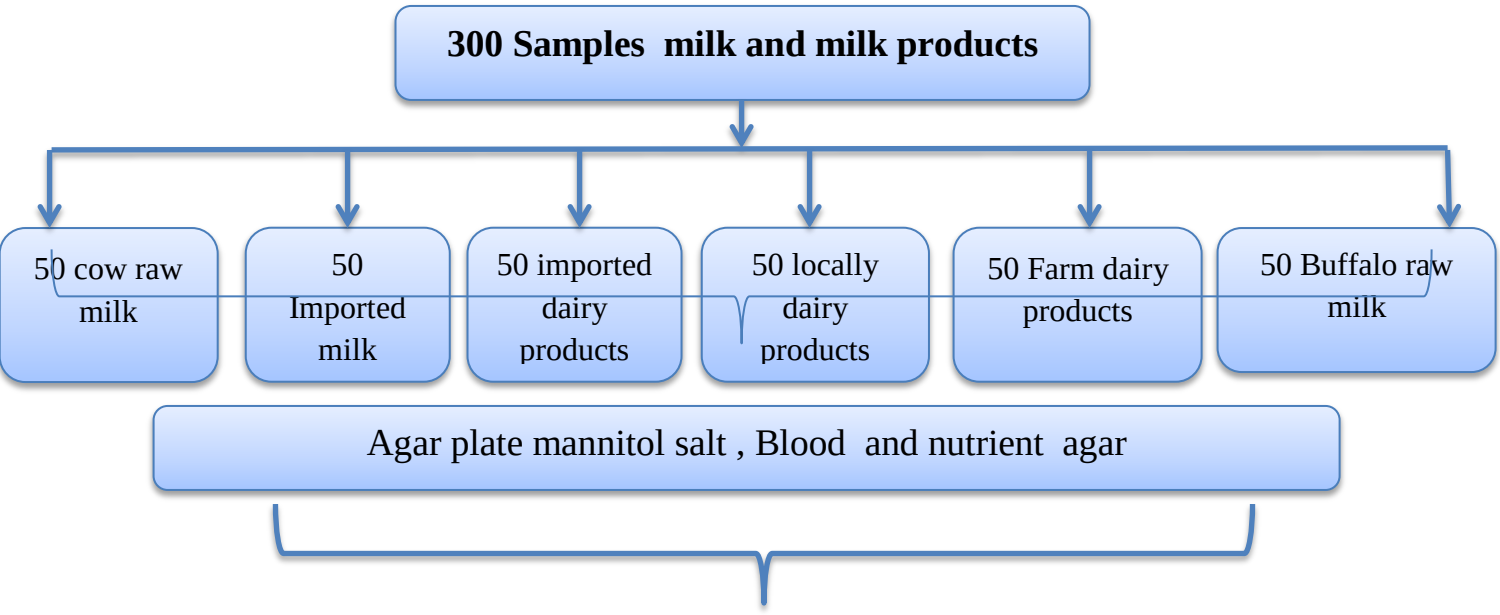
3.2.2. Study design and Specimens collection

Study was performed to collect a total of 300 samples ,Divided into six groups from milk and milk products 50 samples for (cow raw milk ,imported milk ,imported dairy products ,locally dairy products(Factory) , farm dairy products and buffalo raw milk), the study was beginning from October 2022 to February 2023. The samples were collected randomly from different locations in Karbala city . The samples were collected using sterile plastic tubes used for the collection of milk and milk products and all samples were transported in an ice box to the laboratory within 24 hour. the sample immersed in a nutrient broth tube (10ml raw milk and 10 gram of milk products) . then incubated for 24 hour in 37c and cultured on mannitol salt agar directly.

Table (3-8) summarized the number of the specimen from each sample type. Total n(300)

groups	Number of samples
Cow raw milk	50

Imported milk	50
Imported dairy products	50
Locally dairy products(Factory)	50
Farm dairy products	50
Buffalo raw milk	50
Total	300



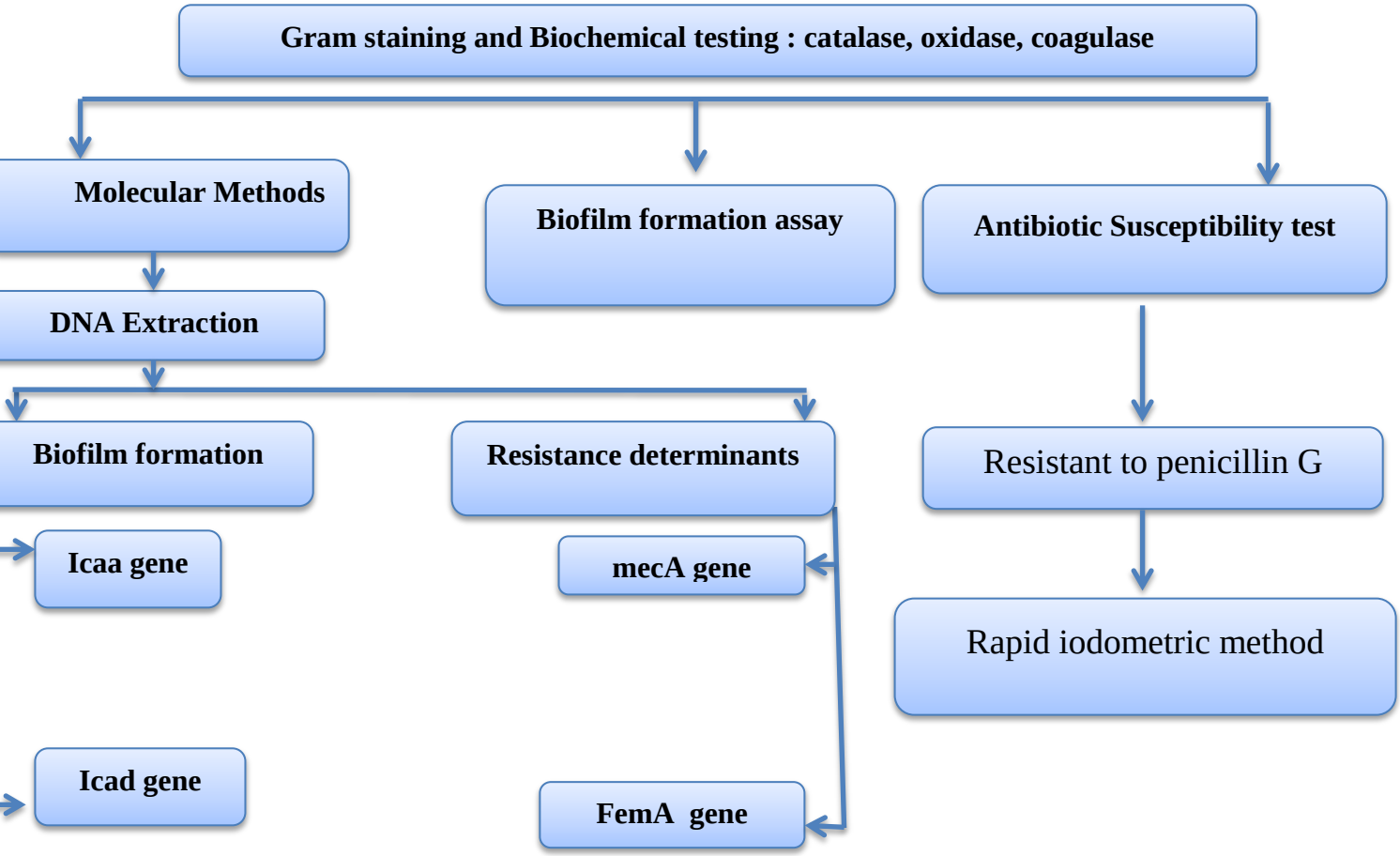


Figure (3-1): Schematic diagram of isolation and identification procedures of *S.aureus*

3.2.3. Preparation of Culture Media

The culture media used in the study were ready use medium (except for blood agar) formulated according to the manufacturer's Company, sterilized by autoclaving at 121ºC for 15min and poured in a sterile Petri dish,

moreover, blood agar was prepared by adding 5% of blood for the routine mixture after autoclaving (Daka et al., 2012).

3.2.4. *Staphylococcus aureus* Isolation and Identification

The Staphylococcus aureus isolation, identification was based on morphological examination on the culture medium and microscope, in addition to biochemical tests (MacFaddin ,2000).

3.2.5. Characteristics of Bacterial Culture

All samples were inoculated for 24 hours at 35°C-37°C on blood agar and mannitol salt agar , to identify zone of hemolysis ,type of colonies,mannitol fermentation,and biochemical test (MacFaddin 2000).

3.2.6. Microscopic examination

The microscopic observation was conducted after the isolates were stained using a Gram staining procedure, using AmScope40X2500Microscope with LCD Touchpad Screen to determining the shape, color, and arrangement of the examined isolates (MacFaddin 2000) .

3.2.7. Solutions and Reagents Preparation

3.2.7.1. Gram stain reagent preparation

These reagents prepared according to the instruction of (Goldman and Green (2015), Gram stain includes crystal violet stain, iodine solution, alcohol acetone solution, and safranin stain was used to determine whether the bacteria were Gram-positive or negative.

3.2.7.2. Catalase reagent preparation

This compound was made like 3% hydrogen peroxide (H2O2) and preserved for the detection of bacterial catalase enzyme in a dark tube

3.2.7.3. Oxidase reagent preparation

The Oxidase reagent was formulated by dissolving 1 g of tetra methyl-p-phenylene-diamine dihydrochloride in 100 ml of distilled water, which is used to detect bacterial oxidase enzyme production (Collee 1996)

3.2.7.4. McFarland standard solution

McFarland's 0.5 is the standard turbidity solution and is the most commonly used in the inoculum preparation process, has specific optical density to provide turbidity equal to 1.5×108 CFU/ml bacterial suspension. This solution was prepared by dissolving 1.175 g of barium chloride dihydrate in 100 ml of distilled water (wt/vol), then 0.5 ml of this solution was added to 99.5 ml of 1% (vol/vol) Sulfuric acid. The solution was stored in a parafilmed test tube for 6 months at room temperature (Benson 2002).

3.2.7.5. Lysozyme enzyme

This solution was prepared by suspending 20 mg of lysozyme powder in 200 µl distilled water according to manufacturer company instructions and stored at -20°C. This enzyme was used to break the bacterial cell wall by the degradation of polymer cell substances responsible for cell rigidity. (intron, Korea)

3.2.7.6. Ribonuclease A enzyme

It was prepared by dissolving 3 mg of RNase A lyophilized powder in 300 µl distilled water and stored at -20°C according to the directions of the

manufacturer company, this solution was used for degrading the RNA of the bacterial cell.(intron, Korea).

3.2.7.7. Proteinase K enzyme

It was prepared by adding 1 mg of proteinase K powder to 88 µl distilled water and processed at -20 C° according to the directions of the manufacturer company, this enzyme was used for the digestion of any kind of proteins, therefore to purified the DNA extract

3.2.7.8. Tris-borate-EDTA (TBE) buffer stock

Tris-borate-EDTA buffer was used at a concentration of 1 X (1: 10 dilution of the concentration stock). The stock solution was diluted by distilled water and stored at room temperature.(intron. Korea)

3.2.8. Identification tests

3.2.8.1. Catalase Test

It was performed on a clean dry microscopic slide rubbed by an inoculating loop loaded by fresh (24 hours culture) bacteria then covered by few drops of 3% H2O2, the formation of bubbles indicates a positive test (Reiner 2013).

3.2.8.2. Oxidase Test

A small part of filter paper was immersed in 1% tetramethyl-p phenylenediamine dihydrochloride and rubbed by a proper amount of well isolated culture was collected using a wooden applicator stick, the change of color to dark purple indicates a positive test (Shields and Cathcart, 2010).

3.2.8.3. Slide Coagulase Test

It was performed according to the coagulase test protocol of Sue Katz (2010), using a microscopic slide, one drop of EDTA-treated rabbit plasma was added and a proper amount of fresh bacterial suspension, and mixed well by a wooden stick, therefore the presence of bound coagulase proteins (clumping factor) on the bacterial cells will lead to clot formation...

