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Evalution of Virulence Genes in *Staphylococcus aureus* Isolated from Eye Infected Patients

A Thesis

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DEDICATION

To:

Light of my eyes, the soul of my heart, Absentees, Attendants and the source of compassion…

To my father's pure soul May Allah have mercy on him

To My dear mother, may Allah Preserve her as an asset for us

The best that supported and encouraged me to reach my dreams…

My brothers and sisters

Everyone who encouraged me and helped me to complete this work.

My beloved family, I present the fruit my effort….

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Summary

Staphylococcus aureus are among the most common pathogens causing ocular bacterial infection. It can damage the anatomic structure of the eye at multiple levels. It includes (conjunctiva, cornea, eyelids, lacrimal system, sclera and retina) with possible blindness. It is capable of producing several virulence factors which help to establish infection by facilitating tissue attachment, tissue invasion and evading from host immune response. The objective of the study included isolation and identification of *Staphylococcus aureus* from ocular infection and investigate the molecular presence of some virulence genes.

Eye swabs specimens are collected from 160 patients who had signs and symptoms for external ocular bacterial infections by using sterile swabs from conjunctiva and eyelids for both sexes with age ranging from (4 - 74 years) who attended to hospitals, including Ibn Al-Haitham Teaching Eye Hospital in Baghdad, Al-Imam Al-Sadiq Hospital and Hilla Teaching Hospital in Babylon. After clinically diagnosed by consultant physician during the period (from November 2021 to March 2022). The percentage of males 71%, while the percentage of females in this study is 29%. It is observed conjunctivitis was the dominant type of clinical manifestation of ophthalmic patients, the rate of conjunctivitis was the highest (75%), followed by the rate of blepharitis (20%), keratitis (4%) and finally the rate of dacryocystitis (1%).

All swabs are cultivated for the isolation and diagnosis of *Staphylococcus aureus*, in different culture media (blood agar, nutrient agar, mannitol salt agar and MRSA Chromogenic Agar Base) and incubated aerobically at the temperature of 37°C for (24-48) hours. The results of cultured medial reveal that 139(87%) specimens gave positive bacterial growth, whereas 21(13%) specimens had no bacterial growth. 57 (41.07%) *S. aureus* isolates obtained from positive growth eye swabs, detecting by morphological and biochemical test and confirmed by using Vitek 2 compact system. The MRSA chromogenic agar base is used to detect methicillin-resistant *Staphylococcus aureus*. The results for bacterial isolates are confirmed by a cefoxitin susceptibility test, which is 100% complete resistance to cefoxitin to determine methicillin resistance. The results of this study show that all isolates are resistant to methicillin (MRSA). Also done antibiotic susceptibility is tested to 18 different types of antimicrobials determined according to Kirby-Bauer disk diffusion method on Mueller Hinton agar which are: Cefoxitin 30, Clindamycin 2, Ciprofloxacin 5, Chloramphenicol 30, Doxycycline 30, Erythromycin 15, Gatifloxacin 5, Gentamicin 10, Levofloxacin 5, Linezolid 30, Nitrofurantoin 300, Norfloxacin, Oxacillin 1, Ofloxacin 5, Pencillin G 10, Rifampin 5, Trimethoprim 10, Vancomycin 30 the results of antibiotic susceptibility shows the highest resistance among the antibiotics at the resistance rate (100%) to Cefoxitin, (96.49%) to Pencillin G, (94.73%) to Oxacillin while the lowest resistance rates were found with Nitrofurantoin (1.75%),(3.5%) for Vancomycin and Linezolid, (8.77%)for Rifampin, Chloramphenicol and Gatifloxacin.. To investigate the multidrug resistance pattern that usually associated with Methicillin Resistance Staphylococci the percentage of antibiotic resistance of isolates show 45(79%) isolates multi-drug resistant.

The study also includes the detection of biofilm formation test by using tissue culture plate method (TCP), and the results show that 43(75%) isolates have the ability to produce biofilm. Polymerase chain reaction (PCR) technique is used to confirmed isolates by amplify the specific primer of the 16S rRNA gene from *Staphylococcus spp*. all 57 isolates give positive result for this gene. PCR is also used to identify some of the genes responsible for the virulence of the isolated bacteria. The detection of staphylococcal enterotoxigenicity according to five classical enterotoxins genes which are *Sea*, *Seb, Sec, Sed* and *See*. 50 *S. aureus* isolate (88%) found to harbor one or more enterotoxin gene. The *Seb* gene is the most frequent enterotoxin coding gene among the others tested; *Seb* accomplished 75% of the detected enterotoxin genes followed by the *Sec* gene, which constituted 67%, and then the *Sed* coding gene by 61% and sea coding gene by 40% while the *See* gene was 32%.

The results obtained show that 6 (10%) isolates of *Staphylococcus* contained only one gene coding for enterotoxins, while 7 (12%) of the isolates possessed two toxin genes, which were mostly *Seb* and 16 isolates of *staphylococcus* (28%) harbored three toxin coding gene,15 isolates (26%) contain four toxin-encoding genes, and 6 *staphylococcal* isolates (11%) harbored all toxin-encoding genes.

For genotypic detection of antimicrobial susceptibility of bacterial isolates two types of genes are utilize in the present study which including *erm B* gene, *tet M* gene. The result shows that the percentage genes in *S. aureus* isolates (*erm B* gene 68%, the *tet M* gene19%). The transfer of antibiotic resistance genes (ARGs) in bacteria plays an essential role in the progression of multidrug resistance (MDR) by the horizontal gene Transmission (HGT), While the result of genotypic detection for *S. aureus* is appeared formed biofilm with high percentage. According to PCR results out of 57 isolates presence of the *Wzm* gene is noticed in 39 isolate with percentage 68% and the result is almost identical to the phenotypic detection of biofilm formation by using tissue culture plate method (TCP).

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Abbreviations

Symbol	Description
ABC	ATP-binding cassette
ARGs	Antibiotic Resistance Genes
CA	community associated
CALT	Conjunctiva-associated lymphatic tissue
CA-MRSA	CA-MRSA community-acquired MRSA
CCR	cassette chromosome recombinase
CHIPS	Chemotaxis-inhibitory protein of S. aureus
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase-negative Staphylococci
Eap	Extracellular adherence protein
EPS	Extracellular polymeric substances
ETs	Exfoliative toxins
HA	hospital-associated
HAI	Health care-associated infections
HA-MRSA	healthcare- associated methicillin resistant
HGT	Horizontal Gene Transmission
LG	lacrimal gland
LPS	Lipopolysaccharide
MDR	Multi Drug Resistance
MetS	metabolic syndrome
XDR	Extensively drug resistant
MGEs	Mobile genetic elements
MRSA	MRSA Methicillin-resistant *Staphylococcus*
MSA	Mannitol salt agar
MSCRAMM	Microbial Surface Components Recognizing adhesive matrix molecules
MSSA	Methicillin sensitive *Staphylococcus*

Symbol	**Description**
NIH	National Institutes of Health
PBP2	penicillin-binding protein 2
PCR	Polymerase Chain Reaction
PIA	polysaccharide intercellular adhesion
PVL	Panton Valentine leukocidin
RPE	retinal epithelial pigment
RPPs	ribosomal protection proteins
SAB	*Staphylococcus aureus* bacteremia
SAgs	superantigens
SaPI	S. aureus pathogenicity islands
SCCmec	staphylococcal cassette chromosomes
SEA	Staphylococcal Enterotoxin A
SEB	Staphylococcal Enterotoxin B
SEs	Staphylococcal enterotoxins
SSSS	Staphylococcal Scalded Skin Syndrome
TCP	tissue culture plate method
TSS	Toxic Shock Syndrome
TSST-1	toxic shock syndrome toxin
UTIs	urinary tract infections
VISA	vancomycin-intermediate *S. aureus*
VRSA	vancomycin-resistant *S. aureus*

CHAPTER ONE

INTRODUCTION

1. Introduction

Ocular infections are a common presenting problem in primary care may result in severe vision and eye loss. Visual loss may represent a handicap ranging from minimal to severe. sight-threatening infections occur worldwide. Red eye conjunctivitis and corneal ulcer/ keratitis are among the top five problems most commonly referred to ophthalmology departments. An infection may be the primary cause of ocular tissue damage and visual loss, or it may arise as a secondary factor that complicates a wide spectrum of injury and disease processes. In the world there exist differing classes of etiology such as bacterial, viral and fungal or parasites for eye infections (Laver and Specht, 2016; Akrae *et al*., 2022).

Bacterial ocular infections are the major contributor of ocular infections worldwide. *Staphylococcal* species Coagulase-negative *staphylococci*(CoNS) and *Staphylococcus aureus* [Both methicillin-sensitive(MSSA) and methicillin resistant (MRSA)] are the most commonly isolated species among bacterial ocular infections they are responsible for many intraocular and corneal infections (Moreno *et al*., 2020; Das and Jhanji, 2021). In a recent meta-analysis by Manente *et al*., were identified as the most commonly reported culture-derived corneal pathogens. It can damage the anatomic structure of the eye at multiple levels. Includes (conjunctiva, cornea, eyelids, sclera and retina) with possible blindness(Manente *et al*., 2022). They usually colonize the skin and nasal nares of healthy individuals, as well as livestock and other animals (Hansen, 2019) .

Staphylococcus aureus (S. aureus) is the most virulent and best known member of the genus Staphylococci, causing opportunistic infections and a wide spectrum of life-threatening systemic diseases(Masood *et al*., 2018).This bacterium is associated with many types of ocular infections such as conjunctivitis, keratitis, blepharitis, and dacryocystitis manifestations *Staphylococcus aureus,* is responsible for approximately 70% of ocular infections (Kowalski *et al*., 2020; Johnson *et al*., 2021). It is a bacterium residing in the mucous membrane and skin which has deadly pathogenic capabilities leading to different hospitalized and community infections (Oliveira *et al*., 2018; Altaee *et al*., 2020).

It is capable of producing several virulence factors which help to establish infection by facilitating tissue attachment, tissue invasion and evading from host immune response such as enterotoxins, hemolysis, invasions, and surface factors that inhibit its phagocytic engulfment enable the organism to be successful as pathogen that causes wide range of human infections (Ge *et al*., 2017). The genome of *staphylococcus. aureus* consists of the core genome of the cell survival and accessory genomes for adaptation in a different environment Circumstances. Mobile genetic elements (MGEs) are parts of DNA encoding a number of virulence and horizontal gene transfer (HGT) transmission between cells. *S. aureus* includes many types of MGEs, for example: Plasmids and staphylococcal cassette chromosomes (Ågren *et al*., 2012; Matuszewska *et al*., 2020).

Some virulence factors such as enterotoxins, biofilm forming as well as The ability to acquire resistance to multiple antibiotics classes currently consider as major virulence factors and have an important role in increasing tissue damage and failure in antibiotic therapy (Meroni *et al*., 2019).

MRSA isolates are commonly resistant to a wide range of antibiotics such as aminoglycosides, erythromycin, tetracycline and fluoroquinolones (Chaieb *et al*., 2007; Aboubakr *et al*., 2019; Chen *et al*., 2021). Activity of efflux pumps such as msr (A/B) and methylation of the ribosomal drug binding site by methylase enzymes encoded by *erm* (*A, B, C*) genes are important resistance mechanisms to macrolide antibiotics(Bagnoli *et al*., 2017; Sedaghat *et al*., 2017).

The importance of biofilm production in pathogenesis of *S. aureus* and development of Multi Drug Resistance (MDR) strains has been documented (Konduri *et al*., 2021). Apart from other adhesion factors, a polysaccharide intercellular adhesion (PIA) which is encoded by ica operon is essential for biofilm formation in staphylococci(Havaei *et al*., 2017) .

The present study aims to determine key virulence genes among *Staphylococcus aureus* isolated from patients with eye infections and the achievement of this aim by the following objective:

1. Isolation and Identification of *Staphylococcus aureus* from ocular infections by Vitek Technique
2. Antibacterial susceptibility tests to determine the MRSA
3. Biofilm formation assay
4. Detection of virulence factors by PCR such as enterotoxin genes

CHAPTER TWO
LITERATURE REVIE

2. Literature Review

2.1. A Brief History

The ancient Egyptian manuscripts on papyrus cane paper dating back to about 1600 BC indicate that ophthalmology was advanced compared to the rest of the medical departments, as many eye diseases were diagnosed, including (blepharitis, retinitis and other physiological diseases).In the second century AD, Galen wrote some approaches to ophthalmology describing the eye as a very sacred organ, and the first operation to remove cataracts from the eye was performed about 2000 years ago by an Indian surgeon, but this process was not used in Europe until the middle of the century nineteenth(Salmon, 2019) .

Ophthalmology witnesses many achievements, including the invention of the ophthalmoscope, which contributed to the advancement of ophthalmology and its treatment. In 1864, the American Ophthalmological Organization was established as the first eye health organization in the United States, and in 1917, ophthalmology became the first branch of medicine. As a result of the continuous studies, a number of medical achievements occurred in this field, including the first successful use of antivirals in the treatment of eye diseases, corneal transplants, the use of lasers, and delicate operations, which added many challenges to moving forward in the field of ophthalmology.(Prajna, 2019)

2.2. External Eye Structures

2.2.1. The Eyelids

Eyelids are thin, flexible layers or folds of tissue that help protect the orbital structures. They are thinner covering the skin of the body. The action of eyelid blinking helps the conjunctiva keep the cornea lubricated and solid and removes debris and potential pathogens. The human eyelid contains a row of eyelashes that help enhance the protection of the eye from dust and foreign particles, as well as from perspiration and prevent dry eyes. Sleeping eyelashes are thrown as a filtering and control system that alerts the brain to the possibility of this happening. occurrence of harmful factors. The eyelids possess two anatomical and protective barriers that prevent the penetration of pathogens outside the anterior surface of the globe. The first is the orbital septum, a thin, multi-layered fibrous tissue that acts as a physical barrier to prevent infections and the second is the conjunctiva that reflects back on itself, preventing material on the anterior surface of the globe from freely moving backward along it surface(Mahon *et al*., 2018; Papadakis *et al*., 2019) .

2.2.2. Orbits

The orbits are conical bony cavities that contain balls, eye muscles, nerves, fat, and blood vessels. In anatomy, the orbit is the cavity or socket of the skull in which the eye and its appendages are located. The "orbit" can refer to the bony cavity, or it can also be used to refer to the contents. The orbital volume in an adult human is 30 milliliters (Amrith *et al*., 2019) .

2.2.3. The Conjunctiva

The conjunctiva is a mucous membrane that covers the inside The lining of the eyelids and the white covering of the eyeballs (sclera). They are similar to the membranes in the mouth and nose, and it constitutes the front line of defense against invading organisms. It consists of a stratified columnar epithelium and an underlying layer of loose connective tissue(Walker *et al*., 2020) .Many cells are found in the conjunctival epithelium and include goblet cells that produce mucin that allows the tear film to adhere to the surface of the eye, melanocytes, T lymphocytes, and B cells(Gibson *et al*., 2017). They also contain macrophages, neutrophilic granulocytes, mast cells, and a few small accessory lacrimal glands located in the orbit and conjunctiva. The accessory lacrimal glands constantly secrete a watery portion of the tears into the conjunctiva, which contributes to immune control and helps prevent microbes from entering the eye(Cecil *et al*., 2012). The conjunctiva produces IgA, like the lacrimal glands (Knop and Knop, 2005).

2.2.4. The Cornea

The cornea is the window to the eye. Its function looks like a crystal on a wristwatch. The inner eye structures can be seen through the cornea. Function: The curved surface of the cornea is the refraction, gathering and focusing of light in the retina. A layer of tears (tear film) covering the cornea provides visual clarity, lubrication and nourishment. Much of the free nerve is located at the endings in the corneal epithelium. When there is a coil intrusion or injury to the epithelium, the patient usually complains of severe pain. Corneal infections and injuries are real visual emergencies (Mahon *et* al., 2018; Fenta and Derbie Alemu, 2022).

2.2.5. The Lacrimal System

It consists of the lacrimal gland (LG), accessory glands, and the excretory system. The lacrimal gland is found in the lateral upper eyelid margin. The human LG consists of many plasma cells located around the secretory acini. They have formed circulating lymph tissue with other white blood cells, especially lymphocytes(Knop *et al*., 2008) .It produces around 10mL of tears a day, the act of blinking serving to smear the tear film from the lateral to the medial edge of the eye surface(Török *et al*., 2017) . The lacrimal gland secretes tears that pass through the cornea and enter the lacrimal excretory system at the lacrimal points. Point tears drain into the duct that leads to the lacrimal sac. The tears drain into the lacrimal sac in the nose so that the lacrimal system forms a direct passage from the anterior ocular appendix to the nasal cavity. With complete obstruction of the nasolacrimal duct, the infected substance in the sac may reflux to the surface of the eye(Klotz *et al*., 2000; Lin, 2019) .