3.2.8.4.Coagulase Test (plasma coagulant enzyme test) tube

It is done by taking colonies and stabbing them in tubes containing the liquid culture medium (brain-heart infusion broth) and incubating them for (18-24) hours at (37) C, then transferring (0.1) ml of the growing bacterial culture onto The liquid culture medium was transferred to sterilized tubes in an autoclave at (121) C and a pressure of (15) pounds / inch 2 for (15) minutes containing (0.3) ml of rabbit plasma prepared from drawing blood from the rabbit heart muscle into a tube containing anticoagulant, then centrifuging it The filtrate was taken, and a tube containing only plasma was left without adding bacteria as a control. After that, the tubes were incubated with the control tube in the incubator at (37) C for a period of (4) hours, then the tubes were left at room temperature for a period of (18-24) hours. The Results based on the bacteria's ability to coagulate or solidify the plasma or their inability to do so.

3.2.8.5.Hemolysis Test

The medium of blood agars was prepared by drawing blood from sheep ,after that this blood was added at a rate of (5%) to the blood agar base, which was prepared according to the manufacturer's instructions, and sterilized by autoclave At a temperature of (121) C and a pressure of (15) pounds / inch2 for a period of (15) minutes, then blood was added to the

medium before pouring at a temperature of (50-55) C. The bacteria were grown by the planning method and incubated at a temperature of (37) C for a period of (24-48) hour the results was confirmed based on the presence or absence of hemolysis.

3.2.9.Solutions for detecting beta-lactamase enzyme and measuring its effectiveness:

3.2.9.1 Preparation of solutions to detect beta-lactamase enzyme by iodine method, : (Rapid Iodometric method)

According to the method of Collee et.al., (1996):

1. Iodine solution:

Prepared by dissolving 2.03 grams of iodine and 5.32 grams of potassium iodide in (100) ml distilled water .`

2.A solution of soluble starch at a concentration of (1%):

It was prepared by dissolving (1) gm. of soluble starch in (100) ml of distilled water, and the bottle was placed in a water bath at (100) C for (10) minutes, and the solution was kept at (4) C. This solution was prepared immediately .

3.2.9.2. Preparation of solutions to measure the activity of the purified beta-lactamase enzyme:

According to the method of Novick, (1962):

Solution No. (1):

Phosphate buffer solution (0.05) molar with a pH of 7. This solution consists of mixing two solutions (Na2HPO4) and (NaH2PO4), where:

1. A solution of (0.2) molar of Na2HPO4 was prepared by dissolving 28.392 g of anhydrous (Na2HPO4) in (900) ml of distilled water, then completing the volume to a liter and sterilizing in an autoclave at a temperature of (121C) and a pressure of 15 pounds / inch2 for a period of 15 minutes

2. A solution of (0.2) molar of (NaH2PO4) was prepared by dissolving 31.202 g of anhydrous (NaH2PO4) in (900) ml of distilled water, then completing the volume to a liter and sterilizing in an autoclave at a temperature of 121 at a pressure of 15 pounds / inch2. for a period of 15 minutes

3. Was mixed (92) ml of solution (1) with (8) ml of solution (2)and prepared in the previous two steps, then complete the volume to (200) ml with distilled water to obtain an initial solution (0.1) M and adjust the pH to (7), then dilute half-dilution using distilled water to obtain a concentration of (0.05) M and adjust the pH to (7).

Solution No. (2):

Penicillin G solution with a concentration of (2.5) micromolar, which was prepared by dissolving (0.0089) g of antibiotic Penicillin G in (10) ml of a phosphate buffer solution (0.05) M, then keeping the solution in the refrigerator, prepare this solution immediately .

3.2.9.3. Detection of the Beta-Lactamase Enzyme:

The rapid iodine method was used as stated in WHO, (1978) to find out the presence or absence of the enzyme in the diagnosed isolates of

Staphylococcus aureus bacteria after isolating them and diagnosing them with biochemical tests. And as follows :

1. Bacterial colonies 24 hours old were prepared by growing them on the medium of blood agars .

2. (0.1) ml of penicillin solution was added in microtitration plates and mixed with (5-7) colonies of the germ growing on blood agar media, leaving the last two horizontal rows without mixing with the colonies for comparison.

3. Incubate the mixture for (30) minutes at a temperature of (37) C, until it forms a thick, cloudy suspension, taking into account that the plate used is covered with (Parafilm) paper to prevent contamination, and then left for one hour at room temperature. (0.2) ml of starch solution is added to the mixture. At a concentration of (1%), to which (0.1) ml of iodine solution was added, and the blue color was formed.

4. The results was read and calculated as positive on the basis of the rapid color shift from blue to white within a minute of adding the reagents. It must be noted that positive results appear after (15) minutes and are considered late positive results.

3.2.10. Preservation and maintaining the *S. aureus* isolates

3.2.10.1.Short-term storage method

It was a storage method to maintain the pure culture for one month by preparing slant of nutrient agar media in screw-capped tubes and streaked by

a charged loop of a single colony of *S. aureus* bacteria Then rolled up with parafilm, held at 4°C (Vandepitte *et al.*, 2003).

3.2.10.2. Long-term storage method

Using this maintaining method, for storing the pure isolates for more than 6 months in LB media supplemented with 20% glycerol, 0.2 ml for every 1 ml of LB was added into cryogenic tubes and inoculated by a single colony of *S. aureus* bacteria and stored in the freezer at -20°C (Vandepitte *et al.*, 2003) .

3.2.11. Susceptibility test for antimicrobials using disk diffusion method (CLSI 2020)

Step 1: Preparation of Inoculum

Few colonies of the fresh isolate were selected from mannitol salt agar medium and suspended with BHI broth medium to make direct colony suspension and compared visually with McFarland standard 0.5%

Step 2: Culturing of Petri-dishes

A sterile cotton swab was inserted into the direct suspension and dried well on the tube's inner wall, then the Muller-Hinton agar plate was inoculated using the streaking method across the whole agar surface more than three times.

Step 3: Application of the antibiotic discs

The discs were placed using a disc dispenser, spread over equal distances between each disc (28 mm distance from center to center) on the agar plate with a size of 90 mm. Then, incubated in an inverted position at 37°C.

Step 4: Reading the Results

The calculation of inhibition zone diameter was after incubation for 18 hours, while oxacillin disks needed 24 hours of incubation before being identified as susceptible.

3.2.12. Testing the biofilm production ability

The biofilm production test was conducted with few modifications according to a method performed by(Piechota *et al.*,2018). The experiment was applied on all *S. aureus* isolates, each isolate was grown on BHI agar supplemented with dextrose 0.5% at 37°C for 24 hours, after incubation, the bacterial colony was transferred to BHI broth supplied with 0.5% of dextrose to prepare bacterial suspension matched to McFarland's standard solution 0.5 % that equal to 108CFU/ml. 200 µm of the suspension transferred into wells of 96-well polystyrene plate and incubated without shaking at 37°C for 48 hours, after second incubation the excessive medium was removed and washed 2-3 times with normal saline solution. The next step was a fixation that performed using an oven at 60°C for one hour, then 200 µm of crystal violate (1%) was added for 5 minutes. after this time, the plate was rinsed with normal saline and dried with air for one hour. Colorant was solved in 96% ethanol and absorbency was measured by absorbance microplate reader at 490 nm, each assay was conducted in triplicate to calculate the average results. Absorbance values were considered to be

positive for biofilm formation at absorbency rate ≥0.12, weak biofilm producers at <0.2, moderate at 0.2-0.4, and strong producers at >0.4

3.2.13. Molecular investigation technique

The DNA of all isolates was extracted directly from colonies aged 24 hours, as instructed by the DNA extraction kit manufacturing company that mentioned in Table (3-4), the preparation of the primers was according to the procedure of each primer depending on the manufacturer instruction by suspending the lyophilized product with nuclease-free water. Moreover, the PCR design and amplification conditions were as in.

Table (3-9) Optimization PCR Protocol of 3 genes

Genes	Phase	Temperature	Time	Cycle
Mec A Icaa	Initial denaturation phase	95°C	5 Min	1X
	Denaturation phase	95°C	30 Sec	X35
	Annealing phase	60 ºC	30 Sec	

Icad	Extension phase	72 ºC	1 Min	
	Final extension phase	72 ºC	5 min	1X
	Hold	12ºC	infinite	

Table (3-10).Optimization PCR protocol of Fem A gene

Gene	Phase	Temperature	Time	Cycle
Fem A	Initial denaturation phase	95°C	5 Min	1X
	Denaturation phase	95°C	30 Sec	X35
	Annealing phase	50 ºC	30 Sec	
	Extension phase	72 ºC	1 Min	
	Final extension	72 ºC	5 min	1X
	Hold	12ºC	infinite	

3.2.13.1.Conventional PCR method

The Conventional PCR was achieved according to the manufacturing company of the master mix and the reaction mixture was prepared in a total volume of 25µl. All coagulase-positive sample were subjected to detection of the mec A and fem A genes to identifying the MRSA.While not present

Mec A ,Fem A MSSA .Furthermore were identified for biofilm gene detection icaA ,icaD.

3.2.13.2. Preparation of agarose gel and DNA loading

The procedure of gel electrophoresis was conducted according to Jegasothy *et al.* (2000). The preparation of the gel was by dissolving two grams of agarose in 100 ml of 1X (TBE) buffer. The mixture then was applied in a boiling water bath until all the powder melts and clarified, then allowed to cool down to add 5µl of ethidium bromide to the combination, and the gel has been poured in a balanced gel template with two combs at the end and middle of it.

The two ends of the gel template were sealed. After half an hour, the gel hardens, the combs are lifted, and the seal is released. The comb created wells that were used to load DNA into it. Five µl of DNA leader marker was loaded in one well of each row, followed by the same amount of PCR product loaded into other wells. The gel template is then set in the chamber of the electrophoresis and poured with a TBE buffer. The procedure was conducted at 70 volts for one and a half hours .

3.2.14. Statistical analysis

Statistical Package for the Social Sciences (version 21) was used to analyze the current data. Differences were obtained by applying the Chi square test. Differences were setting as significant at 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$)(Sahu2016).

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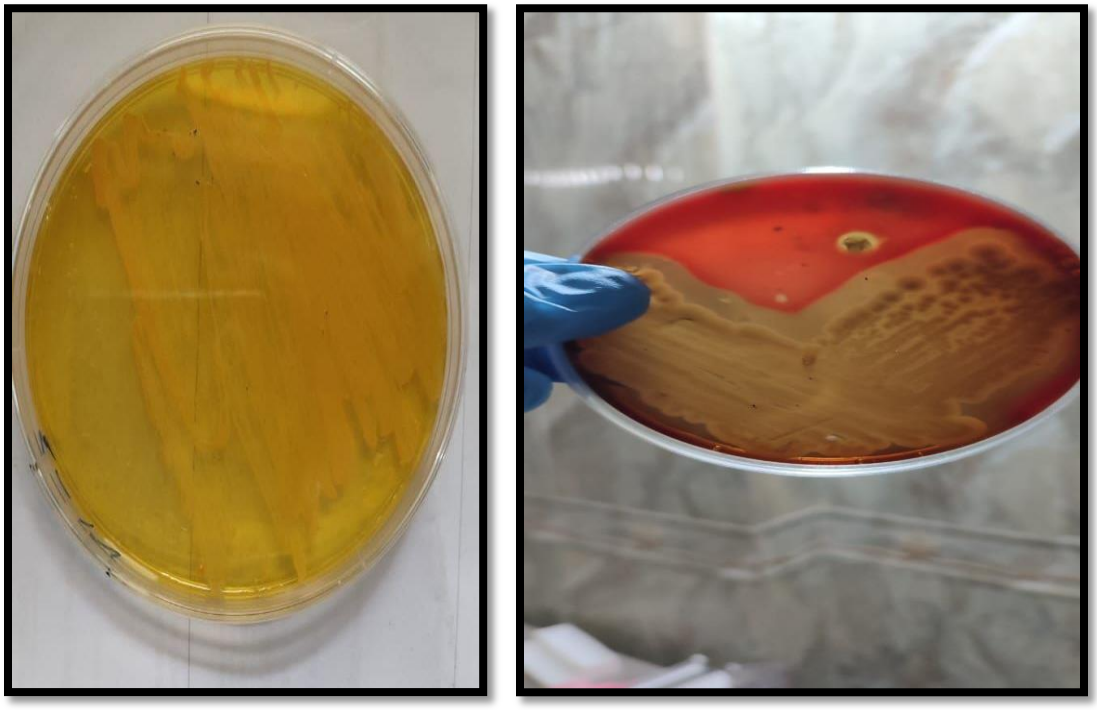
4. Results and Discussion

4.1. Prevalence of *S. aureus*

Staphylococcus aureus isolates from milk and clinical isolates were determined by using conventional standard bacteriological and biochemical tests. All specimens are cultured on blood agar and selective media (MSA) for confirmation of the mannitol fermentation(Figuer 4-1), then subjected to Gram's staining, catalase, oxidase, and further slide coagulase test(Figuers 4-2and 4-3)

Totally 121 (40.3 %) isolates were detected *staphylococcus aureus* and 179 (59.6%) as other bacteria .All *staphylococcus aureus* bacteria have ability to grow in MSA media, oxidase positive and catalase-positive and showed positive results with a slide and tube coagulase test .

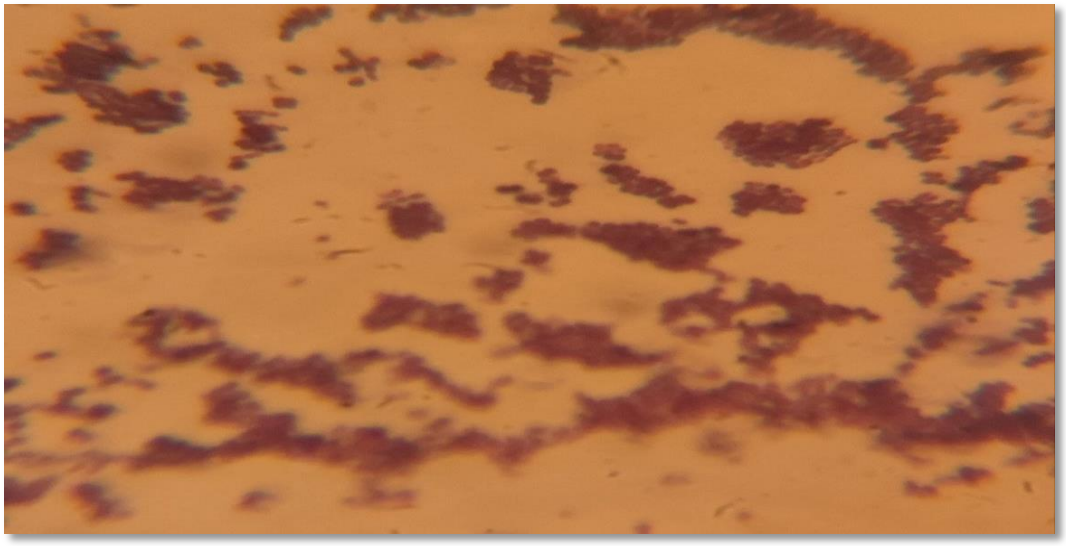


A **B**

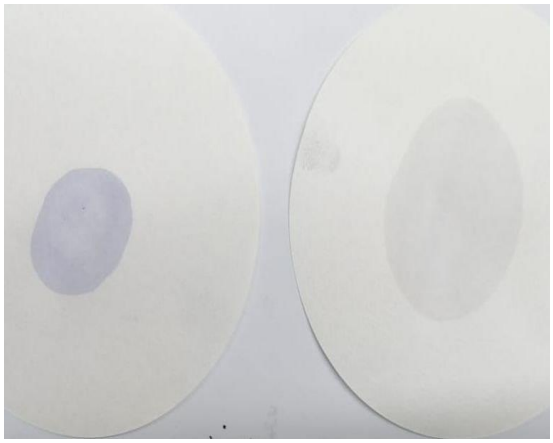
Figuer (4-1) *S.aureus* colony on different agar

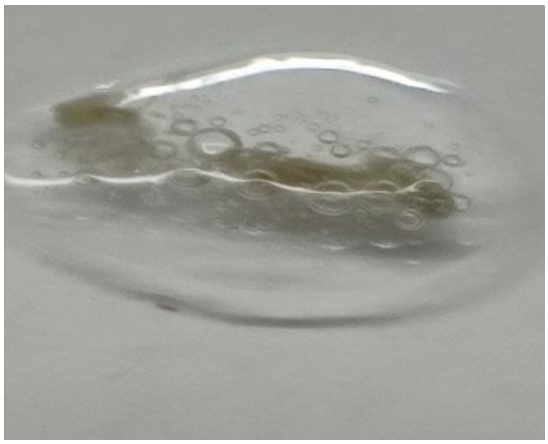
A- *S.aureus* colony on mannital Salt Agar(Yellow coloni with yellow zone)

B-*S.aureus* on blood agar (thick shiny with hemolysis zone)

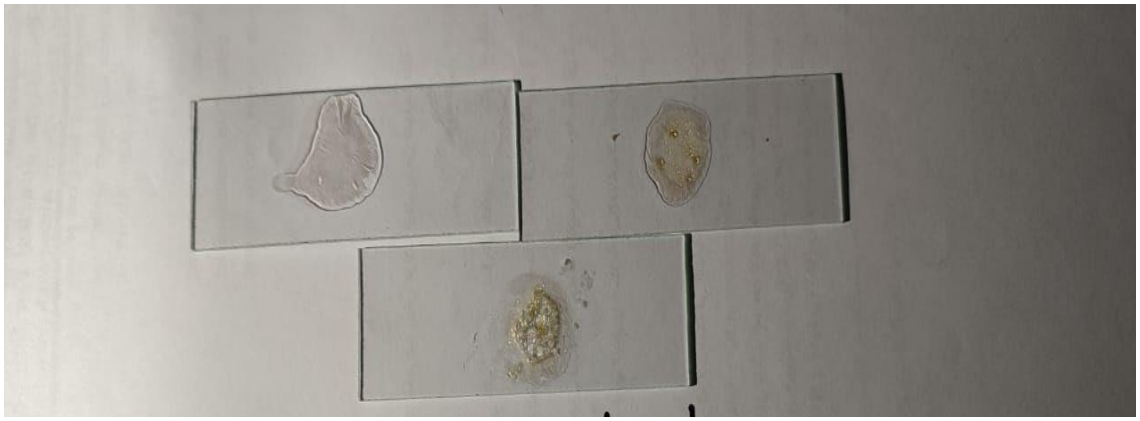


Figuer (4-2) microscopeof *S.aureus* picture after Gram stain(cluster of graps)





A **B**



C

Figuer (4-3) Biochemical test of *S*.aureus

A) Oxidase test B) Catalase tes c) Coagulase slide test

4.1.1.the isolation rate of *S.aureus* among milk and milk products samples

milk is one of the foods that people use daily. It is rich in valuable nutrients. Therefore, it is an excellent growth medium for different microbes (Joanne *et al*., 2011). Furthermore, milk may act as a transmission vehicle for *S. aureus* and other microorganisms from animals to humans. generally the study was conducted and *S. aureus* was isolated from different milk and milk products samples of cow and buffalo in different regions in Karbala city. As shown in Table (4-1), the isolation rate of *S. aureus* from raw milk and milk products samples of cow and buffalo was 40,3%, which was higher than recorded by other studies performed in Baghdad and Basra that showed low isolation rate of *S. aureus* in the same type of samples 31% and 30%, respectively (Abbas 2011; Zakary *et al*.,2011), and lower the results of other researchers in Nineveh and Al Muthanna governorate that shown the isolation rate of *S. aureus* in raw milk were (55%) (Sheet 2010; Aziz *et al*., 2019), and agreed to what some studies have shown in Poland and china

record as (Rola *et al*., 2016; Wang *et al*., 2018). That show the result of isolation of *S.aureus* from raw milk and cheese (50,1%)and (46,2 %) respectively.

Table (4-1), the isolation rate of *S. aureus* from raw milk and milk products samples

Group type	Number of sample	Positive sample	Percentage 0f positive sample	Negative sample	Percentage of Negative sample
Cow raw milk	50	17	34%	33	66%
Imported milk	50	13	26%	37	74%
Imported dairy product	50	18	36%	32	64%
Locally dairy products (Factory)	50	23	46%	27	54%
Farm dairy products	50	30	60%	20	40%
Buffalo raw milk	50	20	40%	30	60%
Total number	300	121	40,3%	179	59,7
Statistical analysis	X^2=14.19, DF=5, P=0.014(S)				

X^2=chi squair ,DF=degree of freedom and P=p value

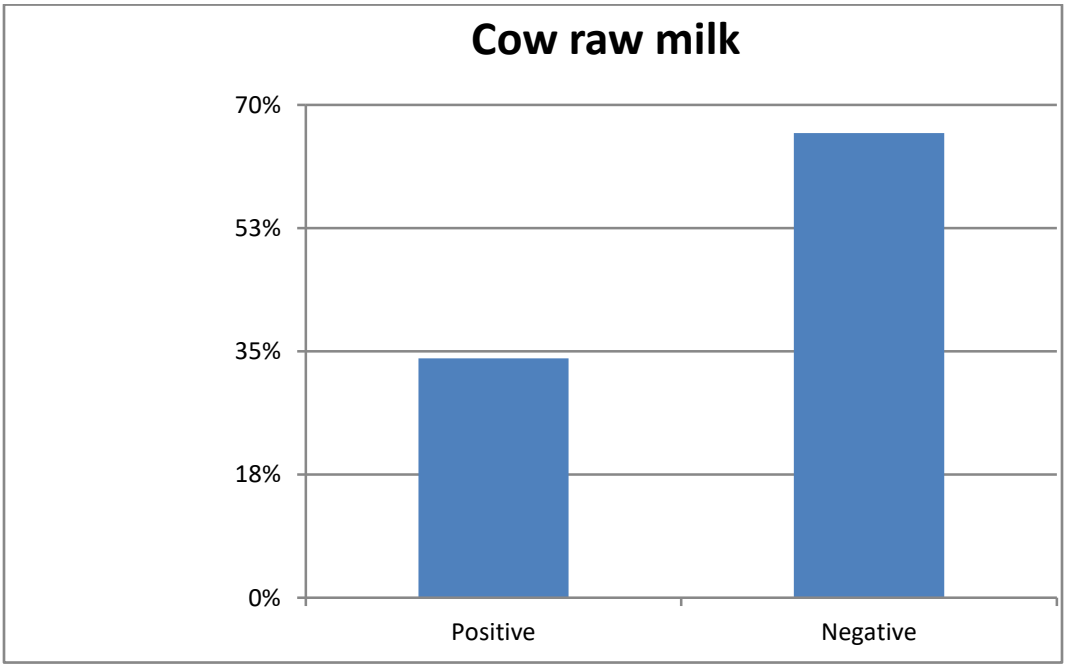
S= significant

This high isolation rate and different isolation rate of *S.aureus* among different regions of study may be due to poor personal hygienic measures during milking, transportation, and presenting raw milk for sale. However, other causes of bad management and the possibility of mastitis cannot be

excluded, geographical origin, manufacturing technology, use of pasteurized or raw milk, sample storage, and handling.