2.3. Normal Flora

The eyes are only sterile for a few hours after birth and then invaded by microorganisms. The exposure of the eye to the atmosphere throughout waking hours and its continuity with the skin and nasopharynx makes it vulnerable to pollution and keeps it polluted. Organisms are found on the skin of the eyelid, lid margins and conjunctiva in abundance. The cornea rarely produces microorganisms unless it is diseased. Microorganisms can be either symbionts or non-symbionts; Both groups of organisms do not produce disease until the host's immunity is perfect. Both groups tend to cause opportunistic infections when conditions are favorable to the organism and against the host. The normal ocular flora is divided in two broad groups resident flora and transient flora. The resident floras are the same organism grows on repeated culture. They represent true colonization. Transient ocular floras are those organisms that are not found consistently when serial cultures are taken under the same condition from the same eye. All the cultures do not yield same microorganism (Amrith *et al*., 2019; Fagehi, 2021; Long *et al*., 2022).

The normal flora of the eye consists primarily of bacteria that under normal circumstances do not cause infection, but may be a primary source of infection after ocular surgery, trauma, or immune-compromised. Bacteria have colonized the eye surface as commensals, forming their normal flora (Sthapit and Tuladhar, 2014). Depending on the various locations of the human body, normal flora varies. Normal eye flora is similar to that of the skin and upper respiratory tract. The Gram-positive bacterium is considered being the majority, most commonly such as Staphylococcus spp.(Kumpitsch *et al.*, 2019). Coagulase-negative staphylococci and Corynebacterium spp. Make up 80% to 90% of the indigenous microbiota recovered from uninflamed eyes and the lowest *Staphylococcus aureus* 0-30% While it is more of a negative bacteria species, *Moraxella* is given at 0-3% which is less common *Escherichia coli* 0-1%(Mahon et al., 2018) . Depending on the age of the individual, season, location, and underlying conditions. Microfloras are generally similar in both eyes at onset. If any organism has been detected in one eye, the other eye is also expected to harbor the same organism. This is true only for non-pathogens (Sthapit and Tuladhar, 2014).

Normal flora may become pathogen due to changed immunity that may be (PK Mukherjee, 2010; Lin, 2019):

- Systemic as in AIDS
- Biochemical change, i.e. diabetes
- Indiscriminate use of antibiotic, steroid
- Chemotherapy and radiation.

- Local ocular conditions like abnormal tear status, ocular surface defect, contact lens, prolonged uses of antibiotics or steroids are some of the examples.

2.4. Eye Infection

Eye infections are among the most common diseases, although they contain many factors that protect them from inflammation, such as their mechanical hydration with tears that contain the enzyme lysozyme that kills bacteria and phagocytic cells, the mechanical barrier of the mucous membrane and other immunity factors. It can affect the functional structures of the eye. The organisms known to cause eye infections are bacteria, followed by viruses, fungi, chlamydia, parasites, arthropods, and larvae (Tummanapalli and Willcox, 2021; Manente *et al*., 2022)**.** While the ocular infection is considered to be a minor infection, it can threaten vision, which leads to irreversible vision loss or blindness. The factors that help in the occurrence of injury are personal hygiene, living conditions, socio-economic status, trauma, usage of contact lenses, fever, systemic diseases, immune suppression, and swimming. Eye infection symptoms include discomfort, redness, discharge, blurred vision, and photophobia, compared to other types of infections the emergence symptoms of bacterial infections are usually shorter(Watson *et al*., 2018) . The conjunctiva, eyelid, and cornea are the most commonly infected areas of the eye. According to international statistics, the incidence of conjunctivitis in the United States is 1.35% annually (Perween *et al*., 2016; Aseffa *et al*., 2019).

2.4.1. Bacterial Eye Infections

Bacteria is the most common cause of eye infections in the world. Infections such as blepharitis, conjunctivitis, keratitis, endophthalmitis, orbital cellulitis and dacryocystitis, which are usually associated with bacteria. The infection may be monomicrobial or polymicrobial and is associated with many causes, including surgery, dry eyes, and persistent obstruction of the nasolacrimal duct (Galvis *et al*., 2014). Approximately 70% of ocular infections are caused by bacteria (Tummanapalli and Willcox, 2021). Gram-positive bacteria such as *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* (CoNS), *Streptococcus pneumonia*, and *Streptococcus pyogenes* are the main causative agents of eye infections worldwide. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Escherichia coli, and *Haemophilus influenza* are the main Gram-negative bacteria isolated from eye infections as shown in table (2-1) (Gronthoud, 2020; Grandi *et al*., 2021).

The skin covering the eyelids is the thinnest on the body. any living creatures able to start skin infections can also cause blepharitis. It is an inflammation of the eyelid caused by bacteria or a blockage of the Meibomian oil glands at the base of the eyelashes (Long *et al*., 2022). Red or pink eye(conjunctivitis) is one of the most common complaints seen by clinicians and a source of frequent referral to ophthalmology. They include all age groups and occur worldwide. An estimated 6 million people in the United States have conjunctivitis each year, and the condition is associated with 1% of all primary care visits. Symptoms may include itching, tearing, foreign body sensation, and discharge (purulent or watery), hyperemia, or increased blood flow resulting in 'redness of the eye'. Red eyes make up more than 50% of clinic visits to ophthalmologists and are the most common in the field of ophthalmology. Reason for microbiological evaluation (Laver and Specht, 2016; Udomwech *et al*., 2022).

Keratitis is an infection of the cornea, considered a true ocular emergency. Estimates in the United States range from 25,000 to 71,000 cases annually, while global rates may exceed 2.0-3.5 million cases every year (Das and Jhanji, 2021). Dacryocystitis is an infection of the lacrimal sac usually due to congenital or acquired obstruction of the nasolacrimal system. It may be acute or chronic and occurs most often in infants and people over the age of forty (Eshraghi *et al*., 2014).

Table (2-1) Bacteria associated with ocular infectious disease(Mahon *et al*., 2018)

Gram-Negative		*Gram-Positive*		*Aerobic Actinomycetes*	*Mycobacteria*
(Aerobic)	*(Anaerobic)*	*(Aerobic)*	*(Anaerobic)*		
Alcaligenes spp. *Acinetobacter spp.* *Actinobacillus actinomycetemcomitans* *Achromobacter xylosoxidans* *Aeromonas hydrophila* *Bartonella henselae* *Borrelia tularensis* *Brucella spp.* *Capnocytophaga spp.* *Chlamydia trachomatis* *Chlamydophila pneumoniae* *Chlamydophila psittaci* *Coxiella burnetii* *Eikenella corrodens* *Enterobacteriaceae* *Flavobacterium spp.* *Francisella tularensis* *Haemophilus aegyptius* *Haemophilus influenzae* *Haemophilus parainfluenzae* *Kingella spp.* *Neisseria gonorrhoeae* *Neisseria meningitidis* *Moraxella catarrhalis* *Moraxella lacunata* *Pseudomonas aeruginosa*	*Bacteroides spp.* *Fusobacterium spp.* *Prevotella spp.*	*Bacillus cereus* *Bacillus spp.* *β-Hemolytic streptococci (A, B, C, F, G)* *Coagulase-negative staphylococci* *Corynebacterium spp.* *Enterococcus faecalis* *Listeria monocytogenes* *Micrococcus spp.* *Staphylococcus aureus* *Staphylococcus epidermidis* *Streptococcus pneumoniae* *Streptococcus viridans group*	*Actinomyces israelii* *Actinomyces spp.* *Clostridium spp.* *Peptostreptococcus spp.* *Cutibacterium (Propionibacterium) acnes* *Propionibacterium propionicus*	*Nocardia spp.* *Streptomyces spp.*	*Mycobacterium abscessus* *Mycobacterium avium-intracellulare* *Mycobacterium chelonae* *Mycobacterium fortuitum* *Mycobacterium gordonae* *Mycobacterium leprae* *Mycobacterium mucogenicum* *Mycobacterium nonchromogenicum* *Mycobacterium triviale* *Mycobacterium tuberculosis*

2.5. *Staphylococcus aureus*

Staphylococcus. aureus bacteria belong to the genus *Staphylococcus*, which is a genera of the family called *Staphylococcaceae*. This bacterium was first seen by Koch in 1878(Ananthanarayan, 2006) . And the name *Staphylococcus* is derived from the Greek term staphyle which means (a bunch of grapes) and coccus which refers to (a bean or berry) given to these bacteria because their cells divide into several divisions and at different levels, while remaining grouped with each other in the form of cluster-shaped groups (Orenstein, 2011; Khan *et al*., 2018).

These cocci are described by the scientist Ogston in 1880, which is members of a group of invading Gram-positive bacteria known as Pyogenic (pus-producing) cocci, as the mentioned scientist is the first to distinguish two types of pyogenic cocci, one of them arranged in clumps or groups called *staphylococci*, and the other arranged in chains called *streptococci* (Peacock, 2006; Joanne M. willey, 2017).

The scientist Rosenbash in 1984 named the staph bacteria based on the color of their colonies, whose colonies are golden yellow in color called staph. aureus whose colonies are white in color called *staph. albus*, later called *staph. Epidermidis*(Kloos *et al*., 1998; Le *et al*., 2018) .

Staphylococcus aureus is a medically important and one of several bacteria that most common causes of health care-associated infections (HAI). *S. aureus* is a normal part of the skin microbiota. Approximately 30% of people carry the bacteria in their noses. In health care settings, the bacteria are spread through direct contact, usually through contaminated hands from a carrier's nose(Anderson, 2020). *Staphylococcus aureus* is responsible for many infections ranging from relatively mild to life-threatening. Infection can be classified as a purulent or toxic-mediated disease. *S. aureus* can be recovered from almost any clinical specimen which is an important cause of nosocomial infections. (Achmit *et al*., 2021) .

2.5.1. General Characteristics

Staphylococci have a typical Gram-positive cell wall structure, catalase-positive, spherical cells with a diameter of about (0.5 to 1.5 μm), that tend to be grapelike irregular clusters, single cells, pairs, and short chains are also seen. They can grow easily on different types of media and are metabolically active, capable of fermenting carbohydrates and producing pigments varying from deep yellow to white, are also seen in liquid cultures. They are non-flagellate, non-motile, non-spore-forming, and aerobic or facultatively anaerobic, although a few strains can be obligate anaerobes. Colonies produced after 18 to 24 hours of incubation are medium sized (4 to 8 mm) and appear cream-colored, white or rarely light gold, and buttery-looking (Peeters and Sarria, 2005; Riedel *et al*., 2019).

Staphylococcus aureus form round colonies and grows at an appropriate temperature of 37 ° C although it may grow between 10 ° C and 46 ° C. The species is selective anaerobic with enhanced growth in the presence of CO2 and O2 (Talaro *et al*., 2013).

The bacterium is hemolytic on blood agar because of its ability to produce four types of hemolysins (alpha, beta, gamma and delta). Practically, almost *S. aureus* isolates are coagulase enzyme producers, which is a virulence factor that contributes to the recognition and diagnosis of the organism. The ability of *S. aureus* to form a coagulase separates it from other, less virulent species. It is common for the other species to be grouped together as coagulase-negative staphylococci (CoNS) (Ryan and Ray, 2004). *S. aureus* is salt durable, which can thrive (grow) with 7.5% NaCl on mannitol salt agar also it is oxidase negative and positive to catalase and commonly produce extracellular polysaccharides capsule (Arumugam *et al*., 2018).

2.5.2. Epidemiology

The primary reservoir for *S. aureus* is the human nares. With colonization also occurring in the vagina, pharynx, axillae, and other skin surfaces. *S. aureus* transmission can occur by direct contact with unwashed, contaminated hands and by fomites (David and Daum, 2010).

Nasal transfer for hospitalized patients common. Because communication between patients and hospital staff not uncommonly, translocation of organisms occurs frequently. Subsequently, increased colonization in patients and hospital staff occurs often. Hospital outbreaks can develop in patients in nurseries. Most places in hospitals at risk for serious infections are baby nurseries, intensive care units, operating rooms, cancer treatment centers, and prolonged or repeated exposure to antibiotics and burn units(Carr *et al*., 2018) .

Significant problems have arisen as a result of the increase in *S. aureus* strain infections in the population named MRSA (methicillin-resistant *S. aureus*). Both health care and community acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are a major health care concern. Several outbreaks were reported in prisoners, sportsmen and primary school children. The infections were transmitted by touch with skin lesions and be extremely difficult to treat and manage (Talaro *et al*., 2013; Gomi *et al*., 2018).

2.5.3. Pathogenesis

Despite the general fact that nose is a favorite place for *S. aureus* but it is can also live in other environments such as mucous surfaces, mouth, vaginal wall, and gastrointestinal tract for humans and other animals. The entrance to pathogens may be a hair follicle, mouth, nose, ear, but they are often cracks in the skin that can be a minute needle-stick or a surgical wound clinical infections of *Staphylococcus aureus* are Classified into community and hospital categories based on the origin of the infection. these two types characteristic in clinical manifestations of infection(Uhlemann *et al*., 2014) .

It also has a great ability to cause opportunistic infections that vary from relatively simple skin infections to life-threatening systemic diseases for example: osteomyelitis, bacteremia, pneumonia, meningitis, and a variety of toxin-mediated diseases, especially when appropriate conditions are available for them, such as the presence of a defect in the immune defense, injuries to the skin, infection with other pathological organisms such as viruses, and the presence of chronic diseases such as cancers(Muñoz-Gallego *et al*., 2020) .

The pathogenesis of *S .aureus* is caused by many virulence factors and their mechanisms into invasion and inflammation which include colonization, synthesis of extracellular molecules which promote adherence, and the ability to avoid host defenses; secreted virulence factors such as toxins(Al-Mebairik *et al*., 2016). The S. aureus infection process involves five stages. They are (1) colonization, (2) local infection, (3) systemic spread and/or sepsis, (4) metastatic infection and (5) toxinosis. The organism is in a carrier state in the anterior orifices and can remain so without causing infections for weeks or months(Farhadi *et al*., 2019) .

2.5.4. Virulence Factors

Staphylococcus aureus possesses a group of virulence factors. These factors enable the organism to be successful as a pathogen it causes a wide range of human and animal infections. Virulence factors help in attachment to host cells, breaking down the host's immune shield, and invading tissues, causing sepsis Toxin-mediated syndromes are provoked(Cheung *et al.*, 2021). This is the basis for persistent staphylococcal infection without a strong host immune response. Based on its mechanism of action and its role in pathogenicity, cluster virulence factors are classified as listed follows (Gor *et al*., 2021)**.**

2.5.4. 1. Helping Attachment to Host Tissues

- **Microbial Surface Components Recognizing adhesive matrix molecules (MSCRAMM):** Cell surface proteins that interact with host molecules Such as collagen, fibronectin and fibrinogen, thus, facilitates tissue attachment. staphylococcal protein A, Fibronectin-binding proteins A and B, which bind to collagen Protein and clustering factor A and B belong to this family. They also participate in host immune evasion(Foster *et al*., 2014).

2.5.4. 2. Breaking(evading) the Host Immunity(Foster, 2019)

- **Polysaccharide microcapsule:** Resist the phagocytosis and killing by polymorph nuclear phagocyte.
- **Cell Wall Constituents:** Its wall contains teichoic acid and peptidoglycan They play an important role in giving rigidity to the cell wall, as well as stimulating the production of antibodies that are believed to contribute to bacterial virulence.
- **Protein A:** Binds to the Fc portion of immunoglobulins acting as a super antigen and limiting the host immune response, prevents opsonization.

- **Coagulase enzyme:** Coagulase is an enzyme that coagulate the blood plasma of humans and rabbits, and this characteristic is one of the most important characteristics that differentiate between the types of *staphylococci*. Negative Coagulase (*Staphylococci*) is a type of coagulase-producing staphylococcus. Besides *Staphylococcus aureus*, there are bacteria *S. intermedius* and *S. hyicus* that are isolated from animals. The importance of the Coagulase enzyme is that it coats the cocci cells with fibrin, thus preventing phagocytic cells from being eaten. This enzyme is bound and free (Tam and Torres, 2019).
    - **bound coagulase:** is found on the outer cell wall of most species of *Staphylococcus aureus* and interacts with fibrinogen causing the aggregation of staphylococci, which is detected using the glass slide.
    - **free coagulase:** it is secreted during bacterial growth, especially during the logarithmic phase, and causes plasma clotting. This enzyme is detected in the coagulase test using the tube coagulase test.
- **Panton-Valentine leucocidin (PVL):** PVL is found in most community-associated MRSA (CA-MRSA). PVL belongs to the group of membrane Pore forming proteins. It consists of two types of protein Components (Luks-PV and LukF-PV) that work together as subunits and form porins on the cell membrane of the host cells, resulting in the leakage of the contents of the cells and their death(Castellazzi *et al*., 2021).
- **Alpha hemolysin(Alpha-toxin) and the other hemolysins (beta, gamma, and delta):** *S. aureus* produces four hemolysins: alpha, beta, gamma, and delta, α-Hemolysin was the first bacterial exotoxin to be identified as a Cell membrane former pore that causes extracellular leakage and death. These extracellular proteins that affect red blood cells and leukocytes are cytolytic toxins in addition to lysing erythrocytes, can damage platelets and macrophages and cause severe tissue damage(Astley *et al*., 2019).