4.1.1.1.The Isolation Rate of *S.aureus* from Cow Raw Milk

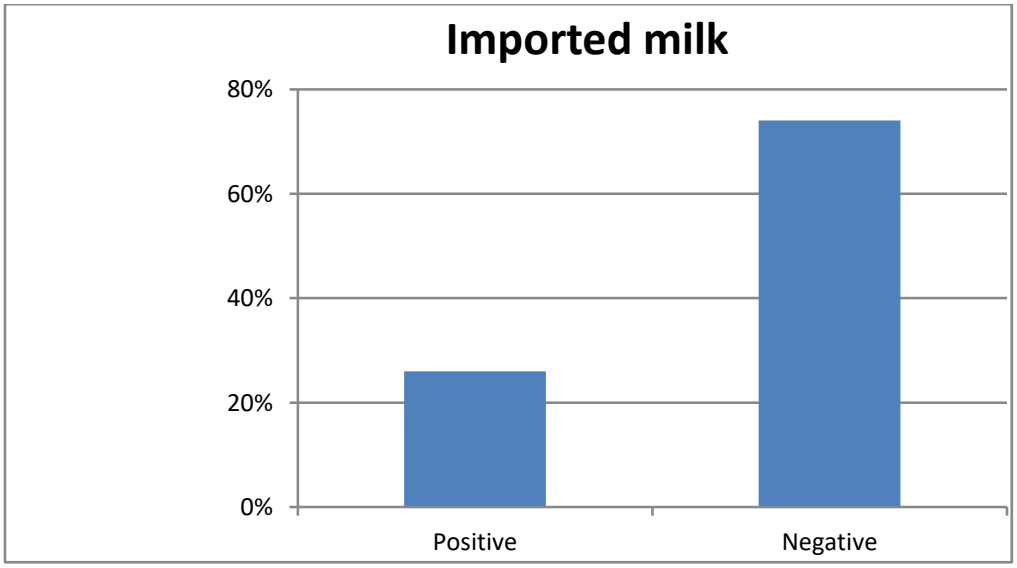
At the contamination of the raw milk, *S. aureus* considered the most common contaminant microorganism incriminated in the staphylococcal food poisoning , that milk and the products derived from it may present a suitable environment for proliferating *S. aureus* and their enterotoxin as well, thus, passing pathogens to consumers , The rate of isolation of *S.aureus* in cow raw milk in this study was 34% Figure(4-4) its lower as compared with other studies in Diyala 52% (Mohammed *et al*.,2021).and Kufa 50% (Almousawi andAlhaatami.,2020). And higher than the study in Misan which show 25% (Hassain and Abbood .,2019). and this result agreed with the previous studies in the same type of samples 31% and 30%, respectively (Abbas 2011; Zakary *et al*.,2011).



Figure(4-4) percentage of isolation of *S.aureus* from cow raw milk

4.1.1.2. The isolation rate of *S. aureus* among imported milk

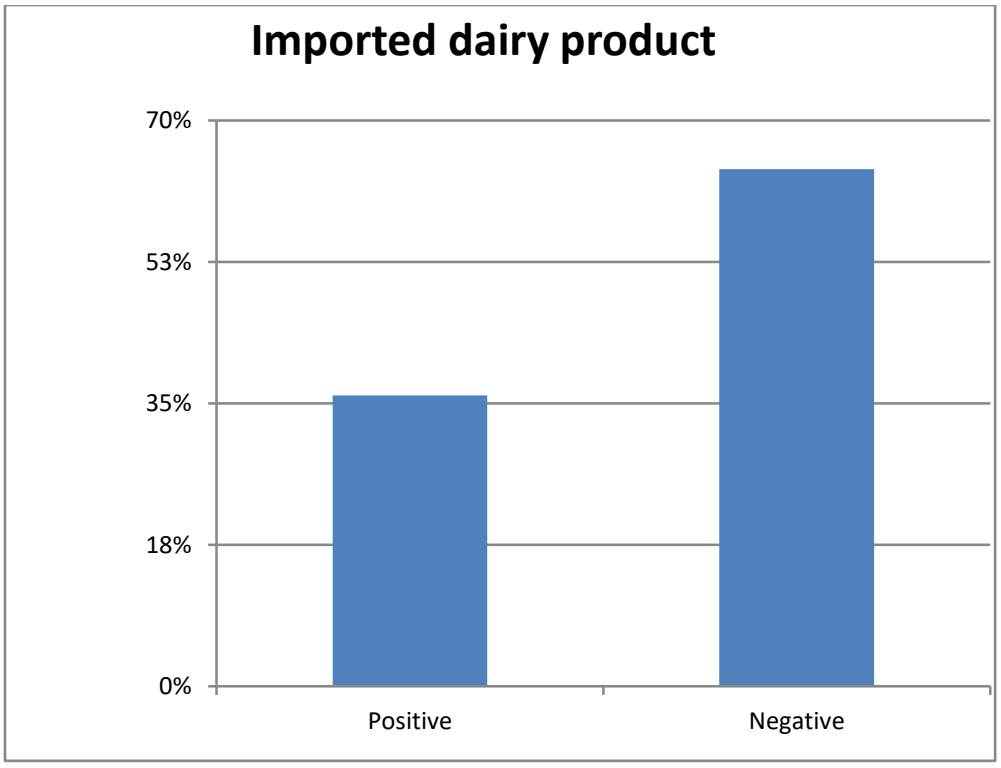
The percentage of isolation of *S.auerus* in imported canned milk was 26% Figure(4-5) its higher than some studies in Iran such as: 10.6 % (Torki *et al*.,2020). and this result agreed with the previous in Misan city 25%(Hassain and Abbood., 2019) .and lower than other study in Baghdad, Iraq that s show percentage 48.5% of *S.aureus* isolation from similer samples (Al-Khafaji *et.al.,2014*)



Figuer(4-5) Percentage of *S.auerus* in Imported Milk.

4.1.1.3. The isolation rate of *S. aureus* among imported dairy products

The percentage of isolation of *S.aureus* imported dairy products was 36% Figure(4-6) its nearly from(41%) isolation in Eygpt(Ahmed *et al*.,2019) .and 32% in Iran (Imani *et al*.,2010) and the present study disagrees with(Rasul *et al*.,2019). in Iran that show result was 18.75% .and lower than anther result in Egypt(Al-Ashmawy *et al*., 2016)that show result was 53% in the same sample.



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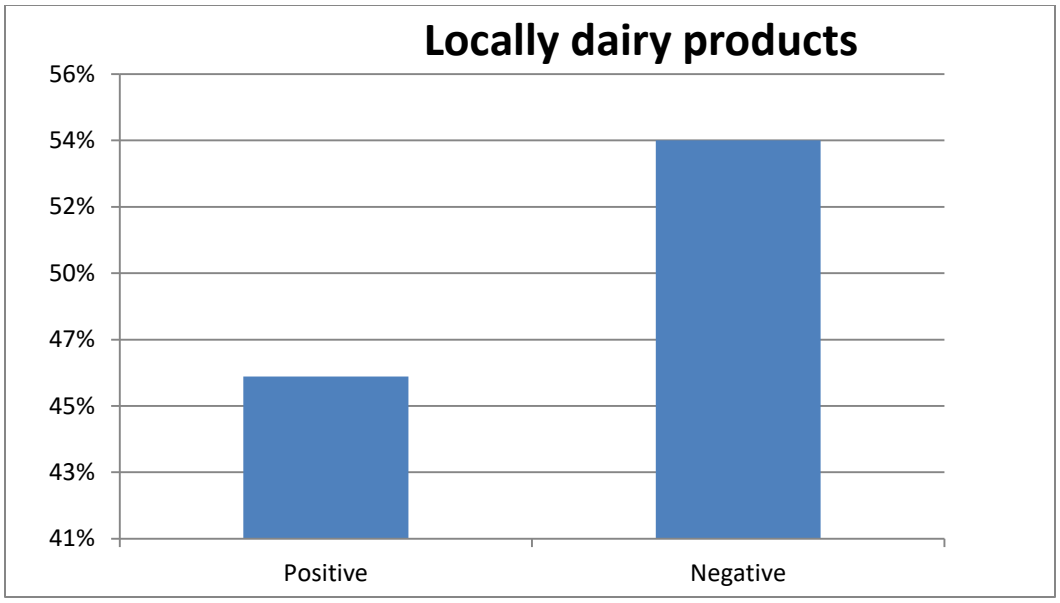
Figuer(4-6) Percentage of *s.auerus* in Imported Dairy Products

4.1.1.4. The isolation rate of *S.aureus* among locallydairy products (factory)

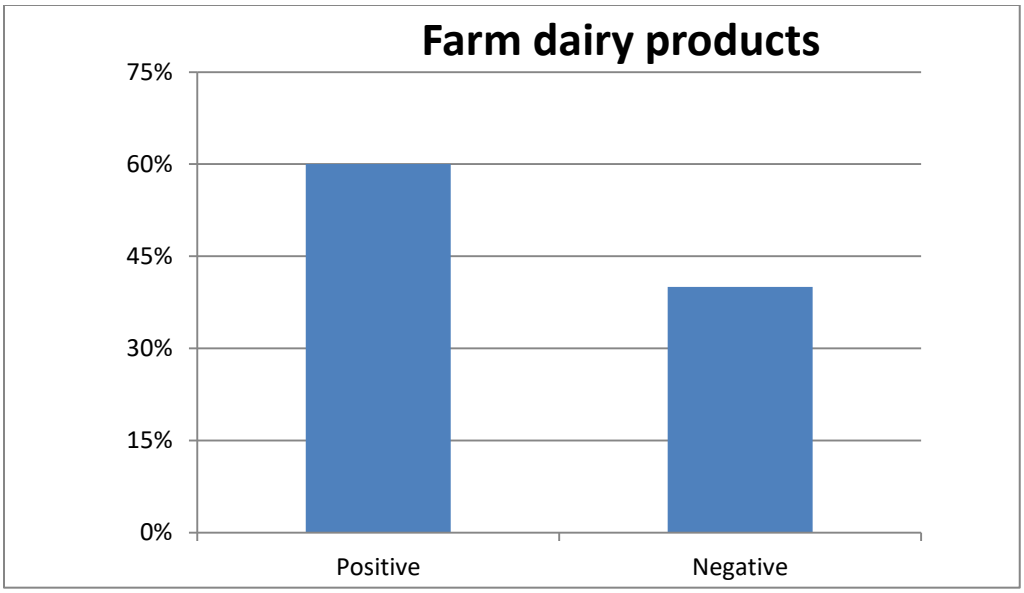
The percentage of isolation of *S.aureus* from this products was 46% Figure (4-7) this result agree with another study in University of Baghdad(Abed Rabba and Saab,2022)that show the result of *s.aureus* was(45%).However, the present study disagrees with(Sasidharan *et al*;2011) in Malaysia that show result the incidence of *S.aureus* from milk products was(10%).



Figure(4-7) Percentage of Isolation of *S.aureus* from locally dairy products (factory)

4.1.1.5. The Isolation Rate of *S. aureus* among farm dairy products

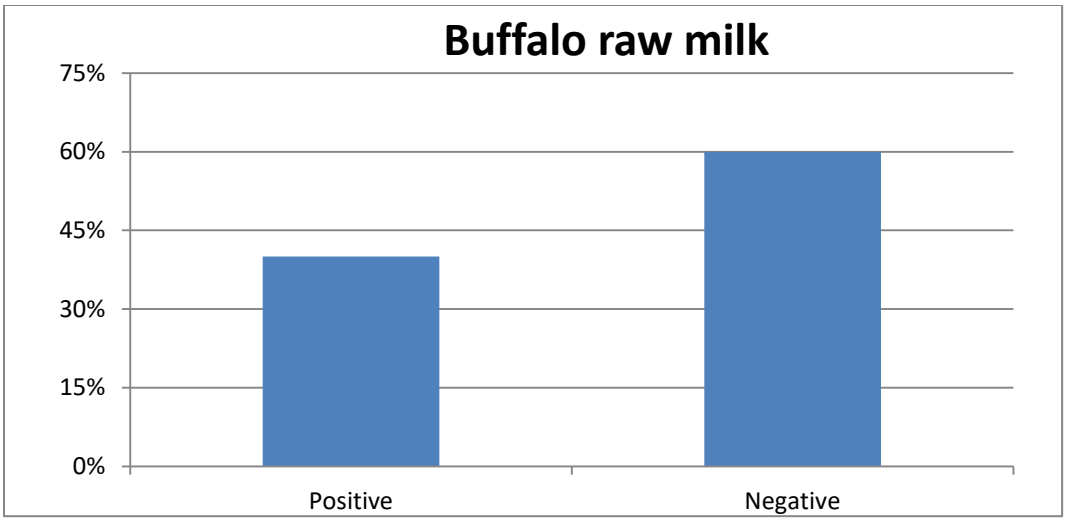
This study show high rate in isolation of *S.aureus* 60% Figuer(4-8) may be a result poor personal hygienic measures during milking, transportation, However, other causes of bad management ,Its higher than other study in market and farm in Al-Qadisiyah and Bagdad that showed low isolation rate of *S. aureus* in the same type of samples 40% and 33.33% respectively **(**Alhasnawi et al .,2018**;** Kanaan and AL-Shammary.,2013**),** while close to the results of other researchers in Basrah that shown the isolation rate of *S. aureus* in the same type of samples 50% (Aboud and Khudaier .,2018) .and lower than study in Diyala show 80%(Mohammed *et al.,2021*).



Figuer (4-8) Precntage Isolatin of *S.aureus* in Farm Dairy Products

4.1.1.6.The Isolation rate of *S.aureus* from Buffalo Raw Milk

The percentage of isolation of *S.aureus* in buffalo raw milk in this study was 40% which was higher than recorded by other studies performed in Qadissiya and Basrah cities that showed low isolation rate of *S. aureus* in the same type of samples 16% and10,23% , respectively (Hiba 2017 ; Bassam *et al*.,2014), and agreed to what some study have shown in Basrah,40,54%(Weam, and Bassam 2018) ,and close to the results of other researchers in Nineveh that show 78% in the same type of samples(Sheet., 2010),and 94% in Bagdad(Hassan.,2017)

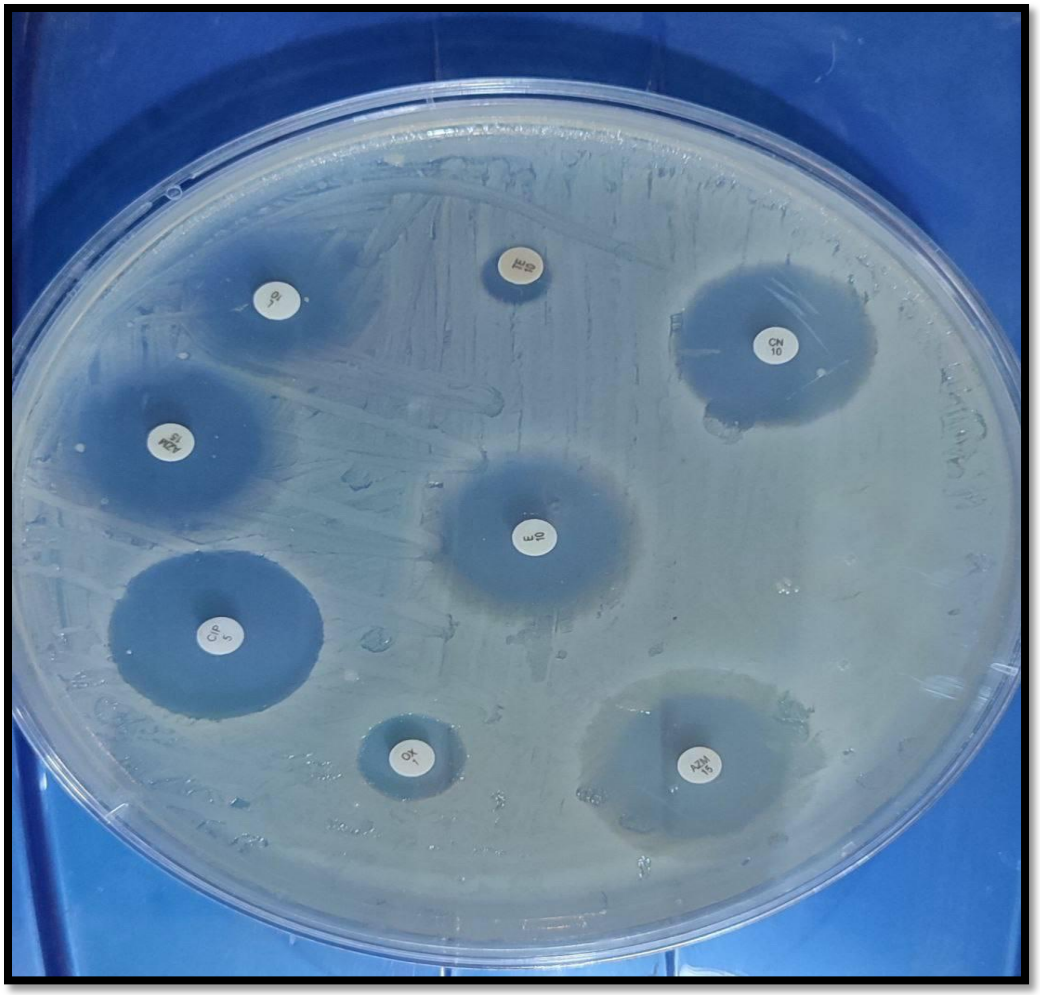


Figure(4-9)percentage of isolation of *S.aureus* from buffalo raw milk

4.2. Evaluation of antibiotic susceptibility test

This study was designed to highlight the current antibiotic-resistant profile of 121 *S. aureus* isolates in order to guide the veterinarian and human All 121 isolates were tested for their susceptibility to 8 antimicrobial drugs and classified as resistant, intermediate, and susceptible according to CLSI (2020)Figuer(4-10). The prevalence of susceptibility to each antibiotic tested is presented in Table (4-2).

The 121 *S. aureus* isolates were most frequently resistant to penicillin (50.76%), oxacillin(40.89%), but more susceptible to gentamycin (100%), ciproflxacine(100%), Azithromycin (73.66%)and Erythromycine(63.24%)



Figuer (4-10) Antibiotic suscptability of *S.aureus*

Table (4-2): Antibiotic Susceptibility Result of 121 *S. aureus* of Milk and Milk Products

Antibiotic	R F(%)	I F(%)	S F(%)	P-value
PG 10	61(50.77 %)	0(0%)	60(49.23%)	p-value=0.064 ns DF= 2 X^2=90.7
OX	49(40.89 %)	0(0%)	72(59.11%)	p-value=0.092 ns DF= 2

				X^2= 100.5
E 10	20(16.3 %)	24(19.54 %)	77(63.24%)	p-value=0.124 ns DF=2 X^2= 75.92
L 10	24(19.75 %)	43(35.23 %)	54(45.01%)	p-value=0.198 ns DF= 2 X^2= 17.13
CN 10	0(0%)	0(0%)	121(100%)	_
CIP 5	0(0%)	0(0%)	121(100%)	_
AZM 15	8(6.58%)	24(19.75 %)	89(73.66%)	P-value=0.13 ns DF= 2. $x^{2=}$ 136.88
TE 10	12(9.88 %)	57(47.23 %)	52(42.9%)	P-value=0.11 ns DF= 2 X^2=45.5

F= Frequency M=, Ns= non-significant, S= significant, pG=Pencilline ,OX=Oxacilline, E10=Erythromycine,L10=Lincocine,CN=Gentamycine,CIP5=Ciproflaxacine,AZM 15=Azithromycin and TE 10=Tetracycline

In table(4-2) all antibiotics was show non significant between different type of antibiotics.

4.2.2 Resistance pattern among milk and milk products

Among milk and milk products samples, there are varying degrees of resistance for each antibiotic and in the following sequence: penicillin G 61 (50.76%), erythromycin 20 (16,29%), tetracycline 12 (9,87%), oxacillin 49

(40,89%), Lincomycine24 (19,75%), ciprofloxacin 0 (0%), gentamicin 0 (0%), and Azythromycine8 (6.58%) show Table(4-3).

In this study show no resistant to ciprofloxacin and gentamycin These results were agreed with (Sahar *et al*.,2019)in Egypt who recorded resistance rates of 0 % and 9.9%, to ciproflaxacine and gentamycine respectively.

The resistance rate to tetracycline of this study was compatible with resistant of *s.auerus* isolated from milk and milk products (Shengjuan *e t al.,* 2019) in China who recorded resistance rates of (0%) to tetracycline , The resistance rate of oxacillin among milk and milkproducts (40,89%) this result agree with (Almousawi and Alhatami., 2020) that show the resistant of oxacilline in the raw milk was (36%) ,

The Resistance of Pencilline G , Erythromycine and lincomycine in this study was compatible with (Hossein *et al.,* 2015)Iran who recorded resistance rates of , 47.3 %, 7.9 %and 11.9 % to Pencilline G,Erythromycine and Lincomycine, respectively. The resistance of Azithromycine show result near to the (Sharm *et al*.,2011)in India that show result (0%) .

Table (4-3) percentage of the resistant of *s.aureus* among different group in this study.

groups	**PG**	**OX**	**E10**	**L10**	**CN10**	**CIP5**	**AZM10**	TE10

Cow raw milk	52.94	39.13	16.67	23.53	0	0	5.88	11.76
Imported milk	53.85	40	15.38	15.38	0	0	7.69	7.69
Imported dairy products	50	46.15	16.67	22.22	0	0	5.56	11.11
Locally Dairy products(factory)	47.83	38.89	17.39	17.39	0	0	8.7	8.7
Farm Dairy products	50	40	16.67	20	0	0	6.67	10
Buffalo raw milk	50	41.18	15	20	0	0	5	10
Average	50.76	40,49	16.3	19.75	0	0	6.58	9.88

PG=pencilline,OX=Oxacilline,E10=Erythromycine,TE=Tetracycline,CN=gentamycine,L=Lincocine,AZM15=Azythromycine, CIP 5=Ciproflaxaci

4.4. Characterization of *S. aureus* and Molecular typing

4.4.1. Molecular prevalence of MRSA

The antibiotic susceptibility test revealed that 49 of *S. aureus* isolated from milk and milk products were resistant to oxacillin. All 121 isolates were assessed for the presence of mecA and fem A genes by PCR. The mecA gene was amplified with a molecular size of 310 bp in 121 isolates while fem A gene was amplified with amolecular size of 134 bp in 121 isolated . Therefore, the prevalence rate of MRSA isolates was 40,49 %. The remaining 72 (59,50%) isolates were mecA-negative (MSSA), as photographed in Figures(4-11)

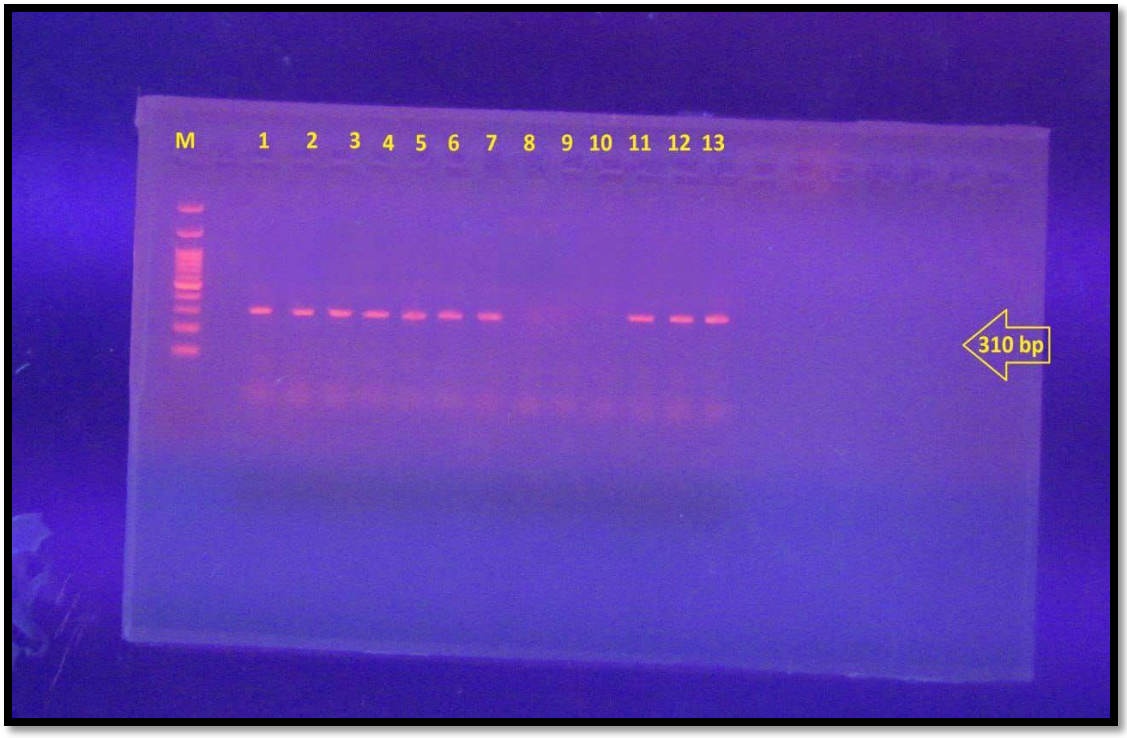


Figure (4-11): Gel electrophoresis of the mecA gene among *S. aureus* isolated from milk and milk products. size 310 bp