- **Chemotaxis-inhibitory Protein of *S. aureus* (CHIPS):** CHIPS is an extracellular protein that inhibits Chemotaxis action of neutrophils and monocytes(Cheung *et al*., 2021).

2.5.4. 3. Tissue Invasion

- **Extracellular adherence protein (Eap):** An exogenous protein that binds to the host cell matrix, the plasma Proteins and endothelial cell adhesion molecule in addition to the roles of adhesion and invasion, it also has immune modulation activity(Raineri *et al*., 2022)
- **Proteases, lipases, nucleases, hyaluronatelyase, phospholipase C, metalloproteases (elastase), and Staphylokinase:** These extracellular enzymes cause tissue destruction and, Thus, it helps in the penetration of bacteria into the tissues. In connection with the aforementioned virulence factors, there are other enzymes and toxins produced by staphylococcus bacteria that have a role in their pathogenesis, such as the production of the enzyme **catalase**, which converts hydrogen peroxide (H2O) to oxygen and water(Alrifaai and Al Jebory, 2016) , and the production of **staphylokinase**, which its ability to degrade fibrin clot, allowing bacteria to spread through host tissues affected by burns, cuts, scrapes and skin infections(Guerra *et al*., 2017). As well as producing the DNA degrading enzyme **DNase**, which breaks the DNA of host cells through its hydrolysis of the phosphodiester diester bond. The main building blocks of nucleic acid and its transformation into liquid (Alrifaai and Al Jebory, 2016) , as well as the **Protease** enzyme, which has the ability to destroy peptides produced by host cells and analyze the protein materials and elements involved in the composition of cells and thus contribute to the pathology of *staphylococci*(Otto, 2014) .

Other enzymes secreted by *staphylococci* are Hyaluronidase, or the so-called diffusing factor, that breaks down hyaluronic acid in connective tissues(Seiti Yamada Yoshikawa *et al*., 2019) , and Lipase, which breaks down lipids. helping *staphylococcus* bacteria to invade fatty tissues and **Penicillinase** that destroys the beta-lactam ring in the penicillin molecule(Peacock, 2006) . And other products of bacteria are **urease** and **gelatinase**, which are considered to be pathogenic factors that contribute to the spread of infection to neighboring tissues, and thus play an important role in the pathogenesis of this bacteria(Seiti Yamada Yoshikawa *et al*., 2019) .

2.5.4. 4. Induces Toxinosis

- **Enterotoxins:** *S. aureus* produces a group of enterotoxins enterotoxin *staphylococci* heat stable exotoxins that cause symptoms, including diarrhea and vomiting. Several serologically distinct enterotoxins have been identified with the majority in groups A through E and G through J. These toxins are produced by 30% to 50% of *S. aureus* isolates. enterotoxins are stable at 100°C for 30 minutes. *Staphylococcus* Food poisoning is most commonly caused by enterotoxins A, B, and D. Enterotoxins B and C the cause of poisoning Consume foods that contain an adequate amount of preformed enterotoxins. And sometimes G and I associated with Toxic Shock Syndrome (TSS). Enterotoxin B has been linked to *staphylococcus* pseudomembranous enteritis. These toxins are super antigens and they have the ability to interact with many T cells, activating aggressive activity, excessive immune reaction (Schelin *et al*., 2017; Oliveira *et al*., 2018).
- **Toxic shock syndrome toxin -1 (TSST-1):** TSST-1 is a 22,000-Da heat- and proteolysis-resistant. TSST-1 and some enterotoxins are called thermophilic

Super toxic antigens, exotoxins mediated by chromosomes. It is expected that 90% of the strains of *Staphylococcus aureus* responsible for menstruation-associated TSS and half of the strains responsible for the other TSS forms produce TSST-1. It is causes toxic shock a special syndrome in women during menstruation(Murray *et al*., 2020) .

- **Exfoliative toxins (ETs) A and B:** Serine proteases that selectively recognize and The breakdown of desmoproteins in the skin and cause it to peel dramatically. ETs causes staphylococcal scalded skin syndrome; a disease it mostly affects infants (Imanishi *et al*., 2019; Cossart, 2020).

2.5.5. Biofilm Formation

Antoine Von Leeuwenhoek, in 17th century, observed for the first time a type of creature on his own teeth, a discovery considered to be a biofilm(Percival *et al*., 2011). Heukelekian and Heller noted the "bottle effect" of marine microorganism promotes growth and activity when attached to the surface, named for the first time as a biofilm by J. W. Costerton in 1978, as in the days Louis Pasteur and Robert Koch, generally checkup bacteria through growth them in pure culture in a rich liquid media, circumstances perfect to put growth known as "plankton" growth. However, this culture conditions often completely different of bacteria natural adjective growing conditions (Heukelekian and Heller, 1940; Cossart, 2020).

A biofilm is a colony of microorganisms, within a matrix of extracellular polymeric substance that they produce. Its contains adherent microbial cells on a stationary surface (living or nonliving). Bacterial biofilms are usually pathogenic in nature and can cause nosocomial infections. The National Institutes of Health (NIH) revealed that, of all microbial and chronic infections, 65% and 80%, respectively, associated with the formation of biofilms (Jamal *et al*., 2018; Jenkins and Maddocks, 2019). The process of biofilm formation consists of several steps, starting with the binding to The surface that will lead to the formation of a micro-colony, which gives rise to three-dimensional structures and ends after maturation with detachment. Insertion of a prosthetic device into the human body it often leads to the formation of biofilms on the surface device (Joanne M. willey, 2017; Panda and Singh, 2018).

Microorganisms in biofilms are causative agents in a variety of eye infections. These include attached contact lenses and crystalline keratitis, orbital implant infections, and late postoperative endophthalmitis. Biofilms can be formed on a variety of eyes biomaterial or on damaged eye tissue. eye biomaterials the most prepared for the formation of biofilms include contact lenses, corneal, intraocular lenses and orbital transplants, Common pathogens include *Staphylococcus aureus*, coagulase-negative *staphylococcus*, *C. albicans*, *Propionibacterium*, and *P. aeruginosa* (Akrae *et al*., 2022).

Staphylococcus aureus has the ability to form a biofilm the extracellular polysaccharide network which leading to colonialism and insistence on prosthetic material. Polysaccharide intracellular adhesin (PIA) is synthesized by the ica operon. In general, bacterial biofilms showed resistance against the human immune system, as well as against antibiotics. Health concerns speak louder due to the potential for biofilms cause diseases (Török *et al*., 2017; Jamal *et al*., 2018).

The ATP-binding cassette (ABC) transporters a common type of active primary transporter with a wide variety of substances, from small ions to large macromolecule four structures of ABC transporters that mediate substance transport. Wzm-Wzt which one of them mediates O antigen transport for LPS synthesis, a polysaccharide is built on a lipid acceptor. *Wzm* gene mediates O- antigen transport for LPS synthesis also involved in biofilm production (Bi *et al*., 2018; Bi and Zimmer, 2020).

2.5.6. Clinical diseases caused by *Staphylococcus aureus*

As with most infections, *staphylococcus* develops infection is determined by the virulence of the strain, the extent of infectious, the condition of the host's immune system. Infections begin when penetration of the skin or mucous barrier is permitted the access of *staphylococci* to adjacent tissues or the bloodstream (Mahon *et al*., 2018).Today, in any hospital in the world *S. aureus* still heads the list of pathogens isolated from the bloodstream of seriously ill patients. Includes patients at risk of developing certain diseases infants (scalded skin syndrome), young children with poor personal hygiene (impetigo and other skin infections), patients with intravascular catheters (bacteremia and endocarditis) or shunt (meningitis), and patients with suspicion previous pulmonary or viral function respiratory infection (pneumonia)(Murray *et al*., 2020) .

Clinical features of some *Staphylococcus aureus* diseases are almost as a result of the activity of toxins (toxin-mediated diseases) include (e.g., Staphylococcal Scalded Skin Syndrome(SSSS) and staphylococcal food poisoning), while other diseases are caused by the multiplication of organisms that cause for abscess formation and tissue destruction (e.g., skin infections and pneumonia,) Significant clinical manifestations caused by *S. aureus* they can be divided into two groups: disease resulting from direct organ invasion by the bacteria, pyogenic (pus-producing) diseases or invasive Infections and diseases caused by exotoxin release toxin-mediated diseases as shown below, the most important diseases caused by these bacteria(Warren, 2016) .

2.5.6. 1. Toxin-mediated Diseases

1. **Gastroenteritis (food poisoning)**: *Staphylococcus* can grow in food it produces an external toxin. The victim will eat afterwards Food containing preformed poison, which then stimulates peristalsis of the intestine with nausea, vomiting, diarrhea, abdominal pain, sometimes fever; The episode lasts from 12 to 24 hours (Gladwin *et al*., 2019).
2. **Toxic shock syndrome (TSS):** Staphylococcal TSS is a clinical syndrome caused by toxic shock syndrome toxin-1 or -2, which are super antigens produced by some strains of S. aureus(Kulhankova *et al*., 2014) . TSS results in a marked life-threatening condition via severe rash it can lead to peeling of the skin as well as low blood pressure. Maybe It also affects the kidneys, digestive system, liver, central nervous system, and It causes thrombocytopenia(Talaro *et al*., 2013) .
3. **Staphylococcal Scalded skin syndrome(SSSS):** SSSS is a fatal disease caused by certain strains of *Streptococcus aureus* that carry Peel Toxin A or B (ETA or ETB). The condition has a low incidence 0.09–0.56 per million and is more common in young children (Handler and Schwartz, 2014).

2.5.6. 2. Pyogenic Diseases or Invasive Infections Resulting from Direct Organ Invasion by the Bacteria

1. **Pneumonia:** *S. aureus* pneumonia can be severe and can lead to necrosis of lung tissue, respiratory failure, empyema, bacteremia, and death(Aliberti *et al*., 2016).
2. **Meningitis:** Meningitis, subdural empyema, and other central nervous system infections caused by *S. aureus* rare in the absence of previous surgery or foreign bodies. It was appreciated *Staphylococcus aureus* is the cause of 0.3-8.8% of cases of meningitis(Aguilar *et al*., 2010) .
3. **Osteomyelitis:** Osteomyelitis caused by *S. aureus* can occur as a complication of bacteremia, with bone seeding by blood spread, or by direct extension from the site infection in adjacent tissues(Tyagi, 2016) .

4. **Acute bacterial endocarditis:** Acute endocarditis caused by *S. aureus* is dangerous with a mortality rate approaching 50% unless It is diagnosed immediately. Although patients with *S. aureus* Endocarditis may initially be similar to nonspecific influenza symptoms, their condition can rapidly deteriorate and Includes disturbance of cardiac output and peripheral evidence of septic embolism. The patient's diagnosis is Poor unless appropriate medical and surgical intervention is taken immediately(Ruebush *et al*., 2019).
5. **Septic arthritis:** *Staphylococcus aureus* is a common cause of septic arthritis of large and small joints. It was an estimated 16,382 patients were treated for septic arthritis in US emergencies in 2012 This infection can be a complication adjacent SSTI (e.g. bursitis) or bone infection, can be after surgery (Singh and Yu, 2017).
6. **Skin infections:** Skin and soft tissue infections are very common. These include the abscess, impetigo, furuncles, carbuncles, paronychia, cellulitis, folliculitis, Hidradenitis suppurativa, eyelid infections (blepharitis), and postpartum breast infections. Lymphadenitis can occur, especially on the associated forearm with an infection in the hand(Riedel *et al*., 2019) .
7. **Bacteremia/sepsis:** *Staphylococcus aureus* is the second most common cause of bacteremia after *Escherichia coli*. At a study of Olmsted County, Minnesota in 1999-2005(Bagnoli et al., 2017). *Staphylococcus aureus* bacteremia (SAB) is significantly associated with morbidity and mortality rates. Often leads to metastases infections that lead to significant clinical complications. Bones, joints, kidneys, and lungs are common sites in diffuse infection, sometimes SAB affects the eyes(Jung *et al*., 2016).
8. **Urinary tract and Urogenital Infections:** urogenital infections caused by *S. aureus* occur at a variety of specific sites, including cystitis. pyelonephritis; prostatitis, epididymitis and testis in men. Abscesses in the female reproductive

system. Uncomplicated lower urinary tract infections (UTIs) are not usually caused by *S. aureus*(Wada *et al*., 2021).

9. **Conjunctivitis:** Conjunctivitis *Staphylococcus aureus*, an infection of the conjunctiva, is a condition. It is usually easily treated with topical antimicrobial therapy, but may progress to the deep tissues of the eye and surrounding structures (Alshamahi *et al*., 2020; Mohamed *et al*., 2020).
10. **Orbital Infections:** orbital cellulitis is an infection of the soft tissues surrounding the orbit. Not almost as common as conjunctivitis, it is the most common eye infection caused by *S. aureus* bacteria (Amato *et al*., 2013).
11. **Endophthalmitis and Panophthalmitis:** endophthalmitis is an infection of the ball of the eye, often identified after observation in an eye examination for deficiencies. Panophthalmitis is endophthalmitis with inflammation of all structures in the globe, while endophthalmitis is the inflammation of the interior structures of the globe (i.e., uvea and retina with pus in the vitreous and aqueous humors)(Mei *et al*., 2019) .

2.5.7. *Staphylococcus aureus* Genome

Staphylococcus aureus is a bacterium that has gained a bad reputation in recent years due to its ability to develop new virulent and drug-resistant strains, genomes of this species carry genes encoding virulence factors such as enterotoxins, exfoliative toxins, toxic shock syndrome toxin and immune evasion factors. These genes are often carried on mobile genetic elements(MGEs) including bacteriophages. The *Staphylococcus aureus* genome consists of a single circular chromosome (2.7-2.8 Mbp) plus a variety of additional extrachromosomal genetic elements (Mlynarczyk *et al*., 1998; Robinson and Enright, 2004).

Approximately 78% of the *S. aureus* genomes consist of a core component consisting of genes are present in all breeds. The remaining 22% of genes are made up of (accessory genome), which contains genes that encode a variety of non-essential functions, ranging from virulence and resistance to drugs and minerals to substrate use and versatility metabolism(Magni, 2010).

Many of the regions that make up the accessory genome are mobile genetic elements (MGEs) that can be transferred horizontally between bacteria, these elements can be transferred by conjugation, mobilization, or phage-mediated ways between microbes including *S. aureus* pathogenicity islands, bacteriophages, staphylococcal cassette chromosomes (SCC), transposons and plasmids(O'Brien *et al*., 2015) .

Most virulence genes tend to be found in genomic islands and phages resistance genes depend on SCC, transposons, and plasmids for transmission. At the same time, clinical isolates of epidemic strains can vary greatly in their transmission of MGEs(Shang *et al*., 2020) .

2.5.7. 1.*Staphylococcus aureus* Mobile Genetic Elements(MGEs)

- **Plasmids**: One or more plasmids are usually found in clinical isolates of *S. aureus*. Plasmids are defined as small pieces of mobile DNA that is simply transferrable between hosts there at least three families of *S. aureus* plasmids(O'Brien *et al*., 2015) .Which have been classified to fifteen incompatible groups . Most plasmids that have it has been described as coding determinants of antimicrobial resistance, and some others have also been described as determinants encoding other clinically important properties, such as the production of toxins (eg. enterotoxins SED, SEJ, and exfoliate toxins ETB)

(Projan and Archer, 1989). Some research indicates that sequencing of Staphylococcus aureus plasmids Created from different environments showed new resistance genes, for example, the *apmA* and *vgaC* genes, which encode resistance to abramycin and streptogram A, respectively(Monecke *et al*., 2011)

- **Bacteriophages:** In Staphylococcus aureus, phages are incorporated into chromosome (prophages) are common, with most strains carrying between one and Four jets(Pantůček *et al*., 2004). Temperate phages it has also been widely used in phage typing, a routine diagnostic tool for differentiation of clinical strains. At present, there are more than 80 complete genomes of *staphylococcus* and phage it can be retrieved from gen bank database, knowledge about it opening up new possibilities for understanding their role in pathogenesis Staphylococcal strains(M. Wang et al., 2021) .
- ***S. aureus* pathogenicity islands (SaPI):** *S. aureus* pathogenicity islands mobile elements about 15-16 kb that encode several virulence genes, including superantigens, enterotoxins B and C (seb and sec) and toxic shock syndrome Toxin-1 (tst)(Baba *et al*., 2008) .
- **Staphylococcal Cassette Chromosomes(SCCs):** The mobile elements Staphylococcal cassette chromosomes are 21-67 kb in size. They often encode antibiotic resistance genes, such as the Mec operon of methicillin Resistance (SCCmec)(Elements, 2009) .
- **Transposons:** *Staphylococcus aureus* transposons are small transposons that often encode resistance genes (B-lactamase, resistant to erythromycin and tetracycline). all transposons encoding a transposase gene, the product of this gene inducing excision or Element repetition, as well as integration (Santiago *et al*., 2015).

2.5.8. Antimicrobial Resistance in *Staphylococcus aureus*

Many antibiotics are given against *S. aureus* infection but eventually, the spread of Multi-Drug Resistance (MDR) from *Staphylococcus aureus* began to appear. Since then methicillin-resistant very common strains of *Staphylococcus aureus* (MRSA) used to cause hospital illness infections. Microorganisms for the need to survive are subject to mutations either changes in the DNA or RNA chromosomes confer resistance. One famous example is methicillin resistance in *Staphylococcus aureus*; *S. aureus* successfully developed multiple resistance strains. Plasmids are capable of carrying resistance genes and many toxic genes. Studies have demonstrated that MRSA strains were resistant to macrolides, chloramphenicol, tetracycline(John *et al*., 2019; Mwangi *et al*., 2019) .

Staphylococcus aureus is resistant to penicillin due to beta-lactamase production enzymes. The first case of resistance was seen in the hospital. There are two primary mechanisms of beta-lactamase resistance: the expression of a unique penicillin-binding protein (PBP2a or PBP2) in the cell wall is encoded by the mecA gene responsible for the increased level Beta-lactam resistance, including penicillinase-resistant antibiotics such as methicillin. Second, expression of the beta-lactamase enzyme encoded by the *blaZ* gene hydrolyzed beta-lactamases such as penicillin. Methicillin resistance confirms resistance to all beta-lactams antibiotics, such as carbapenems and cephalosporins. This also leads for greater resistance of staphylococcus to other antibiotics such as erythromycin, tetracycline and intermediate strains appear to be resistant Vancomycin(Jafari-Sales *et al*., 2018) .

Vancomycin resistance was very clear observed, its transmission among the population and the rise of resistant strains was it has become a major threat globally(Mukherjee *et al*., 2021) . However, Taha *et al*. (2019) reported that linezolid and vancomycin the most effective antibiotic against methicillin-resistant

Staphylococcus aureus(Taha *et al*., 2019). Multi-drug resistance may enhance mortality and morbidity indicating infection agents as (super bugs). The survival of microorganisms increased due to gradual development of widespread resistance against diverse antimicrobials(Tanwar *et al*., 2014).

2.5.8. 1. Mechanisms of Antimicrobial Resistance

Resistance to all of these antimicrobial agents against pathogenic microorganisms are rising in some patients due to prolonged use of antimicrobial agents by these patients. Bacteria use two strategies to avoid being killed by antimicrobials factors: tolerance and resistance. Tolerance is a property of inactivity, non-reproducing (persistent) bacteria in which drug targets are inactive, allowing bacteria to avoid damage and survive. persevering cells are subpopulations of bacteria in the depths of a biofilm which can differentiate into resistant state phenotype(Mukherjee *et al*., 2021; C. Wang *et al*., 2021)

Antimicrobial agents can be natural products, semi-synthetics variants of natural products, and synthetic chemicals. The most commonly used antimicrobial agents interfere with cellular processes to block key biochemical pathways or with cellular structures, resulting in growth inhibition or bacterial death. It can be categorized into six target categories depending on the Targets and Pathways (Yamaguchi and Nishino, 2018; Cossart, 2020; Marta *et al*., 2020).

- Bacterial cell walls biosynthesis such as β-Lactamase (Penicillins (e.g. penicillin, ampicillin, and oxacillin), cephalosporins (e.g. cefazolin, cefoxitin, cefotetan, ceftriaxone, and cefuroxime)
- Bacterial cell membranes such as Lipopeptides (e.g. Daptomycin and polymixin B)

- Bacterial protein biosynthesis Aminoglycosides (e.g. Gentamicin, tobramycin, streptomycin, and kanamycin)
- DNA replication and repair such as Fluoroquinolonesa (e.g. Nalidixic acid, ciprofloxacin, levofloxacin, and norfloxacin)
- RNA synthesis such as Rifamycins (e.g. Rifampin, rifapentine, rifaximin, and rifabutin)
- Folate biosynthesis such as Trimethoprim, Sulfonamides, Trimethoprim and sulfamethoxazole.

Resistance to antimicrobial agents is easily divided into mechanisms that are inherent or acquired resistance. Inherent resistance mechanisms are an innate characteristic of microorganisms are transmitted to the progeny vertically. Like resistance is always natural and inherited characteristic of a particular microbial group, genus, or species(Gronthoud, 2020) . There are a number of ways in which microorganisms become resistant antibacterial agents include these:(Török *et al*., 2017) .

- Production of enzymes (More than 80% of isolates of *Staphylococcus aureus* produce active penicillinase (β-lactamase enzyme) against penicillin and amoxicillin. Semi-synthetic penicillins such as methicillin, naphcillino, Oxacillin)(Gronthoud, 2020) .
- Modify target locations (MRSA has PBP2a instead of PBP2 in its cell wall. This gives resistant to almost all β-lactams including penicillins and cephalosporins)(Petrillo *et al*., 2021).
- Change in the permeability of the outer membrane (Gram-negative bacteria may become resistant to β-lactam antibiotics by change permeability barriers. Mutations that lead to loss of porine channels in the outer membrane, antibiotic particles are no longer allowed to enter and pass into cell.

- Alteration of metabolic pathways(microorganisms develop an altered metabolic pathway to bypasses the reaction inhibited by antimicrobials)
- Efflux pumps (erythromycin resistance in staphylococci the *msrA* gene codes for an efflux mechanism, which results in resistance to erythromycin), three genes including, *erm A*, *erm B* and *erm C* were responsible for this resistance as they encoding methylase enzymes that methylation of the ribosomal drug binding site (Murray *et al*., 2020; Bispo *et al*., 2022).
- Protection of ribosomes by the synthesis of ribosomal protection proteins (RPPs), this mechanism one of two bacterial resistance mechanisms to tetracyclines. This resistance is mediated by tetracycline resistance gene (*tet* gene), *RPP* gene phylogeny classification for nine chapters: *Tet M* gene one of them(Aminov *et al*., 2001; Melville *et al*., 2001)

2.5.8. 2.Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA was first discovered in 1961, a few months after the introduction of methicillin in clinical practice. However, it wasn't until the 1980 endemic strains of MRSA with MDR are becoming a global hospital problem(Török *et al*., 2017) . More than 100,000 healthcare-associated infections (HAIs) occur in the United States annually. Produces many multidrug-resistant organisms (MDROs) such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Bearman *et al*., 2018; Bruce *et al*., 2022).

MRSA strains resistant to all β-lactams due to alteration in penicillin-binding protein (PBP2) and consequently, to alteration the structure of the cell wall. Methicillin resistance is due to the *mecA* gene encodes low-affinity PBP2. mecA is usually located on the(MGE) it is called SCC-mec (staphylococcal cassette chromosome)(Ghaffar *et al*., 2022). It is believed the mec gene was acquired from

CoNS some MRSA strains have also acquired other virulence and resistance genes from CoNS. There 6 major SCC-mec clones, identified by *mecA*. gene class and type from the CCR complex (cassette chromosome recombinase). SCC-mec types I to III They are commonly found in healthcare settings and are often resistant to additional factors antibiotics, while types IV and V are more common in the community, and resistance for agents other than β-lactam is uncommon (dos Santos Barbosa *et al*., 2021).

2.5.8. 3.Vancomycin -Resistant in *Staphylococcus aureus*(VRSA)

Between 2002 and 2005, five different isolates of MRSA emerged vancomycin resistance was first discovered. These isolates it looks like he either got a plasmid containing vancomycin resistance gene *vanA*, from vancomycin-resistant *enterococci* gene, or they develop mutations in their cell walls that make them less permeable to vancomycin. Vancomycin resistance (VRSA) and (VISA) isolates great concern because vancomycin is one of the most proven agents to treat serious MRSA infections (Jenkins and Maddocks, 2019).

During an *Enterococcus faecalis* mixed infection isolate of *Staphylococcus aureus* acquired the vancomycin-resistant gene, vancomycin resistance gene was contained within a transposon (Tn1546 on) a conjugative plasmid(Al-Buhilal *et al*., 2021). Plasmid was transferred by conjugation between *E. faecalis* and *S. aureus*, after lysis of the *E. faecalis*, Staphylococcus *aureus* acquired DNA by transduction and transformed by new DNA. Then the transposons jumped from *E. faecalis* plasmid, recombined and incorporated into *S. aureus* multi-resistant plasmid, (Fig. 2.2) (Murray *et al*., 2020).

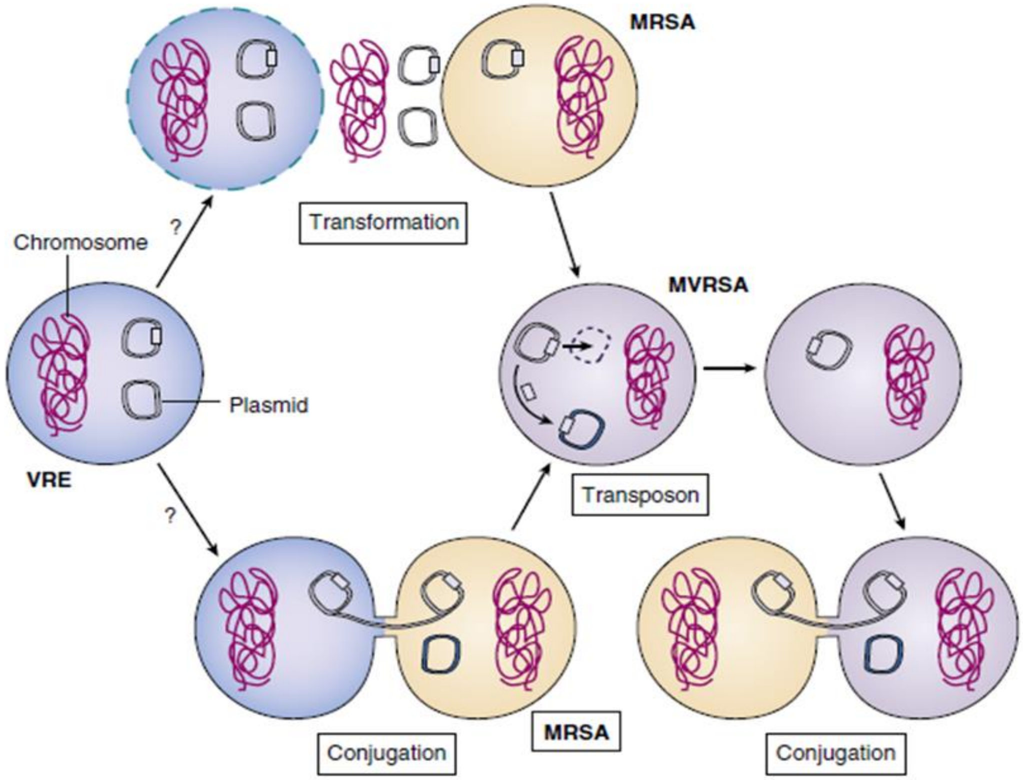


Fig. (2.1) Genetic mechanisms of evolution of methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and MVRSA) MRSA (in pink). Vancomycin-resistant *enterococcus* (VRE) (in blue)(Murray *et al*., 2020) .

Vancomycin is a glycopeptide that inhibits cell wall peptidoglycan synthesis by blocking transpeptidation by a mechanism is different from that of the β-lactams drugs. Vancomycin directly binds to the d-alanyl- d-alanine portion of a pent peptide, which inhibits the enzyme transpeptidase of association, while β-lactams drugs are associated with transpeptidase itself. Vancomycin is also again inhibited an enzyme, a bacterial transglycosylase enzyme, which also acts in the synthesis of peptidoglycan, but this appears to be it is less important than transpeptidase inhibition. Vancomycin is a bactericidal agent effective against certain bacterial gram positive (Warren, 2016; Nadig *et al*., 2021).

MRSA isolates showed different levels of vancomycin resistance. It was obvious that some were resisting the routine susceptibility testing methods, others missed the start by routine testing methods. This important emerging resistance followed the recognition in 1996 of the first MRSA isolate, with reduced susceptibility to vancomycin. Shortly thereafter, several Similar isolates were found to all contain vancomycin MICs 8µg/mL. These isolates of *S. aureus* with low sensitivity to vancomycin called vancomycin-intermediate *Staphylococcus aureus* (VISA)(Chaudhary et al., 2022) .

CHAPTER THREE
MATERIALS
and
METHODS

3. Materials and Methods

3.1: Materials

3.1.1 Equipment of Laboratory and Instruments

The laboratory equipment and instruments are used in the present study are listed in Table (3-1).

Table (3- 1) Laboratory Equipment and instruments

Instruments	Company	origin
Autoclave	Gemmy	Taiwan
Burner	Amal	Turkey
centrifuge	MSE	England
Disposable (Pteri Dish, Syringe, Plane tube and Latex)	Citro	China
Distiller	Ogawa	Japan
Electric sensitive balance	Sartorius	Germany
Electrical Oven	Memmert	Germany
Electrophoresis system	Fisher Scientific	USA
Eppendorf tubes	Eppendorf	Germany
Finn tips with different sizes	Eppendorf	Germany
Hood	Bio LAB	Korea
Hot plat	Biocote	England
Incubator	Binder	Germany
Light microscope	Olympus	Japan

Instruments	Company	origin
Micropipette	Dragonlab	China
Microtiter plate reader	Memmert	Germany
Microwave	Sanyo electric	Japan
Millipore Filter Unit 0.80µm	Chm	USA
Nanodrop spectrophotometer	Optizen	Korea
Para film	BDH	England
PCR Device	Leica	Spain
PCR tube	Eppendorf	Germany
Platinum Wire Loop	Himedia	Indian
Refrigerator	Marubeni	Japan
Screw capped test tubes	BBL	USA
Slides and Cover slide	Sail Brand	China
Sterilize Swab	ATACO	Brand
Transfer swab	AFCO	Jorden
Ultraviolet imaging device	Motic	USA
Vitek 2 system	Biomerieux	France
Volumetric flasks	Jlassco	India
Vortex mixer	Eppendorf	Germany

3.1.2: Chemical Materials

Chemical materials, stains, reagents and solutions are used in the present study illustrated in Table (3-2).

Table (3- 2) Chemical materials

Type of chemical	Company	origin
Acetone	BDH	England
Catalase reagent	BDH	England
Crystal violet	Merk	England
Ethanol 99%	fisher	England
Glycerol ($C_3H_8O_3$)	Merck	England
Oxidase reagent	BDH	England
Human Blood	Imam Sadiq Teaching Hospital	
Agar-agar	Himedia	India
Gram stain solution	Himedia	India
Normal Saline solution	S.D.I	Iraq
Glucose	Fluka	Switzerland
Cefoxitin Supplement (Cat. 6069)	Mast	U.K
Plasma Coagulase EDTA Selectavial	Mast	U.K

3.1.3: Antibiotic Disks

Table (3- 3) Antibiotic disks used in the present study

Antibiotics	Antibiotics Classes	Symbol	µg/ disk	Inhibition zone/diameter Mm			Company/ origin
				S	IN	R	
Cefoxitin	Cephalosporin	CX	30	≥25	-	≤ 24	Himedia/ India
Clindamycin	lincosamides	CD	2	≥21	15-20	≤ 14	Roseto /Italy
Ciprofloxacin	Fluoroquinolones	CIP	5	≥21	16-20	≥15	Condalab/Spain
Chloramphenicol	Phenicols	C	30	≥18	13-17	≥12	Roseto /Italy
Doxycycline	Tetracyclines	DXT	30	≤12	13-15	≥16	Roseto /Italy
Erythromycin	Macrolides	E	15	≥23	14-22	≥13	MAST/U.K
Gatifloxacin	Fluoroquinolones	GAT	5	≥23	20-22	≥19	Roseto /Italy
Gentamicin	Aminoglycosides	CN	10	≥15	13-14	≥12	Condalab/Spain
levofloxacin	Fluoroquinolones	LEV	5	≥19	16-18	≥15	Bioanalyse/ Tur
Linezolid	Oxazolidinones	LZN	30	≥21	-	≥20	Roseto /Italy
Methicillin	Nitrofurans	MET	5	≥14	10-13	≥9	Himedia/ India
Nitrofurantoin	Fluoroquinolones	F	300	≥17	15-16	≥14	Roseto /Italy
Oxacillin	Penicillin's	OX	1	≥22	-	≥21	Roseto /Italy
Ofloxacin	Fluoroquinolones	OFX	5	≥18	15-17	≥14	Roseto /Italy
Pencillin G	Penicillin's	P	10	≥29	-	≥28	Bioanalyse /Tur
Rifampin	Ansamycins	RA	5	≥20	17-19	≥16	Roseto /Italy
Trimethoprim	Folate pathway antagonists	TMP	10	≥16	11-15	≥10	Bioanalyse /Tur
Vancomycin	Glycopeptides	VA	30	≥15	18-20	≥10	Bioanalyse /Tur

3.1.4: Commercial Kits and Other Materials for DNA Extraction and PCR Technique

The commercial kits used in the present study are illustrated in table (3-4).