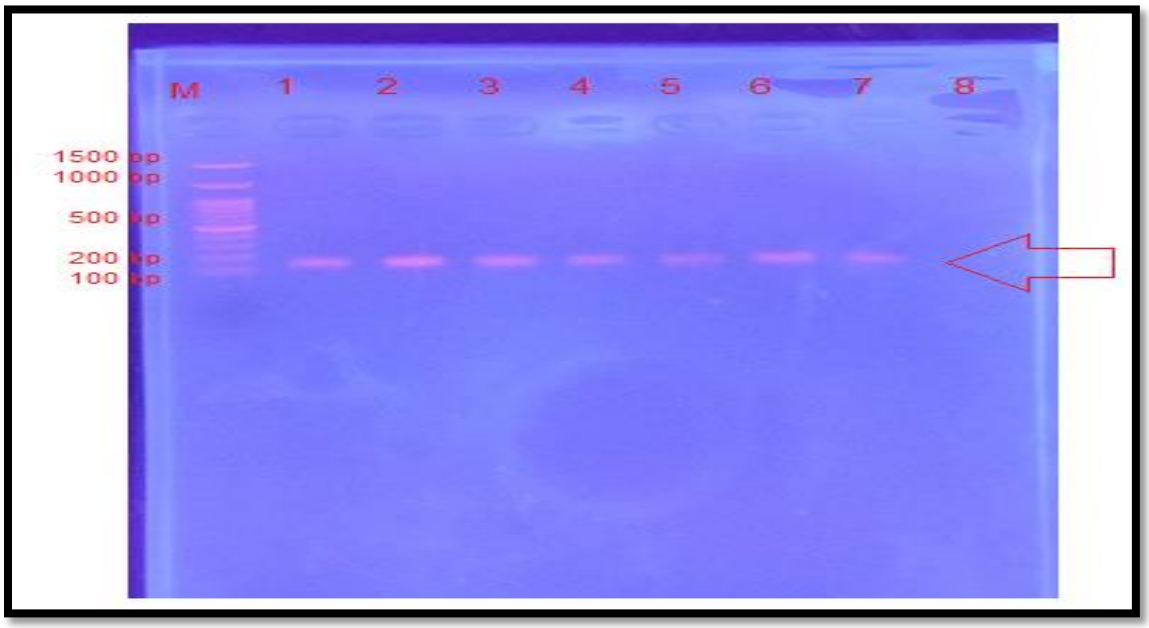


Figure (4-12): Gel electrophoresis of the femA gene among *S. aureus* isolated from milk and milk product size 134 bp

Table(4-4) Number of MRSAand MSSA of *staphylococcus areus* in different source

Group type	MRSA	Percntage	MSSA	percentage	Number
Cow raw milk	7	41,17%	10	58,82%	17
Imported milk	6	46,15%	7	53,84%	13
Imported Dairy products	7	38,88%	11	61,11%	18
Locally dairy products (factory)	9	39,13%	14	60,86%	23
farm Dairy products	12	40%	18	60%	30
Raw buffalo milk	8	40%	12	60%	20
Total	49	40,49	72	59,50	121
Statistical analysis	X^2= 0.218, DF=5 , P-value = 0.99				

The pooled prevalence of MRSA among *S. aureus* isolates was 40,49% and for each type of sample as follows:7(41,17%) among cow raw milk ;6(46,15%)among imported milk 7 (38,88%) among imported dairy products;9 (39,13%) among locally dairy products factory;:12 (40%)among farm dairy products and 8(40%) from buffalo raw milk .The current study revealed that fem a gene MRSA isolate was found among milk and milk products and this result agreed with study in Al-Qadisiyah in Iraq(Neamah*et al*.,2019) that show result (46.7%) positive to fem a Isolation from raw milk

in cattle.and(Weldemelak *et a.*,2020) that show (42.9%) of *S.auerus w*as positive to fem a gene that isolation from dairy products in Ethiopia.

The discrepancy in the results of antibiotic suscptibility and molecular test can be explained by the fact that bacteria may use other methods to combat methicillin and its derivatives without having to possess. a mecA gene that responsible of β-lactam resistance, it can be due to many reasons, including hyper production of β-lactamase enzyme among mecA-negative MRSA strains (Olayinka *et al*., 2009).

Moreover, (Ba *et al*.,2014) reported that there is a specific alternation in different amino acids among the proteins of protein binding cascade (PBP type 1, 2, and 3) and these features were among MRSA strains that lack the mecA gene. Furthermore,(Banerjee *et al*.,2010) reported that there are specific mecA-negative MRSA strains have expressed specific mutation in different amino acids among the protein of PBP4 that may help the bacteria in methicillin resistancee.

In 2003, Yoshida *et al* reported that the loss of a mecA gene among MRSA strains can be compensated for by acquiring a wall three times thicker than normal. These findings demonstrate that there are other mechanisms for resistance to methicillin and its derivatives, and the molecular methods alone are not sufficient for the definitive characterization of MRSA isolates.

4.5. Biofilm formation ability of *S. aureus*

One of *S. aureus'* virulence factors is its biofilm-forming capacity (Archer, 1998). Biofilm is a sessile microbial community embedded in a

protective extracellular polymeric matrix, in which cells are attached to a surface or to other cells. This form of growth displays altered physiology, gene expression and protein production, which enables *S. aureus* bacteria to attach to medical implants and host tissues, and underlies its resistance to therapeutic treatment (Lister and Horswill, 2014).

All 121 isolates were assayed for biofilm formation . In respect to the total ability of isolates to produce biofilm in Figuer (4-13) , the findings showed biofilm formation that classified to strong,modrate and weak , The biofilm formation assay revealed that 74 (62,5%) of *S. aureus* isolates possessed strong biofilm-formation ability, 28 (22,08 %) had moderate, and 19 (15,42 %) isolates had weak biofilm-formation ability, show table(4-5) these results were agree with (Poliana *et al*.,2012).in Brazil that show the result of biofilm formation in *s.aureus* isolation from cow milk was (98.9%) ,(Al-Iedani.,2016) that show result of biofilm formation of *S.aureus* isolation from raw milk in Basrah city was (90.9%) and (Bissong and Ateba ,2020) in the south Africa that show result (90.9%) of biofilm formation of *S.aureus* in same sample .

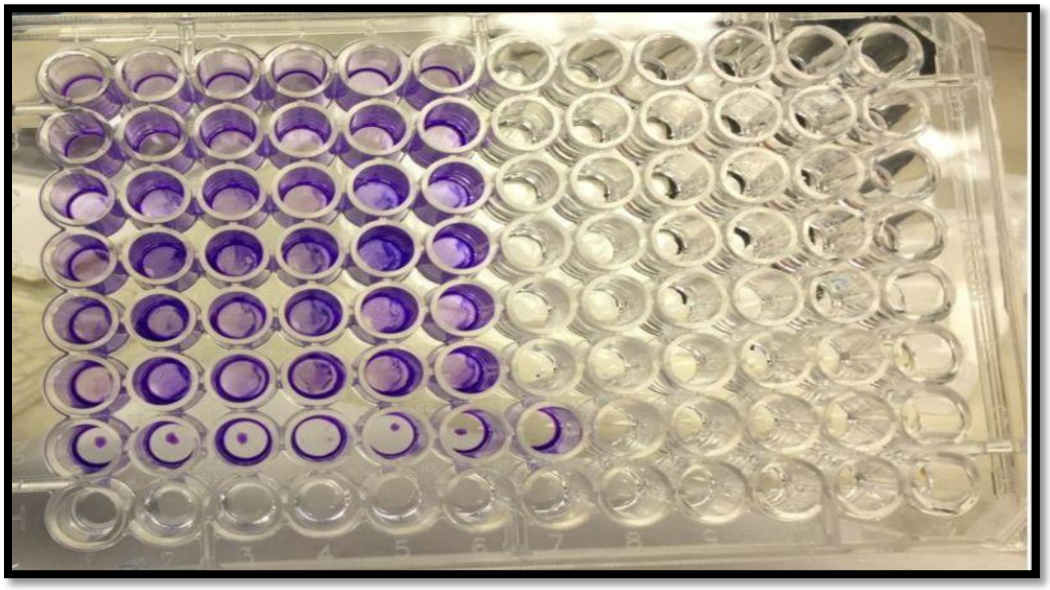
While, these results disagree with studies that showed lower isolation rates , these results were close to (Lee *et al*.,2014) who showed the biofilm production among *S. aureus* isolated from different cow samples was 45.2%, and with(Gajewska and Chajęcka .,2020) who demonstrated that the production ability was 41% among *S. aureus* isolated from cow milk samples, also (Thiran *et al*.,2018) reported that the rate of biofilm production among *S. aureus* isolated from milk samples was 45.8%.

Moreover, the current study showed a high rate of strong biofilm producers among *S. aureus* isolated from milk and milk products 62,5% This result close to (Gajewska and Chajęcka .,2020) results who reported a nearlly ratio among cow milk samples that show results (60%). In a previous study conducted in Basra city no strong biofilm producer *S. aureus* isolates had been reported (Idbeis and Khudor. , 2019).

Table 4-5: biofilm formation test results for *S. aureus* isolated from milk and milk products

Group type	Positive sample	Strong	%	Moderate	%	Weak	%
Cow raw milk	17	11	64.71 %	6	35.29 %	0	0%
Imported milk	13	9	69.23 %	0	0%	4	30.77 %
Imported dairy products	18	16	88.89 %	2	11.11 %	0	0%
Locally dairy products (factory)	23	12	52.17 %	6	26.09 %	5	21.74 %
Farm dairy products	30	18	60%	6	20%	6	20%

Buffalo raw milk	20	8	40%	8	40%	4	20%
total	121	74	62,5 %	28	22.08 %	19	15.42 %
Statistical analysis	X^2= 20.59 , DF= 10 , P-value= 0.024						



Figuer(4-13) Determination of biofilm formation of *S.aureus* By using Specto photometer in 490 nm

4.6. Molecular typing of biofilm formation

Among several defensive mechanisms adopted by *S. aureus*, the biofilm production, mainly mediated by the icaA and icaD genes, is a potential virulence factor involved in bacterial evasion from host immune surveillance and leading to persistent udder infections in dairy animals (Thiran *et al*., 2018). Moreover, biofilm-producing *S. aureus* exhibits reduced antimicrobial susceptibility due to poor penetration of antimicrobials, slower growth of bacteria, and horizontal transfer of antibiotic resistance genes in biofilm (Shin *et al*., 2021) .

Among 121 sample of *S.aureus* the percentage of Icaa(67.86%)and Icad(64.24%) show in table(4-6) this result lower than (Zahid *et al*;2020)in Misan city that show the result of Icaa and Icad of *s.aureus* that isolation from raw milk 92,30%,(Bissong and Ateba.,2020) in South Africa that show the result of biofilm formation was (90.9%) of *S.aureus* isolated from raw milk, .and higher than another study of (Prenafeta *et al*;2014)in United Kingdom that show percentage of Icaa and Icad was 50% from *s.aureus* that isolation from bovine milk.