Table (3- 4) commercial kits

Type of Kit	Company	Country
Agarose	Bio Basic	Canada
Deionized sterile distilled water	Promega	USA
DNA extraction Kit	Geneaid	Korea
DNA ladder (100-1500 bp)	Simgen	China
DNA Loading dye	Intron	Korea
Go Taq® G2 Green Master Mix Including: (Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR).	Promega	USA
Nuclease free water	Promega	USA
Red Safe	Fisher	USA
TBE 10 X	Bioneer	Korea

3.1.5: Culture Media

All media are prepared according to the manufacturers of Hemedia (India) except for MRSA Chromogenic Agar Base according to the manufacturers of Conda (Spain) and 5% fresh human blood was added to the blood agar base after sterilization by the autoclave at 121°C for 15 min to prepare the blood agar. The culture media used in this study are mentioned in Table (3-5).

Table (3- 5) Cultures media and purpose of use

Culture media	Purpose of use
Blood Agar Base	Use it to test the ability of bacteria to blood hemolysis and to detect the type hemolysis
Brain heart infusion agar	Use for activation the isolates and for Short-term preservation
Brain Heart Infusion broth	Use for preserving bacterial isolates by adding 15% glycerol to 85% of the liquid medium after sterilization and stored at (-20C°) for 2-6 months and activation the isolates after preservation
Mannitol Salt Agar	Isolation of *staphylococcus* and differentiation of *staphylococcus aureus*
Muller - Hinton Agar	Use this medium to test for antibiotics susceptibility
MRSA Chromogenic Agar Base	For the detection of methicillin resistant *Staphylococcus aureus* from clinical samples
Nutrient Agar, Nutrient Broth	General purpose medium, use for activation the isolates and for preservation
Tryptic Soy broth	Use to detect the biofilm formed by bacteria

3.1.6: Gene's Selection

nine genes were selected to be amplified PCR technique *16S rRNA*, *Sea*, *Seb*, *Sec*, *Sed*, *See*, *tet M*, *erm B* and *Wzm* gene. And their primers for polymerase chain reaction (PCR) listed in (Table 3-6) with their manufactured by and checked in BLAST for product formation.

3.1.7: Primers Used in this Study

The primer pair for 16S rRNA, *Sea*, *Seb*, *Sec*, *Sed* and *See* gene were purchased from (Realgene/China) While for *tet M* , *erm B* and *Wzm* gene from (Macrogen/Korea) (Table 3-6) which used in this study were dissolved using sterile ddH_2O. Stock solution (100 pmol/µl) was prepared by adding ddH_2O to the vial containing lyophilized primer as recommended by provider and stored in deep freezer until used in Polymerase Chain Reaction (PCR) Technique, while working stock of 10 pmol/µl was made by mixing 10 µL from each primer and add 90µL ddH_2O.

Table (3-6): The primers used in the current study

Gene Name	Primer	Sequence 5`............3`	Amplicon size (bp)	Reference
16S rRNA	Stap 16SF	CCTATAAGACTGGGATAACTTCGGG	791	(Al-Khafaji et al., 2019)
	Stap 16SR	CTTTGAGTTTCAACCTTGCGGTCG		
Sea	SA-Sea-F	GGTTATCAATGTGCGGGTGG	102	(MacFaddin, 2000)
	SA-Sea-R	CGGCACTTTTTTCTCTTCGG		
Seb	SA-Seb-F	GTATGGTGGTGTAACTGAGC	164	
	SA-Seb-R	CCAAATAGTGACGAGTTAGG		

Gene Name	Primer	Sequence 5`............3`	Amplicon size (bp)	Reference
Sec	SA-Sec-F	AGATGAAGTAGTTGATGTGTATGG	451	
	SA-Sec-R	CACACTTTTAGAATCAACCG		
Sed	SA-Sed-F	CCAATAATAGGAGAAAATAAAAG	278	
	SA-Sed-R	ATTGGTATTTTTTTTCGTTC		
See	SA-See-F	AGGTTTTTTCACAGGTCATCC	209	
	SA-See-R	CTTTTTTTTCTTCGGTCAATC		
tet M	tet M-F	AGTGGAGCGATTACAGAA	158	(Strommenger et al., 2003)
	tet M-R	CATATGTCCTGGCGTGTCTA		
erm B	ermB-F	TGGTATTCCAAATGCGTAATG	587	(Malhotra-Kumar et al., 2005)
	ermB-R	CTGTGGTATGGCGGTAAGT		
Wzm	Wzm-F	TGCCAGTTCGGCCACTAAC	148	(Vuotto et al., 2017)
	Wzm-R	GACAACAATAACCGGGATGG		

3.1.8: Preparation of Reagents and Solutions

3.1.8.1: Catalase Reagent

Catalase reagent used to learn about the bacterial potential of generating the enzyme catalase. It was prepared by dissolving (3ml) of H_2O_2 in (100ml) of distilled water (3%) and stored in dark container (Forbes,*et al*., 2007).

3.1.8.2: Oxidase Reagent

It was prepared directly by dissolving (1gm) of Tetramethyl-p-phenyl Diamine dihydrochloride in 100 ml of distilled water in an opaque vial. This reagent was used to investigate the ability of bacteria to produce the enzyme oxidase (Forbes *et al*., 2007) .

3.1.8.3: Coagulase Reagent

Plasma Coagulase EDTA Selectavial was supplied from Mast Group. Contains lyophilised rabbit plasma with EDTA prepare directly depending on the manufacturer's instructions by rehydrate the vial using the diluent specified on the pack label and shake the vial to ensure the plasma coagulase is completely reconstituted. It was used to procedure tube coagulase test and slide agglutination test to investigate the ability of bacteria to produce the enzyme coagulase.

3.1.8.4: Gram Stain Solution

Gram stain solution was supplied from Himedia company. These solutions included: four solutions Crystal violate, Iodine, %96 ethanol, and safranin stain. It has been used to study the morphology and arrangement of cells, to distinguish between Gram-negative bacteria and Gram-positive bacteria (Forbes *et al*., 2007).

3.1.8.5: Normal Saline Solution

It was prepared by dissolving (8.5 g) of NaCl in a small volume of distilled water, then completed to 1000 mL, pH constant at 7.2 and sterilizing in an autoclave at 121°C for 15 min, then keeping at 4°C.(MacFaddin, 2000) .

3.1.8.6: McFarland's Turbidity Standard (0.5)

The fresh 0.5 McFarland's standard tube (1.5× 10^8 CFU/ml) was prepared according to the method described before (Zapata and Ramirez-Arcos, 2015), as it consists of two solutions.

A- Barium chloride solution (1.175 %): Dissolve 1.175 g of barium chloride [($BaCl_2$. $2H_2O$: H_2O (w/v, 1.175g: 98.825 ml)] in an amount of distilled water and after completing the dissolution, complete the volume to 100 ml with distilled water.

B- Sulfuric acid solution (1%): 1 ml of concentrated sulfuric acid [H_2SO_4: H_2O (v/v, 1 ml:99 ml)] was added to 99 ml of distilled water.

Then McFarland's solution was prepared by adding 0.5 ml of solution A to 99.5 ml of solution B and mixing the solution well in order to obtain a barium sulfate precipitate. The solution was used to visually compare the turbidity of the bacteria suspension with the turbidity of the 0.5 McFarland standard. The accuracy of the prepared 0.5 McFarland standard was verified using a spectrophotometer. The optical density was measured at 625 nm which should be between 0.08 and 0.1 (Zapata and Ramirez-Arcos, 2015).

3.1.8.7: 1X TBE Buffer

It was prepared by added 10 ml of 10X TBE to 90 ml of sterile distal water, the solution was used to dissolve agarose in the electrophoresis process (Green and Sambrook, 2012).

3.1.8.8: Agarose Gel (1.5%)

Agarose gel was prepared at a concentration in1.5% for the PCR product, by dissolving 1.5 g of agarose gel in 100 ml of TBE solution (1x) to prepare a concentration of 1.5%. Then melt it in the microwave for a few minutes until the dissolving is complete, then cool it to about 50-60 ° C, after that add 5 microns of safe red and pour the agarose gel into a special tray containing a comb to form wells in the gel. The formed bubbles are removed using a micropipette. After final solidification of the gel, the comb was carefully removed, and the tray was then transferred to an electrophoresis tray filled with 1X of TBE buffer.

3.1.8.9: Lysozyme

It was prepared according to the manufacturer's instructions (Geneaid) by adding 0.8 mg weight of lyophilized lysozyme in g + buffer in a volume of 200 µL. Use as a stock solution and store at -20°C. Used to extract DNA.

3.1.8.10: Proteinase K

According to the manufacturer's instructions (Geneaid) 11 mg of lyophilized proteinase K was dissolved in 1.1 mL of free deionized distilled water. Use as a stock solution and store at 4°C. used to extract DNA.

3.1.8.11: Solutions of Primers

3.1.8.11.1: Stock Solution (100 pmol. µl-1)

The lyophilized primers were dissolved depending on the information of primers manufacture (Realgene/China) for *16S rRNA*, *Sea*, *Seb*, *Sec*, *Sed* and *See* gene (Macrogen/Korea) for Wzm, *tet M* and *erm B* gene, in nuclease free deionized distilled water, then vortex and stored at -20°C until use.

3.1.8.11.2: Working Solution (10 pmol. µl-1)

Prepared by mixing of 10 µl of stock solution (100 pmol. µl-1) with 90 µl of nuclease free deionized distilled water and stored at-20°C until use.

3.1.9: Preparation of Culture Media

The media is prepared according to the instructions on the packages and sterilized by autoclave and to ensure that these media were not contaminated by placing them in the incubator at a temperature of 37 °C for 24 hours. They were placed in the refrigerator at a temperature of 4 ° C.

3.1.9.1: Blood Agar Medium

Blood agar medium is prepared by dissolving 40 g of blood agar base in 1000 ml of sterile water. The medium was autoclaved at 121 °C for 15 min and at a pressure of 15 pounds per square (psi), cooled to 50 °C and 5% fresh human blood was added. This medium was used as an enrichment medium to grow bacterial isolates and to determine their ability to hemolysis(Forbes *et al*., 2007) .

3.1.9.2: Brain Heart Infusion Broth

This medium is prepared according to the instructions of the supplied company, by dissolving 37 g of liquid nutrient medium in a liter of distilled water and sterilized by autoclave at 121ċ for 15 min. It was used for the purpose of developing and activating bacteria as well as for preserving bacterial isolates by adding 15% glycerol to 85% of the liquid medium after sterilization and stored at (-20C°) for 2-6 months(Forbes *et al*., 2007).

3.1.9.3: Brain Heart Infusion Agar

This medium is prepared according to the instructions of the supplied company, use for activation the isolates and for Short-term preservation was accomplished by culturing bacteria on Brain heart infusion agar slant in screw-capped tubes and incubating at 37°C for 24 hours, then storage at 4°C for one to three months(Prescott *et al*., 2007)

3.1.9.4: MRSA Chromogenic Agar Base

This medium is prepared by add 51.7 g of medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Sterilize in an autoclave at 121 °C for 15 min. Cool to 45-50°C and add one vial of cefoxitin aseptically Supplement (Cat.66069). and dispense into petri sterile plates. MRSA chromogenic agar base is a chromogenic, selective and differential medium used to detection of methicillin resistant *S. aureus* from clinical samples (Gurran *et al*., 2002).

3.1.9.5.: Mannitol Salt Agar

This medium is prepared according to the instructions of the supplied company, by dissolving 108 gm in a liter of distilled water and sterilizing it with an autoclave at 121ċ for 15 min. This medium was used to isolate *staphylococci* and diagnose it in terms of its ability to tolerate the salinity of the medium it contains 75% NaCl as well as its ability to ferment mannitol sugar and turn the color of the medium to yellow. It is used as a selective media for the isolation of *Staphylococci* and differentiation of *S. aureus* (MacFaddin, 2000).

3.1.9.6: Muller-Hinton Agar

This medium is prepared according to the instructions of the supplied company, by dissolving 38 g of the medium in a liter of distilled water. Sterilize the medium using an autoclave at 121ċ for 15 min, and after completing the sterilization process, cool it to (45-50) C^{o} and pour it into sterilized petri dishes. The medium was used to grow bacterial isolates for antimicrobial susceptibility testing(Forbes *et al*., 2007).

3.1.9.7: Nutrient Broth

This medium is prepared according to the manufacture company and sterilized by autoclave at 121ċ for 15 min use for activation the isolates (MacFaddin, 2000) .

3.1.9.8: Nutrient Agar Medium

This medium is prepared according to the manufacturer's instructions by dissolving (28) gm of nutrient agar in a liter of distilled water and sterilizing by autoclave at 121ċ for 15 min. It has been used to preserve and sustain bacterial isolates for a short period of 2-3 weeks and in DNA extraction, bacteria are grown in the medium at 24 hours of age, and then extracted from this medium(MacFaddin, 2000) .

3.1.9.9: Trypticase Soya Broth (TSB)

This medium is prepared according to the manufacturer's instructions by dissolving 8 g in 1000 ml of distilled water. TSB with 1% glucose has been used to detection of the bacterial ability to produce biofilm by tissue culture plate method (TCP) (biofilm assay) based on the methods described by(Christensen *et al*., 1985; Mathur *et al*., 2006) .

3.2: Methods

3.2.1: Specimen’s Collection

one hundred and sixty eye swabs specimens collected from patients with a bacterial eye infections from different parts of the eye, one swab for each patient including (conjunctiva, eyelids, cornea and dacryocystitis) for both sexes with ages ranging from (4-74) years who attended to Al-Imam Al-Sadiq Hospital, Hilla Teaching Hospital / Babylon province , and Ibn Al-Haitham Teaching Eye Hospital/ Baghdad province, from November 2021 to March 2022, by using sterile swabs, using the expertise of specialists in the field of ophthalmology, taking into account that the patients did not use antibiotics, the samples were transferred to the laboratory and then inoculated on the differential and diagnostic media.

All swabs were cultivated and incubated aerobically at the temperature of 37°C for (24-48) hours. In culture media, Blood agar, Nutrient agar, Mannitol salt agar, MRSA Chromogenic Agar Base.

3.2.2: Laboratory Diagnosis

3.2.2.1: Isolation of *Staphylococcus aureus* Bacteria

After collecting the swabs and transporting them to the laboratory. The inoculated specimens are incubated at 37 °C for 24 h on blood agar already prepared for this purpose, the isolates are examined for shape, size, color, pigments and hemolytic activity. Then they were transferred, streaked on mannitol salt agar (MSA) and incubated at 37°C for 24 h, which is a selective and differential method for isolating, purifying and identifying *Staphylococcus aureus* and to determine the ability of each isolate to ferment mannitol as the medium contains phenol red as an indicator and a high level of salt concentration (NaCl 7.5%). All colonies from primary cultures were purified by subculture on brain heart infusion agar (BHI) then reinoculated onto(MSA) and incubated at 37 °C for 24 h. Then one pure isolated colony was transferred to Nutrient agar medium for preservation and further biochemical tests Confirm identification of isolates (Brown *et al*., 2005).

3.2.2.2: Microscopic examinations

A portion of the bacterial growth was transferred by a loop and placed on a glass slide, fixed and stained with gram stain and examined under the oil lens of a light microscope, the shape of the bacteria was observed as blue cocci, arranged in grapes like irregular clusters(Gillet *et al*., 2002) .

3.2.2.3: Identification of MRSA on MRSA Chromogenic Agar Base

MRSA Chromogenic Agar Base, selective and differential medium for detection of methicillin resistant *Staphylococcus aureus*. Laboratory diagnosis and susceptibility testing are critical in the treatment, management, and prevention of MRSA infection. This media has been designed and is suitable for screening MRSA. alpha-glucosidase produced by *Staphylococcus aureus* cleaves the chromogenic substrate and gives a blue color to the colony of *Staphylococcus aureus*. Cefoxitin inhibits the growth of methicillin-sensitive *Staphylococcus aureus*(Gurran *et al*., 2002).

3.2.2.4: Biochemical Tests to Identification of Isolates

3.2.2.4.1: Catalase Test

This test was conducted to determining the ability of bacteria to produce catalase enzyme which converts the decomposition of the toxic compound hydrogen peroxide H_2O_2 to oxygen $O_2$and water. A single isolated bacterial colony was looped out of the culture plate and placed on a clean glass slide. after that 2 drops of 3% concentration of H_2O_2 were added to the cells in the slide and mixed. and inferred the positive result by the appearance of bubbles of O_2 gas(Forbes *et al*., 2007)

3.2.2.4.2: Oxidase Test

This test was carried out by transferring a portion of the pure colony to the surface of a saturated filter paper with an adequate oxidative stress reagent. The appearance of the deep purple color during 5-10 seconds indicates the ability of bacteria to produce enzyme oxidase. The test is based on the presence of some bacterial oxidases that would catalyze the transfer of electrons between the bacteria's electron donors and the redox dye (tetramethyl-ρ-phenylenediamine dihydrochloride); The dye has been reduced to a deep purple(Forbes *et al*., 2007).

3.2.2.4.3: Coagulase Test

This test was used to detect the ability of tested bacteria to produce the Coagulase which is an enzyme-like protein produced by *S. aureus* that converts fibrinogen into fibrin and clots oxalated plasma. It is an important method for differentiation between *S. aureus* and other species of *Staphylococcus*. plasma. There are two forms of coagulase enzyme, bound or clumping factor and Free coagulase, the test was performed as the following according to the manufacturer's instructions Mast Group for Plasma Coagulase EDTA Selectavial which was used for this purpose:

A. Tube Coagulase Test (free coagulase)

A volume 0.5 mL of reconstituted plasma (Coagulase reagent) was added to sterile tubes using a sterile pipette using a culture less than 24 hours old, inoculate the plasma Coagulase by emulsifying 1 loop (2 to 4 colonies) of bacteria, from an uninhibited agar plate, into the plasma tube Incubate the tube Inoculated at 37°C for 4 hours examined for clot formation. Results should be read in 4 hours. A positive test for coagulation enzyme production results in coagulation in rabbit plasma. Degree of coagulation is considered a positive test. The test It is considered negative when plasma remains liquid, does not clot. Negative tests should be left at room temperature overnight and checked again.

B. Sl**ide Agglutination Test (bound coagulase or clumping factor)**

Place a drop of rehydrated plasma (Coagulase reagent) on a clean, dry glass slide Place a drop of sterile water on the slide to act as a control using a sterile loop Emulsify the amount of the isolated colony being tested in each drop, and inoculate the water first. Try to create a smooth suspension. Note the clumping in the plasma coagulant and note the appearance in the control. Clumps that do not mix uniformly into Plasma Coagulase represent a positive slide and are indicative of *S. aureus*. A negative reaction is recorded when colonies blend smoothly into a homogenous suspension.

3.2.2.4.4: Hemolysis Test

Hemolysis is determined by lysis of blood cells by stretching the bacterial isolates onto a plate of blood agar and incubating at (37°C) for (24-48 hours) the appearance of a clear area around the colonies referred to as β-hemolysis or color changes The middle (green area) around the colonies indicated partial hemolysis (α-hemolysis), while no change indicates no hemolysis (γ-hemolysis) (Atlas and Snyder, 2006).

3.2.2.4.5: Mannitol Fermentation Test

The test was used to detect the ability of *staphylococcus aureus* for mannitol sugar fermentation and the appearance of colony in yellow color indicated positive results where the acidity of the medium changes as a result of bacterial fermentation of mannitol and the pH of the phenol red indicator turns also; *S. aureus* changes the color of the medium from alkaline (red) to acidic (yellow), the rest of the *staphylococci* grow without changing the color of the medium, so this medium was used for the purpose of selective isolation and cultivation of bacteria, after streaking on mannitol salt agar plate medium and incubated at a temperature of 37 °C for a period of 18-24 hours(Gillet *et al*., 2002).

3.2.2.5: Identification of Bacterial Isolate with Vitek 2 System

The Vitek 2 was used to validate the biochemical and antibiotic assay, which was performed according to the manufacturer's instructions. This machine consists of personal device, reader incubator, which is made up of several internal components including: card filling process, loading process, card cassette, barcode scanner, card sealer, cassette spiral and incubator. Along with optical transport, waste processing, electronic control tools and firm ware. The system is equipped with an expanded identification database for all routine identification tests that provide improved microbial diagnostic efficiency that reduces the need for additional testing to improve safety for both the tester and the user.

The following steps have been planned according to the manufacturers' directions.

I. Preparation of the bacterial suspension: A sterile swab was used to transfer a sufficient number of *S. aureus* bacteria Pure cultured colonies (the colony must be 24 hours of age) were suspended separately in 3 ml of sterile saline in transparent plastic test tubes. The turbidity was modified by inserted test tubes into the colony standardization assay system with McFarlands standard solution (1.5 × 108 cells/ml).

II. Identification card was inoculated with isolated *S. aureus* suspension. The test tube containing the staphylococcal suspension was placed into a special rack and the card was placed in the adjacent slot while the transfer tube was inserted into the corresponding suspension tube. The filled cassette was placed manually or automatically into a vacuum chamber station. After vacuum was applied and air was reintroduced to the station, the suspended staphylococci were pushed through the transfer tube into the microchannels that filled all test wells.

III. Card sealing and incubation: Inoculated card was passed by a mechanism, that cuts the transfer tube and seals the card before being loaded into the carousel incubator. Carousel incubator can accommodate up to 30 or up to 60 cards. All types of cards are incubated online at 35.5 + 1.0 ºC. Each card is taken out of the carousel incubator once every 15 minutes, transmitted to the optical system for reaction readings, and then returned to the incubator until the next reading time, data were collected at 15 minute intervals during the entire incubation period.

3.2.3: Maintenance of Bacterial Isolates

Maintenance of bacterial isolates was performed as follows according to Collee et al., (1996):

3.2.3.1: Short Term Storage

Short-term maintenance was accomplished by culturing bacteria on the surface of nutrient agar medium and incubating at 37°C for 24 hours. The plates were tightly wrapped with parafilm and stored at 4 C., then storage at 4°C for a period of few weeks.

3.2.3.2: Medium Term Storage

The isolates maintained by streaking on Brain-Heart agar slant for a period of one to three months in screw-capped vials containing 10-15 ml of medium. The isolates on Brain-Heart agar slant incubated at 37 °C for 24 h. And stored in the refrigerator at 4 °C.

3.2.3.3: Long Term Storage

bacterial isolate preservation for a long time, by a sterile tube containing 2ml of Brain-Heart broth containing 15% glycerol (15% of glycerol + 85% brain heart infusion broth) glycerol was inoculated with the isolate and incubated at 37°C for 24 hours. When visible bacterial growth was seen (turbidity), the tube was sealed tightly with parafilm and stored at -20 °C in deep freezing for a long period of time.

3.2.4: Antibiotic Susceptibility Test

The antibiotic susceptibility of *S. aureus* isolates to different antimicrobials determined according to Kirby-Bauer disk diffusion method on Mueller Hinton agar (MHA)(Bauer, 1966) .

One of the most common methods used routinely in diagnostic laboratories and is based on inoculating the bacteria under test on a solid culture medium (Muller Hinton agar) in a Petri dish. After cultivate of the bacterial isolate using brain heart infusion broth at (37°C) for (24) hours, and by adding sterile normal saline compared with (0.5) a standard McFarland tube (1.5 x 10^8 CFU/ml), then spread on Muller Hinton agar (MHA) using a sterile cotton swab and leave it to dry, different antibiotic tablets were used in different concentrations such as Cefoxitin 30 ,Clindamycin 2 ,Ciprofloxacin 5 ,Chloramphenicol 30 ,Doxycycline 30 ,Erythromycin 15 ,Gatifloxacin 5 ,Gentamicin 10 ,Levofloxacin 5 ,Linezolid 30,Nitrofurantoin 300 ,Oxacillin 1 ,Ofloxacin 5 ,Pencillin G 10 ,Rifampin 5 ,Trimethoprim 10 ,Vancomycin 30.

With sterile forceps, the selected antimicrobial disks were placed on the surface of the inoculated medium and incubated at 37°C for 24 h, during the incubation period the antibiotic spread from the disc to the medium. If the organism is sensitive to antibiotics, zones of lack of growth appear around the disc, and the higher the sensitivity, the larger the diameter of the area of inhibition. Antibiotic inhibition zones were noted and measured with a ruler or caliper, the antibiotics names and its standard inhibition diameter were used according to the Clinical and Laboratory Standards Institute (CLSI 2021) for sensitivity or resistance of the organism to each antibiotic.

3.2.5: Detection of Biofilm Formation (semi Quantitative Biofilm Assay)

The semi quantitative measurements of biofilm formation test by using tissue culture plate method (TCP) were done according to the method described by (Ghellai *et al.*, 2014) included:

1. After activation of the bacterial isolate using brain heart infusion broth at (37°C) for (24) hours. Bacterial culture, 5 μl was used to inoculate flat bottom wells

Microtiter containing 145 µL of trypticase soy broth (TSB) with 1% sucrose. Control wells contained only 150µl of trypticase soy broth (TSB) with 1% sucrose (0.5 gm sucrose for each 50ml of trypticase soy broth).

2. Microtiter plate was incubated at 37°C for 24 hours and sealed with parafilm during that.
3. then the unattached bacterial cells removed by washing the wells with normal saline (pH= 7.2) for three times.
4. Microtiter plate was incubated at 60 °C for 30 min, then added 200µl of crystal violet solution (0.1%) to the wells for 15 min.
5. Then the crystal violet solution is removed, and all wells are washed three times by distal water to remove unbounded dye, and leave it to dry in the room temperature.
6. Extracted of the crystal violet solution form the biofilm by using 150 µl of 95% ethanol and acetone [8:2 (v/v)] for each walls.
7. By using an ELISA reader, the absorbance of each well was measured at 630 nm. And results classified to absorbance into 4 categories: non, weak, moderate, and strong according to(Stepanović *et al*., 2007) this classification is demonstrated in the table below.

Table (3-7): classification of *S. aureus* as biofilm formation.

"OD" ≤ "ODc"	non-biofilm
"ODc < OD ≤ 2 x ODc"	Weakly biofilm producer
"2 x ODc < OD≤ 4 x ODc"	Moderate biofilm producer
"4 x ODc< OD"	Strong biofilm producer

*"OD" Optical density reader average of Isolate, "ODc" Optical density reader average of control.

3.2.6: Molecular Detection Methods

3.2.6.1: Extraction DNA whole (total) Genomic

Sample Preparation

A large number of bacterial cells leads to a higher yield of DNA. (3-5) pure bacterial colonies were therefore cultured on nutrient agar plate, under aerobic conditions, at 37 °C for 24 h. Then bacterial colonies transferred by sterile loop from plate into eppendorf tube (1.5 ml) containing 1ml normal saline solution(Lema *et al*., 1994).The *Staphylococcus aureus* whole genomic DNA was extracted by using Geneaid bacterial DNA extraction (Korea kit), DNA extraction can be summarized as follows according to manufacturer's protocol.

The Procedure

1. A volume 1.5 mL Eppendorf tube containing suspended cells centrifuged at 14000 rpm for 1 minute. The supernatant was poured off, after precipitated the cells.
2. A volume of 200µl from Gram+ Buffer contains 0.8 mg of Lysozyme (0.8 mg/200 µl) added for each Eppendorf tube, Followed by vortex and incubation for 30 min at 37 °C. During the incubation period, the tubes were inverted every 10 minutes.
3. A volume 20 µl of proteinase k was added to the tube and mixed by vortex then incubated at 60° C for at least 10 min, and the tube was inverted every 3 min.
4. Two hundred µl of GB buffer was added to a 1.5 mL Eppendorf tube and mixed by vortex for 10 s. The tube is left in the incubator for 10 minutes at 70 °C and inverted every 3 minutes.
5. A volume 200 µl absolute ethanol was added and immediately mixed by vortex to prevent precipitation. All of the previous mixture was transferred to a GD column which was placed in a specified sterile collection tube. The GD column was centrifuged at 14,000 rpm for 2 min. Next, the collection was removed along with its content, and the GD column is placed in a new collection tube (this step is the DNA ligation step).

6. A volume 400 µl of W1 buffer is added to the GD column for (washing specific matrix DNA from other contents) and centrifuged at 14,000 rpm for 30 s. The collection tube and its contents are then discarded, and the GD column was transferred to a new collection tube.
7. Six hundred washing buffer is added to the GD column for perfection of washing step, the centrifuged at 14000 rpm for 30 sec.
8. The GD column after the washing step and removing the liquid from the collection tube, was dried by centrifuged at 14,000 rpm for 3 min. Then, the GD column was transferred to a sterile 1.5 Eppendorf tube.
9. A volume 100 µl of pre-heated elution buffer is added into the center of the GD column for elution of DNA step, and left for 3 min at room temperature (to saturate of DNA matrix with elution buffer) and centrifuged at 14000 rpm for 30 secs, to obtain 100 µl of final volume.
10. The GD column was discarded, and the purified DNA (collected at the bottom of the Eppendorf tube) was kept at −20 °C until used.

3.2.6.2: Detection of total DNA by Agarose Gel Electrophoresis

About 4µL of samples were added to each well of (1%) agarose gel after mixing with 1µLof loading dye, an electric circuit was operated at a voltage of 75 volts /cm for 1-2 hr. bubbles arise at the anode when starting the device, dye transmitted through the gel. The result determined under UV light (Bartlett and Stirling, 2003).

3.2.6.3: Measurement of Concentration and Purity of Extracted DNA

The DNA quantity and purity was determined using a spectrophotometer (Nano drop) at the absorbance at 260nm and 280nm respectively. The concentration of DNA was estimated at ng/ml and the purity calculated as 260/280 ratio, when the DNA solution is pure the ratio ranged (1.8 - 2.2).

3.2.6.4: Polymerase Chain Reaction (PCR) Technique

3.2.6.4.1: PCR Amplification

Extracted DNA, primers and PCR premix were thawed at room temperature, vortex and centrifuged briefly to bring the contents to the bottom of the tubes. PCR mixture was prepared with a total volume of 25 µl containing: the DNA extracted, primers and PCR premix listed in the table (3.9) including Nuclease-Free Water and PCR Master Mix. PCR Master Mix is a ready-to-use mixture containing dNTPs derived from bacteria, Taq DNA polymerase, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. Master Mix contains two pigments (blue and yellow). which allows monitoring of progress during electrophoresis. The combined reactions with Master Mix have sufficient intensity for direct loading on agarose gels. The mixture components of the PCR pre-mix and the additives of materials depend according to the manufacturer's protocol (Promega, USA).

Table (3-8) Contents of the reaction mixture for amplification of genes in this study.

PCR mix		Volume
Master mix		12µL
Template DNA		5µL
Primer	Forward Primer	1.5µL
	Reverse Primer	1.5µL
nuclease free water		5µL
Total volume		25µL

3.2.6.4.2: PCR Program

the PCR program done by using DNA template extracted from all isolates of *S. aureus* for detection the genes. The optimum conditions and temperature for the polymerase chain reaction was used for each gene shown in table (3-9).

Table (3-9): Thermal cycler programs are used in this study

Gene	Temperature (°C)Time					No. of cycles
	Ini. Dent.	Cycling condition			Final Ext.	
		Denat.	Anneal.	Ext.		
Staph16S rRNA	95 ºC/ 2min.	95 ºC/ 30sec.	56 ºC/ 30sec.	72 ºC/ 70sec	72ºC/5min.	٣٠
Sea	95 ºC/ 2min.	95 ºC /30sec.	58.3 ºC/ 30sec	72 ºC/ 20sec	72ºC/5min.	29
Seb	95 ºC /2min.	95 ºC /30sec.	56.2 ºC/ 30sec	72 ºC/ 20sec	72ºC/5min.	30
Sec	95 ºC/ 5min.	9٤ ºC/ 2min.	57 ºC /2min.	72 ºC /1min	72 ºC/ ٧min.	35
Sed	95 ºC/ 5min.	9٤ ºC / 2min.	57 ºC /2min.	72 ºC /1min	72 ºC/ ٧min.	35
See	95 ºC/ 5min.	9٤ ºC/ 2min.	57 ºC /2min.	72 ºC١ /min	72 ºC/ ٧min.	35
tet M	94 ºC/ ٣min.	9٤ ºC /30sec.	45 ºC/ 30sec	72 ºC١ /min	72 ºC/ ٨min.	40
erm B	95 ºC/ 5min.	9٤ ºC /30sec.	55 ºC/ 30sec	72 ºC/ 30sec	72ºC/5min.	30
Wzm	95ºC/ 2min.	95ºC/ 30sec.	58ºC/ 40sec	72ºC /30sec	72ºC/5min.	٣٥

3.2.6.5: Agarose Gel Electrophoresis

Five µL of PCR products and DNA ladder (100-1500bp) carefully loaded in the wells. The electrophoresis proceeded for about 1:30 hour at 72, the size of DNA fragment was determined by running (100-1500bp) DNA ladder with DNA samples. DNA bands were visualized by U.V. illumination 350 nm wave –length on U.V. transilluminater system.