Table(4-6) Prevalence of (icaa,icad) genes among different groups in milk and milk products

Group type	Positive sample	Icaa	percentage	Icad	percentage	P value
Cow raw milk	17	12	70.59%	11	64.71%	0.71
Imported milk	13	10	76.92%	8	61.54%	0.39
Imported Dairy products	18	14	77.78%	15	83.33%	0.67
Locally dairy products (factory)	23	15	65.22%	14	60.87%	0.76
farm Dairy products	30	20	66.67%	18	60%	0.59
Buffalo raw milk	20	10	50%	11	55%	0.75
Total	121	81	67.86%	77	64.24%	0.34
Statistical analysis	X^2= 0.387 , DF=5, P-Value= 0.99					

In this study that show the biofilm prevalence genes Icaa was(67.86%) among different groups This outcome was consistence with (Ren

et al:2020) in china that show the rate of Icaa 69.2% from dairy products.and lower than (Unlu *et al* ;2018)in turkey that show the rate of Icaa in *S.aureus* was 90% that isolated from milk samples.and higer than(Kandil *et al*;2020) in Eygpt that show Icaa genes in *s,aureus* was 20% from milk samples.show Figure(4-14)

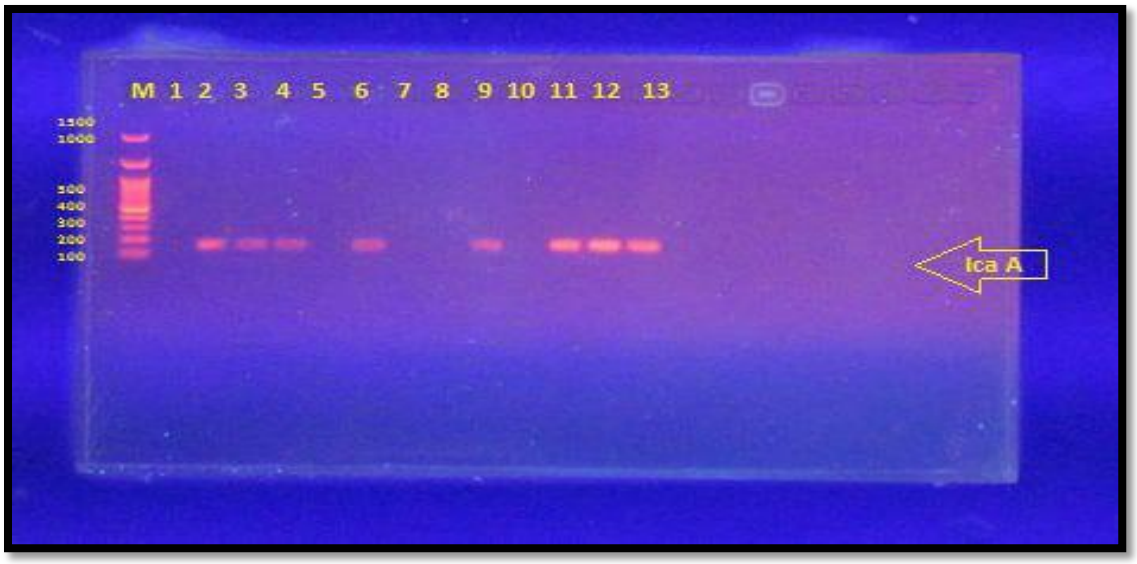


Figure (4-14): gel electrophoresis of the Icaa gene among *S. aureus* isolated from milk and milk products size 151 bp

Prevalencs of Icad genes in this study was (64.24%) among different groups of milk and milk products,This study lower than(Khoramrooz, *et al*.,2016)in Iran that show the prevalence genes Icad of *S.aureus* from bovine milk was (87.5%).and higher than(Hendijani *et al*;2019) in Iran that show the prevalence of Icad gene in *S.aureus* that isolation from raw milk was(25%). Show Figuer(4-15).

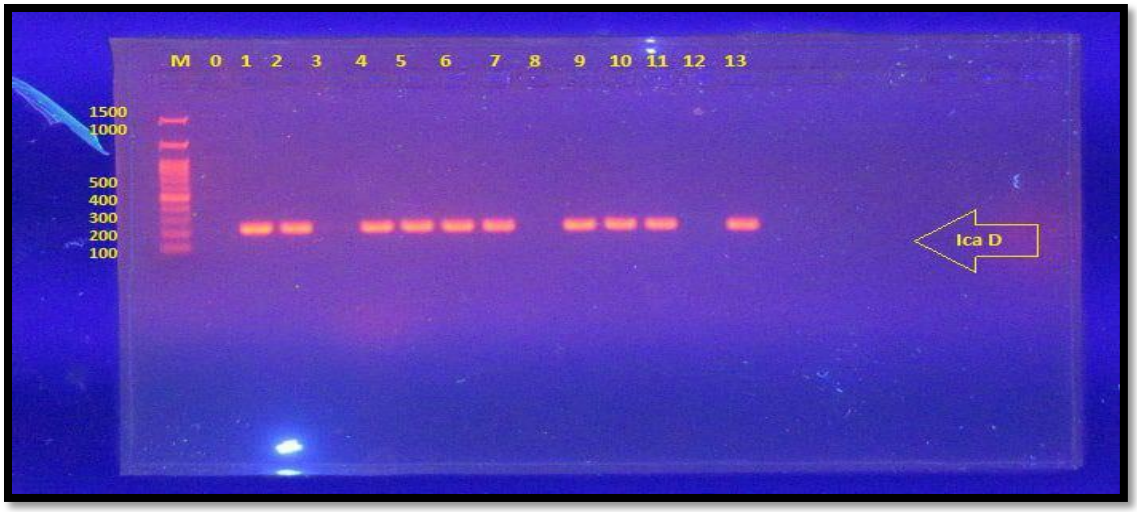


Figure (4-15): gel electrophoresis of the Icad gene among *S. aureus* isolated from milk and milk products size 211 bp

4.7. Detection of beta-lactamase enzyme by a rapid direct iodine method

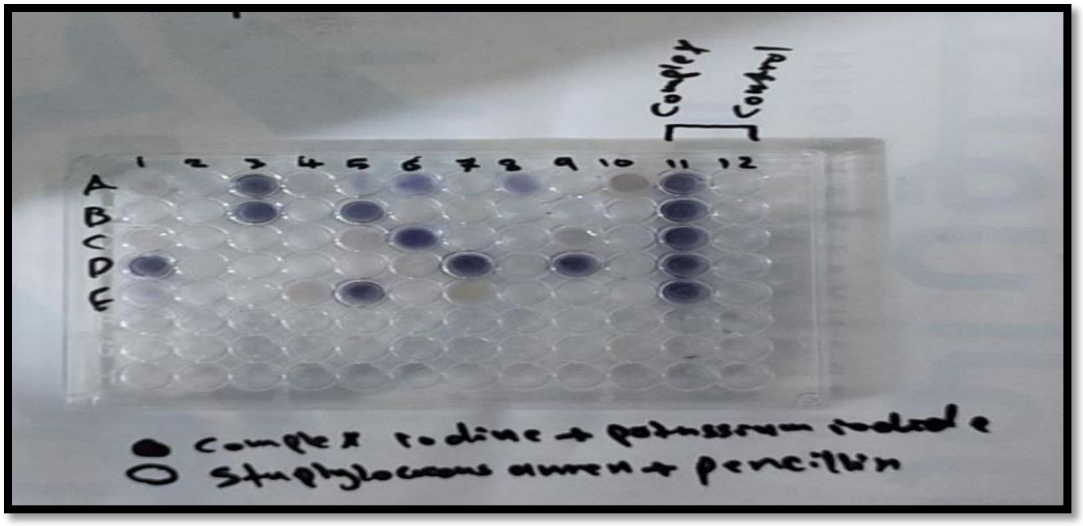
There has been a surge in the emergence of antimicrobial resistance (AMR) in pathogens due to presence of innate or acquired genetic machinery that confers reduced susceptibility to antibiotics(Aslam *et al*.,2018).

Bacterial species have numerous mechanism by which they evade the effect of antibiotics. The most common mechanisms are the production of inactivating enzymes which react with antibiotics to inactivate them. For example β lactamases cleave the beta lactam ring of beta lactam antibiotics, thereby rendering them ineffective. Another mechanism is the increased production of efflux pumps that continuously pump antibiotics out of the bacterial cell.

Decreased permeability to drugs are another factor where in there is a decrease in expression of porins to reduce the amount of antibiotic entering the cell. Transformation of antibiotic target, alteration of the drug target to prevent the drug from binding are some other mechanisms of drug resistance. Amongst these the prevalence of genes for production of antibiotic inactivation enzymes like beta lactamase is reportedly high almost 80% varying slightly in certain geographical regions.(Nasa *et al*.,2012).

In this study used direct method to detected the beta- lactamase Enzyme by using starch-Iodine complex in rapid direct iodine method. Taking into account that the isolate is resistant to most beta-lactam antibiotics in the antibiotic susceptibility test (pencilline G) in Figuer (4-16). The result show within (15 munits) and the percentage of detection of beta lactamase Enzyme was 61(100%) from sample that show resistant to penicillin G in different group of milk and milk products .

This result agree with (Mustafa,2007) in Basra ,(Rajasree *et al* ;2020) in KSA and(Chaudhary *et al*;2021) in india that show the effect of *S.aureus* beta-lactamase was 100% from *S.aureus* isolation from raw milk , poultry and human respectively.and higher than anthor studies (Bouharkat *et al*;2020)in Algeria that show beta-lactamase production from *S.aureus* isolation from human was 18.2%



Figuer (4-16) Detection of beta-lactamase enzyme by the direct rapid iodine method

4.8. Relation between antibiotic susptability and biofilm formation

The results of the current study showed in Table (4-7) that the resistance of *S.aureus* bacteria to antibiotics increases on biofilm production, as we found in our study that there are 104 (85.95%), 42 (34.71%) and 28 (23.14%) resistant *S.aureus* bacteria that have the ability to produce strong, medium and weak biofilms, respectively, except for two antibiotics, gentamicin and ciprofloxacin that we found that there is no significant relationship between them and biofilm production.

Table (4.7) relationship between antibiotic susptability and biofilm formation

Antibiotic	Strong biofilm			Moderate biofilm			Weak biofilm		
	S	I	R	S	I	R	S	I	R
PG	36	0	38	15	0	13	10	0	9
OX	30	0	44	12	0	16	7	0	12
E 10	12	16	46	4	4	20	4	4	11

TE 10	6	31	37	3	15	10	3	11	5
CN 10	0	0	74	0	0	28	0	0	19
L 10	14	25	35	7	12	9	3	6	10
AZM 15	6	14	54	1	9	18	1	1	17
CIP 5	0	0	74	0	0	28	0	0	19
TOTAL	104	86	402	42	40	142	28	22	102

PG=pencilline,OX=Oxacilline,E10=Erythromycine,TE=Tetracycline,CN=gentamycine,L=Lincocine,AZM15=Azythromycine, CIP 5=Ciproflaxacine,R=Résistance, I=intermediate and S=sensitive

4.9. Characterization of MRSA isolates

The coexistence of mecA,femA,Icaa and Icad genes, antibiotic susceptibility testing and biofilm production as well among MRSA, that show in table(4-8)

Isolates code	Origin	Antibiotic resistance pattern	mecA	FemA	Icaa	Icad	Biofilm formation test
S3	Cow raw milk	PG,OX	+	+	+	+	Strong
S15	Cow raw milk	OX,TE,AZM	+	+	+	+	Strong
S20	Cow raw milk	PG,OX	+	+	+	+	Strong
S22	Cow raw milk	PG,OX,TE	+	+	+	+	Strong
S31	Cow raw	OX,L,AZM	+	+	+	+	Moderate

	milk						
S44	Cow raw milk	OX,E,	+	+	-	+	Strong
S49	Cow raw milk	PG,OX	+	+	-	+	Strong
S10	Imported milk	OX,AZM.	+	+	-	--	Strong
S14	Imported milk	PG,OX,	+	+	-	+	Moderate
S28	Imported milk	OX,PG	+	+	+	+	Moderate
S33	Imported milk	OX.AZM.PG	+	-	+	+	Moderate
S47	Imported milk	OX,PG	+	+	+	+	Weak
S50	Imported milk	OX.E	+	+	+	-	Moderate
S2	imported dairy products	PG.OX,L	+	+	+	+	Strong
S9	imported dairy products	OX,AZM,	+	-	+	-	Strong
S17	imported dairy products	OX,PG,TE	+	+	+	-	Strong
S26	imported dairy products	OX	+	+	+	-	Strong
S36	imported dairy products	OX,PG	+	+	-	+	Moderate
S41	imported dairy products	PG,OX.AZM	+	+	+	+	Strong
S46	imported dairy products	OX.E,PG	+	+	+	+	Strong
S1	Locally dairy	OX,PG.TE	+	+	-	+	Weak

	product factory						
S9	Locally dairy product factory	OX,AZM,E,PG	+	+	+	+	Moderate
S11	Locally dairy product factory	OX,TE,L	+	+	+	-	Moderate
S17	Locally dairy product factory	OX	+	-	-	+	Strong
S28	Locally dairy product factory	OX ,PG,AZM	+	-	+	-	Strong
S35	Locally dairy product factory	OX,PG	+	+	+	+	Strong
S37	Locally dairy product factory	OX ,E,PG	+	+	+	+	Strong
S40	Locally dairy product factory	OX,L	+	+	+	+	Strong
S46	Locally dairy product factory	OX,AZM,	+	+	-	+	Strong
S1	farm dairy products	OX,PG.L	+	+	+	+	Strong
S13	farm dairy products	OX,AZM,E	+	+	-	+	Strong
S19	farm dairy products	OX,PG	+	+	+	-	Strong
S20	farm dairy products	OX ,AZM	+	+	+	+	Strong
S25	farm dairy products	OX	+	-	-	+	Moderate
S31	farm dairy products	OX ,PG,E	+	-	+	+	Moderate
S34	farm dairy	OX,PG	+	+	+	+	Moderate

	products						
S35	farm dairy products	OX,L	+	+	-	+	Moderate
S38	farm dairy products	OX,PG.AZM	+	+	-	+	Strong
S40	farm dairy products	OX PG	+	+	+	-	Strong
S44	farm dairy products	OX	+	+	+	+	Strong
S46	Farm dairy products	OX,TE,PG	+	+	+	-	Strong
S3	Buffalo raw milk	OX	+	+	+	+	Strong
S9	Buffalo raw milk	OX,TE,PG	+	+	-	-	Moderate
S15	Buffalo raw milk	OX,PG	+	+	-	+	Moderate
S33	Buffalo raw milk	OX,L,AZM,PG	+	+	+	+	Strong
S41	Buffalo raw milk	OX,PG	+	+	+	+	Strong
S43	Buffalo raw milk	OX	+	+	+	+	Strong
S47	Buffalo raw milk	OX,PG,E	+	+	-	+	Modrate
S50	Buffalo Raw milk	Ox,AZM,TE	+	-	+	+	Strong

***yellow color /strong biofilm and all genes products(meca,fema,icaa and icad)**

*** Red color /moderate biofilm and all genes *Green color/ weak biofilm and all genes products**

***Green color/ weak biofilm and all genes products**

PG=pencilline,OX=Oxacilline,E10=Erythromycine,TE=Tetracyclin,Lincocine,
AZM15=Azythromycine,meca=Fema=icaa= intercellular adhesion icad=inter cellular adhesion

We obtained in the table (4-8) 17(34,69%) that there is a positive sample for MRSA that is resistant to antibiotics and carries all genes (mecA,femA,Icaa and Icad) and produces a strong biofilm

While there was an isolate 4 (8.16%) that carried all the encoded genes(mecA,femA,Icaa and Icad) gave the moderate biofilm

On the other hand, there 1(2.04%) has the ability to produce a weak biofilm and has all the encoded gene(mecA,femA,IcaA and Icad) from imported milk.

Conclusions 5.1.

1.High prevalence of *S.aureus* in farm dairy products rather than from another milk and milk products.

2.MSSA are more prevalence than MRSA.

3.Most of *S.aureus are* isolated from milk and milk product are strong biofilm formation.

4.All *S.aureus* isolated show highly sensivity to Gentamycin and ciprofloxacin as well as have ability to produce beta lactamase enzyme.

5. Icca gene was more superiority over the rest of the virulence genes.

6.MRSA carry all virulence genes , resistant to beta lactam antibiotics and strong biofilm formation was recorded as 34,69%

Recommendations 5.2.

1. Study the pathogenicity of current isolates to interpret the causal association between the environmental variables and virulence factors.

2.No antibiotics are given to animal after an antibiotics sensitivity test was done, in addition supervision and quantification of antibiotic use in veterinary practice.

3.Use of modern techniques like Real time PCR in detection of some genes responsible for virulence of *S.aureus.*

4. Improvement of the management and hygienic measures were used in dairy cattle husbandry and in dairy industry.

5. Conducting periodic checks for farmers and workers in the field of milk production and its derivatives to ensure that they are free from infection with *Staphylococcus aureus* so that they are not a source of transmission of these germs.

6. Educating farmers and workers in the dairy industry to use sterilizers and disinfectants that are not harmful to animal and human tissues during the production period of milk and its derivatives in order to reduce contamination with bacteria.

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Appendix 1: DNA extraction kit procedure

1.Transfer 1 ~ 2 ml cultured bacteria cell into 2 ml tube

2. Pellet bacteria by centrifugation for 1 min at 13,000 rpm, and discard supernatant. Resuspend completely the cell pellet with remnant supernatant by tapping or vigorously vortexing.

3. Add 200 µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by vortexing vigorously

4. Incubate lysate at 56℃ using preheated heat block or water bath for 10 ~ 30 min.

5. When lysis is completed, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70℃ for 5min.

6. Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then carefully transfer 350 ~ 400 µl of the supernatant into a new 1.5 ml tube (not provided).

7. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

8. Add 200 µl of absolute ethanol into the lysate, and mix well by pulse vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid.

9. Carefully apply the mixture from step 7 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at

13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a new 2 ml Collection Tube (additionally supplied).

10. Add 700 µl of Buffer WA (Buffer WB) to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube

11.Add 700 µl of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a new 2.0 ml Collection Tube (additionally supplied), Then again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether

12. Place the Spin Column into a new 1.5 ml tube (not supplied), and add 30 - 100 µl of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elu

الخلاصة

تعد المكورات العنقودية الذهبية هي المسبب للأمراض تلوث الحليب ومنتجات الألبان مسببة للتسمم الغذائي بسبب إنتاج السموم المعوية بشكل أساسي ، ولديها ذخيرة من عوامل الضراوة ، وآليات مقاومة المضادات الحيوية المتعددة ، وقدرتها على تكوين الأغشية الحيوية ، بالإضافة إلى قدرتها على اكتساب جديد جينات المقاومة عبر إنتجرون ، مما قد يؤدي إلى صعوبة العلاج. لهذه الأسباب ، تهدف الدراسة إلى تحديد معدل الانتشار ، ودقة بعض أنماط الفوعة وتكوين الأغشية الحيويةIccd ،mecA, femA, في المكورات العنقوديه الذهبية المعزولة من الحليب ومشتقاته (Icca(

تم إجراء دراسة مقطعية لجمع إجمالي 300 عينة مقسمة إلى ست مجموعات 50 عينه لكل بقري خام، حليب مستورد ، منتجات ألبان مستوردة ، منتجات ألبان محلي(مصانع) ، من(حليب ومنتجات ألبان مزرعة وحليب جاموس خام) ، بدأت الدراسة من شهر تشرين الاول 2022 الى شباط 2023. جمعت العينات عشوائيا من مواقع مختلفة في مدن كربلاء. تم التعامل مع العينات ، ثم تم *S. aureus* باستخدام الطرق البكتريولوجية والكيميائية الحيوية لعزل والتعرف على بكتيريا إجراء اختبارات إنتاج الأغشية الحيوية واختبارات الحساسية للمضادات الميكروبية. تم استخدام) وتم Icad و Icaa، femA، mecA الأدوات الجزيئية لدراسة انتشار بعض جينات الفوعة (Idometric بطريقة(β-lactamase على إنتاج إنزيم *Staphylococcus aureus* فحص قدرة assay(

S. aureus عينة تم استزراعها وتحديدها ، 121 (40.3٪) ، وصفت كعزلات 300 من بين ، وتم توزيعها كحليب بقري خام 17 (34٪) ، حليب مستورد 13 (26٪) ، منتجات ألبان مستوردة 18 (36٪) ، منتجات الألبان مصنعه محليًا 23 (46٪) ، منتجات الألبان الحقلية 30 (60٪) و حليب الجاموس الخام 20 (40٪).

للمضادات الحياتية ، كانت العزلات في أغلب الأحيان *S. aureus* فيما يتعلق بمعدلات مقاومة 61 (50,76%)،والأوكساسيلين 49 (40.49٪) ولكنها أكثر عرضة Gمقاومة للبنسلين للسيبروفلوكساسين (100٪) والجنتامايسين (100٪) ، الأزيثروميسين 89 (73.66٪) ، الإريثرونيسين. (63.24٪) والتتراسيكلين 52 (42.9٪).

اعتمادًا على اكتشاف MRSA علاوة على ذلك ، كان الانتشار الجزيئي والنسبة المئوية لجرثومة بين MRSAوالباقي 72 (50،59). تم تسجيل الانتشار الجزيئي لـ 40,49) (49 mecAجين المجموعات المختلفة مثل حليب البقر الخام. 7 (17،41٪) ، حليب مستورد 6 (15،46٪) ، منتجات ألبان مستوردة 7 (88،38٪) ، منتجات ألبان محلية (مصنع) 9 (13،39٪) ، منتجات ألبان مزرعة 12 (40٪) وحليب الجاموس الخام 8 (40٪).

الكلية 100٪ ، وقد قسمت النتيجة إلى *S. aureus* كان معدل إنتاج الأغشية الحيوية في بكتيريا غشاء قوي ،وغشاء متوسط ،وغشاء ضعيف). كانت النسبة المئوية لهذه ثلاث مراحل (تكوين

المراحل بين منتجات الألبان المختلفة ، قوية لتكوين الغشاء 74 (62،5٪) ، معتدل لنكوين الغشاء (15.42٪) .19 لتكوين الغشاء 28 (22.08٪) و ضعيف

(67,86) S.*aureus* 81 في جميع عينات Icaa من ناحية أخرى ، كان عدد ونسبة جين موزعة على مجموعات الدراسة . حليب بقري خام 12 (70.59٪) ، حليب مستورد 10 (76.92٪) ، منتجات ألبان مستوردة 14 (77.87٪) ، منتجات ألبان محلية (مصنع) 15 (65،22٪) ، منتجات ألبان مزرعة 20 (66.67٪) وجاموس الحليب الخام 10 (50٪) بينما كانت النتيجة جين Icad (64.24٪) بين مجموعتي الحليب ومشتقاته. حليب بقري خام 11 (64.71٪) ، حليب مستورد 8 77 (61.54٪) ، منتجات ألبان مستوردة 15 (83.33٪) ، منتجات ألبان محلية (مصنع) 14 (60.87٪) ، منتجات ألبان مزرعة 18 (60٪) حليب خام جاموس 11 (55٪).

لديها القدرة على إنتاج إنزيم بيتا لاكتاماز بطريقة اليود السريع *S.aureus* جميع بكتريا المباشر بالاضافة الى قدرتها على على تكوين الغشاء القوي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الصحة العامة

التوصيف الجزيئي للمكورات العنقودية الذهبية المعزولة من الحليب ومشتقاته

رسالة

مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة

الماجستير في فرع الصحة العامة

مقدمة بواسطة

محسن باسين طه

بأشراف

أ.م.د. علي جاسم جعفر **أ.م.د حيدر علي محمد**

م 2023 **هـ 1445**