3.2.7.: Statistical Analysis

Statistical analysis performed by using Statistical Package of Social Science (SPSS) program V. 20 analyzed descriptive statistics and Chi-square test ($\chi 2$) used to determine the relationship between the variables, P value < 0.05 was considered statistically significant.

CHAPTER FOUR
RESULTS
and
DISCUSSIO

4. Results and Discussion

4.1. Description of Study Specimens

The current study included a collection of (160) swabs from patients suffering from bacterial infections in the eyes aged between (4-74) years that attended hospitals, Ibn Al-Haitham Teaching Eye Hospital in Baghdad 23 (14%), Al-Imam Al-Sadiq Hospital 106 (66%) and Hilla Teaching Hospital in Babylon 31(20%) for the period from November 2021 to March 2022 as shown in table (4-1). Patient related information was recorded in the hospital, source of collect, gender, age, residence, Chronic diseases they have and type of infection. As in the form shown in appendix figure1.

Table (4-1) Distribution of the eye infections swabs according to hospital

Source	No. of patients	Percentage %	P. value
Al-Imam Al-Sadiq Hospital	106	66%	0.000
Hilla Teaching Hospital	31	20%	
Ibn Al-Haitham Teaching Eye Hospital	23	14%	
Total	160	100%	

A total of (160) specimens (males and females) distributed into five groups according to age, (4-17) years (28) patients, (18 -31) years (46) patients, (32-46) years (52) patients, (47 -61) years (22) patients, (62-74) years (12) patients as shown in table (4-2). The results showed that the highest rate of infection is in the age group (32-46) years in the rate of (32%) and followed by the age group (18-31) years in the rate of (29%), (4-17) years in the rate of (17%), (47-61) years in the rate of (14%)while less percentage with age (62-74) years in the rate of (8%).

Table (4-2) Distribution of the eye infections swabs according to Age/ years

Age/ years	No. of patients	Percentage %	P. value
4 - 17	28	17%	0.000
18 - 31	46	29%	
32 - 46	52	32%	
47 - 61	22	14%	
62 - 74	12	8%	
Total	160	100%	

The percentage of male is 71%, while the percentages of female in this study 29% as shown in table (4-3).

Table (4-3) Distribution of the eye infections swabs according to Gender

Gender	No. of patients	Percentage %	P. value
Male	114	71%	0.000
Female	46	29%	
Total	160	100%	

The tables (4-2,4-3) shows that the infection occurs is high in aged group 32- 46 years and the infection occur in male is high than female in all age group this agree with Ayehubizu *et al.*, (2021) they reported the majority of external ocular infections patients are males (64.7%) while females are (35.3%), and these results did not agree with a study obtained by Teweldemedhin *et al.*, (2017). Those who proved that bacterial eye infections are more common among females compared to males and the percentage is higher in the age group (46-60). This can be attributed to the social, geographic, and climatic differences of the population.

According to gender show that males had a tendency to get ocular infections more than females, this is due to the fact that males are more susceptible to environmental conditions in their lives than females, as well as the nature and type of work specified for males over females and this result is agreed with the result (Perween *et al*., 2016). who recorded that bacterial eye infections are more common among males compared to females and the percentage was higher in the age group (20-40) years, and this variation in gender can differ from country to country (Petrillo *et al*., 2020). In Iraq (Rahama *et al*., 2017) Show that males had a tendency to get conjunctivitis more than females.

There are significant differences between patients according to residential area where urban patient is the most percentage in rate (78%) from rural region which is (22%) as shown in table (4-4). These residential area rate variations may be due to many social factors and lifestyle habits. Residency of patients in urban live in poor overcrowded areas. Patients tend to be exposed to the healthcare environment more frequently, especially considering that the majority of ocular infections occurred in the winter in the current study and hence were most vulnerable to eye infection.

Table (4-4) Distribution of the eye infections swabs according to Residential area

Residence	No. of patients	Percentage %	P. value
Urban	125	78%	0.000
Rural	35	22%	
Total	160	100%	

This result is consistent with the result recorded that residency of patients are urban (92%) and rural (7.5%) with highly significant (P<0.01) (Rahama *et al*.,2017). Residency of patients in urban are more than rural agree with study (Abid and Ewadh,2012). MRSA was detected in 16.9% (25/148) of suburban samples and 36.5% (27/74) of urban samples. No MRSA was found in rural samples. Nursing homes from urban areas had a significantly higher (P < .001) prevalence of *S. aureus* compared to nursing homes from suburban and rural sites. Areas with high nurse touch rates were the most commonly contaminated (Cheatham *et al*., 2019).

With regard to the distribution of samples based on the presence of chronic diseases such as high blood pressure (hypertension) and diabetes, the results showed a significant difference between the samples, as most of the patients did not suffer from these chronic diseases as in the table (4-5).

Table (4-5) Distribution of the eye infections swabs according to Chronic diseases (diabetes, blood pressure)

Condition of patients	No. of patients	Percentage %	P value
With chronic diseases	25	16%	0.000
Without chronic diseases	135	84%	
Total	160	100%	

Micronormal flora even in the eyes they may become pathogenic under certain conditions such as immunocompromised patients with chronic diseases these bacteria can cause infections to the conjunctiva, cornea and eyelids. Obesity, metabolic syndrome (MetS), and diabetes are frequent in surgical populations and can enhance susceptibility to postoperative surgical site infections. Reduced neutrophil function has been linked with diabetes and risk of *Staphylococcus aureus* infection. Therefore, neutrophil function in diabetic and obese subjects (± MetS) was assessed in this prospective serological and cellular surveillance study to determine whether vaccines administered to protect against infections after surgery could be effective in these populations (Scully *et al*., 2017).

Invasive staphylococcal infections are more prevalent in patients with diabetes and obesity than in those without, and are associated with a poor outcome (Befus *et al*., 2015). The underlying mechanisms linking these comorbidities to *S. aureus* infection are not fully defined, but may be linked to impairment in several aspects of the immune response to bacterial infections. These aspects include impaired healing, fibroblast and epidermal cell dysfunction, impaired angiogenesis, damage from reactive oxygen species and advanced glycation end products, and decreased host immune resistance outcome (Guo and Dipietro, 2010).

The primary defense against gram-positive pathogens such as *S. aureus* is engulfment and oxidative killing by neutrophils, a process that is dependent on tissue oxygen tension. Obese patients have decreased tissue oxygen tension and poor blood supply. In those undergoing surgery, this presents a particular problem at the surgical incision site, and increases the risk for surgical site infections outcome (Kabon *et al*., 2004). Decreased serum and tissue concentrations of prophylactic antibiotics and increased rates of perioperative hyperglycemia outcome (Edmiston Jr *et al*., 2004) may further increase the risk of postoperative infection.

After eye swabs are subject for culturing on different types of culture media, to diagnosis the cause agents, 139 (87%) samples gave a positive growth while 21(13%) samples showed no growth as shown in table (4-6). This might be explained by previous antibiotic treatment which was ignored completely by the patients in filling the questionnaire accompanying this study. Other reasons for failure of growth include death of the organisms during transport or storage, or the presence of highly fastidious organism like Chlamydia trachomatis and Haemophilus influenzae which both cause eye infection in humans.

In this study observed conjunctivitis is the dominant type of clinical manifestation of ophthalmic patients119 (75%) followed by, blepharitis 32 (20 %), keratitis 7(4%) and dacryocystitis 2(1%) table (4-6). The reason for the high incidence of infection in the conjunctiva may be due to the conjunctiva is the primary reservoir of stray microbes.

Table (4-6) Distribution of the eye infections swabs according to Type of Infection

Type of Infection	No. of patients	Percentage %	Positive growth	Negative growth	No. of *S. aureus* Isolates	P. value
Conjunctivitis	119	75%	105	14	41	0.000
Blepharitis	32	20%	25	7	12	
Keratitis	7	4%	7	0	4	
Dacryocystitis	2	1%	2	0	0	
Total	160	100%	139	21	57	

From the table that also shows the number of isolates based on the type of infection, (41) conjunctivitis, (12) blepharitis, and (4) from keratitis it is clear that the higher rate of bacterial infection of the eyes due to *staphylococcus. aureus*. The doctrine that the cause of the predominance of *staphylococcus. aureus*. It spreads as part of normal skin in the air and its ability to colonize the respiratory system and other epithelial and mucous surfaces effectively in healthy individuals, which act as trigger for spreading infection and even into the eyes become pathogenic under certain conditions or it may be transmitted through contaminated hands, as it possesses many virulent factors such as enzymes and toxins. This agrees with Ayehubizu *et al*., (2021) where the frequency of culture-confirmed conjunctivitis was common, followed by blepharitis, and dacryocystitis was 125 (60.4%), 67 (55.8%), and 5 (45.5%), respectively.

Shiferaw *et al*., (2015) found that 93.7 % (89/95) were gram-positive and patients clinically categorized as conjunctivitis (n = 69) and blepharitis (n = 47). Conjunctivitis varies in the world from 0.9-21% depending on socioeconomic conditions (Nabat *et al*., 2019). The main causative microorganisms for ocular surface infections were *Staphylococcus spp*. (Petrillo *et al*. 2020). Shiferaw *et al*. (2015). demonstrated that Coagulase-negative *Staphylococci* (CoNS) was the predominant pathogen (57.3 %; n = 51/89) followed by *Staphylococcus aureus* (23.6 %, n = 21/89) this concluded that agreed with this study. This study agreed with other local study obtained by Mahdi, (2009) in Al-Diwaniya city was found that Gram-positive bacteria are the dominance to cause ocular infection, and also agreed with a study obtained by Abid and Ewadh, (2012) that shows that both Gram-positive and Gram-negative share in an Eye infection. The most percentage is Gram-positive bacteria.

Fifty-seven (41.07%) *S. aureus* isolates obtained from positive growth eye swabs, detecting by morphological and traditional test and confirmed by using Vitek 2 compact system. These percentages suggested that eye infections were sustained by *S. aureus*. This agree with Petrillo *et al*., (2021) they reported the majority bacterial conjunctivitis and keratitis pathogens 49.1% were *S. aureus*. This result also approaches to the study Hassan, (2021) that showed *S. aureus* one of the most common ocular isolates in the rate 34.78%. and agreed with a study obtained by

(Wang *et al*., 2015; Petrillo *et al*. 2020). Where the main causative microorganisms for ocular surface infections were Staphylococcus spp.

This is supported by several previous reports from Ethiopia and countries such as Nigeria, Iran and India that also reported Staphylococcus spp. bacteria as the main cause of bacteria that caused external eye infections (Getahun *et al*., 2017; Mohammed *et al*., 2020; Ayehubizu *et al*., 2021). In addition to the local studies obtained by it (Nabat *et al*., 2019).

4.2. Detection of *Staphylococcus aureus* Using Traditional Methods

A total of 57 isolates of *Staphylococcus aureus* among the culture-positive samples, from patients with bacterial eye infection, they are cultured for the isolation and diagnosis of *Staphylococcus aureus*, using differential media (blood agar, nutrient agar, mannitol salt agar and MRSA Chromogenic Agar Base). The Gram staining technique was performed to study the microscopic properties and was observed under an oil immersion lens (100X).

Staphylococcus aureus can be distinguished by producing yellow colonies on mannitol salt agar with surrounding yellow medium (mannitol fermentation, thus changing the color of the medium from red to yellow), in blood agar, all isolates produce clear β- hemolysis around their colonies. Positive form of the coagulation reaction figure (4-1). After isolation of the organisms from eye swabs and culture on different agar used, the organisms were Gram-stained such as staphylococcus seen as Gram-positive cocci appearing in clusters in microscopically diagnosis (Hassan,2021).

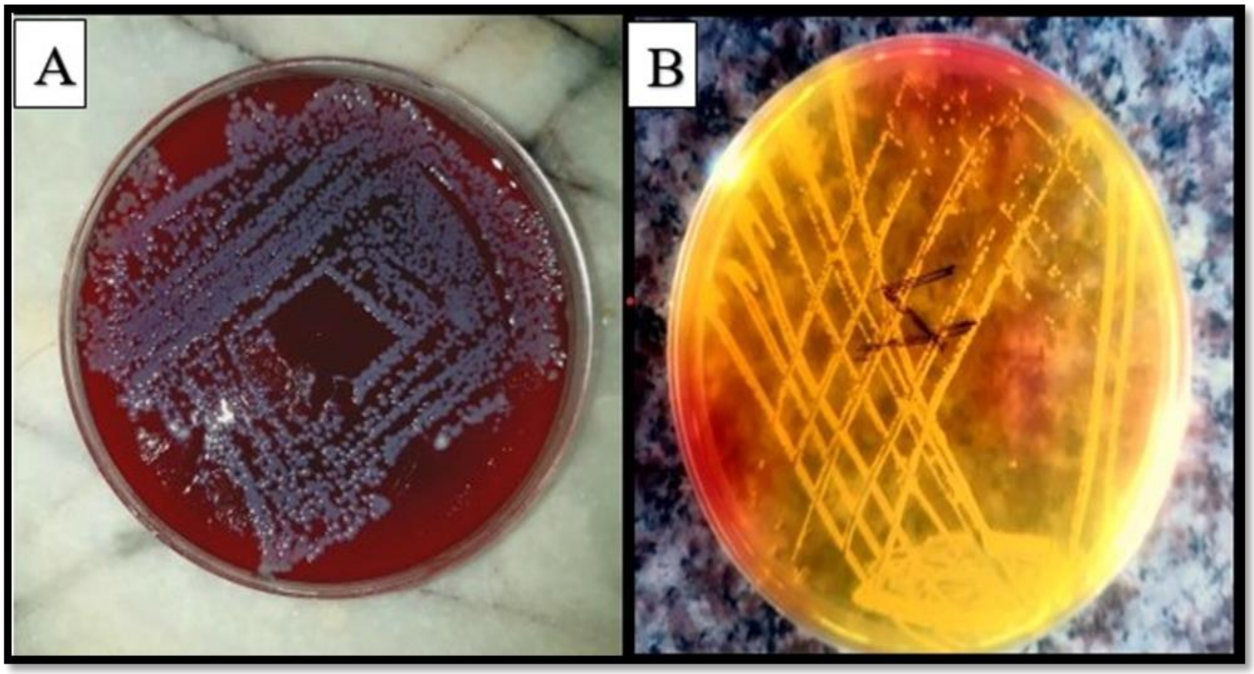


Figure (4.1). ***S. aureus*** **colonies on (A) Blood agar and (B) mannitol salt agar** (A show gray to white colonies with clear zoon (β- hemolysis) around the colony on blood agar, B show yellow colonies on mannitol salt agar with surrounding yellow medium**)**

4.2.1. Detection of *Staphylococcus aureus* by Biochemical Methods

The gram stain films of these isolating cultures revealed single or bi arrangement. All *Staphylococcus aureus* isolates have shown a positive result in biochemical tests for catalase tests, Coagulase, clumping factor hemolysis test and mannitol fermentation while negative results to oxidase test as shown in table (4-7).

Table (4-7): Biochemical tests for identification of isolates

Test	Results
Catalase	Babbles (+)
Oxidase	No Purple color (-)
Coagulase	(+)
Clumping factor	(+)
hemolysis test	(+)
mannitol fermentation	(+)

Abbreviations: (+), positive test; (-), Negative test

Staphylococcus is positive catalase, Coagulase tests and negative oxidase (Appendix Figures 2,3). In addition, for diagnosis using the Vitek 2 compact system (Appendix Figures 5,6,7) final by specific primer for 16S rRNA gene of *Staphylococcus spp*.

4.2.2. Detection of Methicillin-Resistance *S. aureus* on MRSA Chromogenic Agar Base

The MRSA chromogenic agar base is used to detect methicillin-resistant *Staphylococcus aureus*. Alpha-glucosidase produced by *Staphylococcus aureus* cleaves the chromogenic substrate and gives a blue color to the *Staphylococcus aureus* colony figure (4-2). Cefoxitin inhibits the growth of methicillin-sensitive *Staphylococcus aureus* (Gurran et al., 2002).

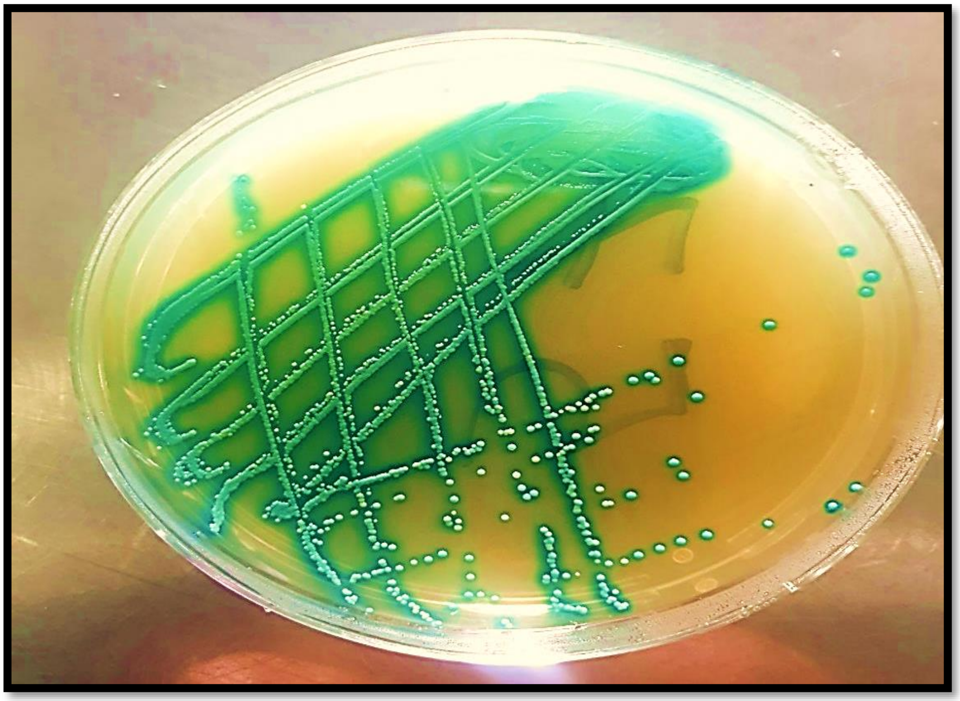
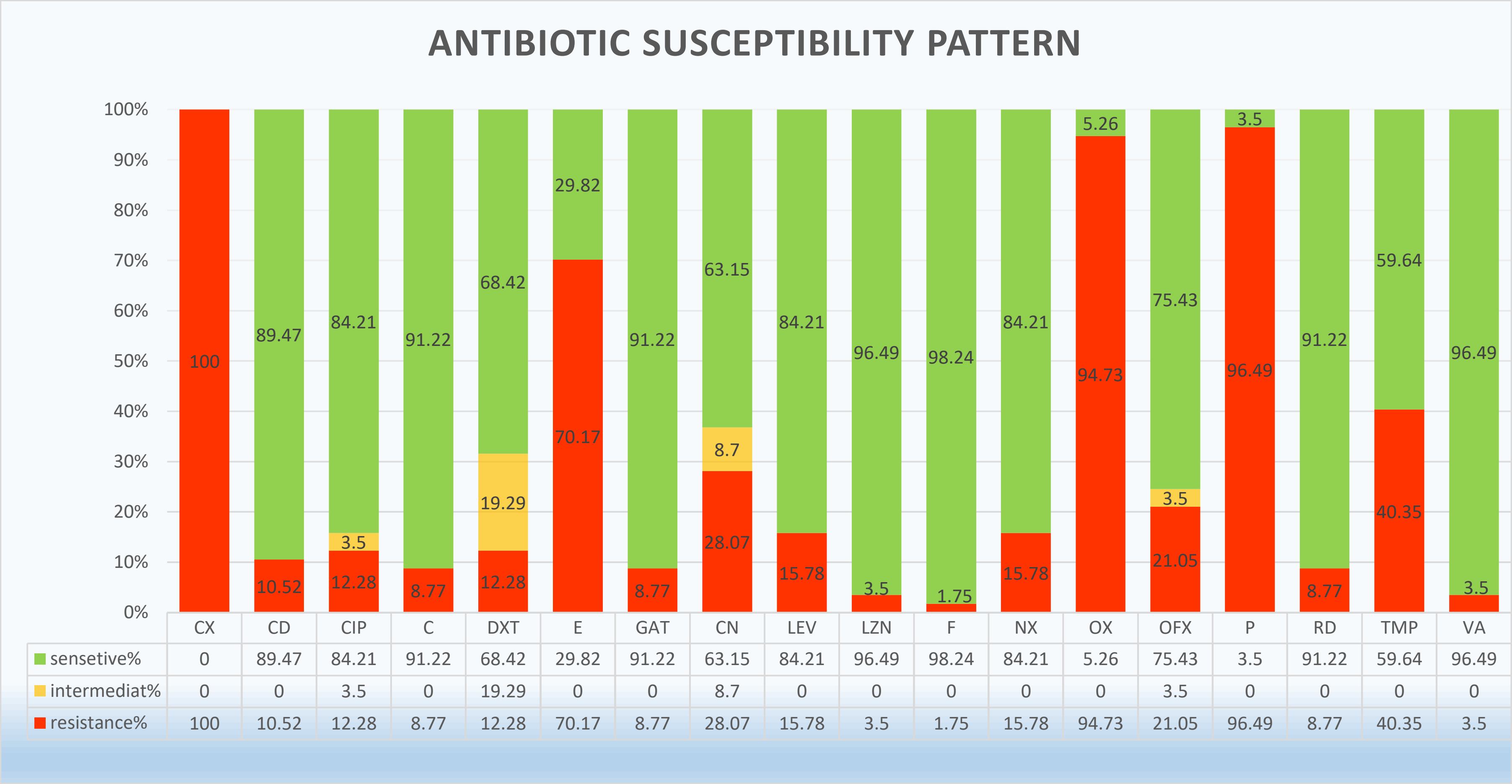


Figure (4.2): *S. aureus* colonies on MRSA Chromogenic Agar Base (The alpha-glucosidase produced by Staphylococcus aureus cleaves the chromogenic substrate and gives a blue color to the Staphylococcus aureus colony).

The results for bacterial isolates are confirm by a cefoxitin susceptibility test, which is 100% complete resistance to cefoxitin to determine methicillin resistance by disc diffusion method based on CLSI 2021. The results of our study shows that all isolates are resistant to methicillin (MRSA). The high prevalence and an increase in distribution of MRSA strains among ocular infections is serious and alarming and may reflect antibiotic overuse. It remains an important pathogen causing nosocomial and community infections. It has also become a principal cause of clinically relevant eye infections which develops mainly in patients within the community and can lead to severe vision loss and poor outcomes for patients, MRSA colonization is prevalent with a high rate of MDR. In addition, the capacity of *S. aureus* to arise resistance to multiple antibiotic classes increase the expansion of epidemic posing a serious public health threat. Methicillin-resistant in staphylococci will influence treatment regimens. we found a high rate of resistance to macrolides, Cephalosporin and penicillin by bacterial isolates. This has an important impact on the choice of empirical therapies in patients with ocular infections. This result is agreed with many others Studies show increased resistance to methicillin-resistant *Staphylococcus aureus* (Enright *et al*., 2002; Stevens, 2007; Kolar *et al*., 2011).The present study are disagreed with study (Alshamahi *et al*., 2020)was found that The prevalence of MRSA was 34.1% and (Petrillo *et al*., 2021) that found among the *S. aureus* strains, 23.7% had an MR-phenotype (MRSA).

4.3. Antibiotic Susceptibility Test

It is shown in figure (4-3), 57 identified *Staphylococcus aureus* isolates (1 to 57) are evaluate against 18 common antibiotics. The majority of isolates exhibited antibiotic resistance, particularly β-lactamase (Oxacillin, Penicillin G) and cephalosporin’s (Cefoxitin) antibiotics. The low percentage of resistance is to Nitrofurantoin 1.75%, Linezolid and Vancomycin 3.50% .



	CX	CD	CIP	C	DXT	E	GAT	CN	LEV	LZN	F	NX	OX	OFX	P	RD	TMP	VA
🟩 sensetive%	0	89.47	84.21	91.22	68.42	29.82	91.22	63.15	84.21	96.49	98.24	84.21	5.26	75.43	3.5	91.22	59.64	96.49
🟨 intermediat%	0	0	3.5	0	19.29	0	0	8.7	0	0	0	0	0	3.5	0	0	0	0
🟥 resistance%	100	10.52	12.28	8.77	12.28	70.17	8.77	28.07	15.78	3.5	1.75	15.78	94.73	21.05	96.49	8.77	40.35	3.5

Figure (4.3): Antibiotic susceptibility pattern ,abbreviations: [Cefoxitin (CX), Clindamycin (CD), Ciprofloxacin (CIP), Chloramphenicol (C), Doxycycline (DXT), Erythromycin (E), Gatifloxacin (GAT), Gentamicin (CN), levofloxacin (LEV), Linezolid (LZN), Nitrofurantoin (F), Norfloxacin (NX), Oxacillin (OX), Ofloxacin(OFX), Pencillin G (P), Rifampin (RD), Trimethoprim (TMP) and Vancomycin (VA)].

we are found that all 57 isolates of *Staphylococcus aureus* test are resistant to Cefoxitin., 55 isolates are resistance to Pencillin G, 54 isolates are resistance to Oxacillin whereas only one isolate of *Staphylococcus aureus* is resistance to Nitrofurantoin, 2 isolates of *Staphylococcus aureus* are resistance to Linezolid and Vancomycin as shown in (Appendix table 1 and figure 4,8,9,10).

Antibiotics are natural products produced by some microorganisms to combat the growth of other microbes (Walsh and Wencewicz, 2016). Antimicrobial resistance, or the ineffectiveness of widely used drugs/antibiotics against specific bacteria, has emerged as the most serious public health issue and a major problem for global drug development efforts. The increasing antibiotic resistance worldwide has been attributed to their widespread systemic use, their over-the-counter availability and patients can access them easily with low price, and their inappropriate use (Spengler *et al.,* 2017). The most used antibiotic classes for eye infection treatment are penicillins, aminoglycosides, fluoroquinolones, tetracyclines, macrolides, phenicols and sulfonamides, in ocular infections factors such as empirical prescription, shortterm exposure, and repeated exposure of antibiotics contributed to the resistance of ocular pathogens (Steinert, 1991).

The *S. aureus* isolates are resist to Oxacillin 54 (94.73%), these findings are close to local studies related to MRSA isolates by Al-Taey, (2021) who found that (100%) of MRSA isolates in Baghdad hospitals resisted Oxacillin. Cefoxitin is resistant to 57(100%) that is agreed with local study of AL-Zengena e*t al.,* (2020) who found the resisted of cefoxitin is (100%), this might be related to the frequent use of this antibiotics, however, they showed high resistance for penicillin's class and erythromycin, among 57 *S. aureus*, 55 (96.49%) and 40 (70.17%) are resistant to Pencillin G and erythromycin respectively, this agree with study Mohammed *et al*., (2020) who found among 74 *S. aureus*, 69 (93.2%) and 57 (77%) are resistant to penicillin and tetracycline respectively. Other study found that among MRSA isolates, cumulative resistance is high for penicillins class (penicillin and oxacillin, 97.8%, respectively), 71.1% for erythromycin (Zheng *et al*., 2021).

The results show that the resistance rate of MRSA isolates to Vancomycin is 2(3.5%). The result of the present study agreed with a study done by Salman and Ali, (2017) who mentioned that the complete low resistance to Vancomycin is (9.1%) in hospitalized patients in Baquba teaching hospital. The establishment and subsequent spread of Vancomycin resistance is seen as a problematic scenario that adds to the difficulty of treating MRSA and most Vancomycin be reserved to treat isolates that are resistant to other antibiotics.

A study performed in a hospital in Al-Najaf, Iraq A. N. Al-Khafaji, (2018) showed that the resistance rate of S. aureus to Gentamycin (10%). Aminoglycoside resistance in S. aureus is mediated by enzymes that alter aminoglycoside. These enzymes can deactivate aminoglycosides by phosphorylating, acetylating, or adenylating hydroxyl groups or amino groups in the antibiotics' structure. Many enzymes that change aminoglycosides have been found, and these enzymes are encoded by plasmid genes and transposons, including those that convey resistance to other antibiotic classes (Ramirez and Tolmasky, 2010). Our study shows that the resistance rate of isolates to Gentamycin (28.07%), and Gentamycin remains the drug of choice in treatment of eye infection.

In the current study, one classes inhibition DNA synthesis contain two types of antibiotics. The resistance of *S. aureus* isolates to ciprofloxacin and norfloxacin is (12.28 %and 15.78%) respectively. In another local study conducted by Saber and Kandala, (2018) who monitored in their study in Baghdad hospitals that the resistance rate of Ciprofloxacin is (37.19%). The availability of a wide range of fluoroquinolone medications namely Ciprofloxacin, allowed for the efficient treatment of infections caused by *S. aureus* strains, these strains quickly developed resistance to these drugs (Pourmand *et al.*, 2014). But it is still low and this may be explained by the fact that local isolates didn't develop high resistance to this antibiotic due to its limited use in comparison with Penicillin and also because Ciprofloxacin is a broad spectrum antibiotic.

The result on the sensitivity of *S. aureus* to Ciprofloxacin are close to the results of Hameed, (2020) who found that the resistance of these bacteria is low to the antibiotic Prescott, (2002), in his study, also found that ciprofloxacin is very active against *S. aureus* strains, because this antibiotic inhibits bacterial growth through the inhibition of DNA gyrase enzyme which is responsible for spiral recoiling of DNA and insures their being apart during transcription process.

Another study found that among MRSA isolates, cumulative resistance is high for Clindamycin (71.1%), which is inconsistent with the results of our study, which is 10.52% for Clindamycin. It is also found that only a small proportion are resistant to Nitrofurantoin (9.1%), Ciprofloxacin (7.1%), Rifampin (2.2%), and Levofloxacin-class fluoroquinolones (2.8%). (Zheng *et al*., 2021). Our result is also approximately similar to it which is Nitrofurantoin (1.75%), Ciprofloxacin (12.28%), Rifampin (8.77%), and Levofloxacin (15.78), Nitrofurantoin continues to provide a low level of resistance in this study with extensive coverage of the empirical treatment, is the most effective antibiotic on the studied bacteria.

Levofloxacin, one of the most commonly used eye drops for pediatrics in China, is found to be more sensitive to ocular MRSA infections than in the United States. An increase in the resistance of ciprofloxacin and levofloxacin with age might be one of the reasons for the difference between pediatrics and the subjects with a broader age range in China. we recommend that prescription of quinolones such as levofloxacin should be cautious to avoid development of resistance from indiscriminate use this agree with (Daum ,2009).

All isolates are tested to chloramphenicol and Doxycycline, the results showed that the resistance rate for chloramphenicol (8.77%) and Doxycycline (12.28%) The result of the present study agreed with a study done by Petrillo *et al*., (2021) found all isolates are susceptible to chloramphenicol (100%) topical chloramphenicol is an excellent first line choice of therapy for suspected or proven MRSA.

Other study found that The results of antibiotic susceptibility shows that *S. aureus* isolates are susceptible to Doxycycline 21(84%) then Chloramphenicol 20(80%) (Khalil *et al*., 2017).The susceptibility to the other antibiotics decreased in the following order: gentamicin (98%), chloramphenicol (76%), oxacillin (74%) and ciprofloxacin (46%), most of the microbial keratitis strains from Australia (isolated between 2006 and 2018) are more commonly susceptible to ciprofloxacin (57%) and oxacillin (93%) compared to microbial keratitis strains from the USA (isolated in 2004) for ciprofloxacin (11%; $p = 0.04$) and oxacillin (11%; $p = 0.02$). (Afzal *et al*., 2021). The results reveals that isolates sensitive 41% to chloramphenicol, 26% sensitive to gentamicin, 15% sensitive to erythromycin, 13% sensitive to ciprofloxacin (Baz *et al*., 2021).

It’s possible that the great prevalence of resistance to the antibiotics mentioned is attributable to their widespread usage in the treatment of human diseases. This suggests that these antibiotics are no longer effective as an empirical treatment for *S. aureus* infections in the research field. The low activity of these antibiotics could be related to earlier exposure to these drugs that would have accelerated the development of resistance. The rise in antibiotic abuse in our region, which stems from self-medication, failure to react to care, and antibiotic-sale actions, can bolster this assertion (Baz *et al*., 2021).

The present study reveals significant differences between multi-drug resistant (MDR) and Non- multi-drug resistant (Non-MDR) isolates where 45(79%) of isolates are MDR while the remaining isolates (21%) are Non-MDR as presented in table (4-8). Multiple antibiotic resistant *Staphylococcus aureus* is one of the common causes of severe nosocomial infections, and the gastrointestinal tract is an important source of its transmission (Onanuga and Temedie, 2011). Most strains (79%; 45/57) are multidrug-resistant (MDR), which is defined as being resistant to three different classes of antibiotics (Zhang *et al*., 2011).

Table (4-8): Percentages of multi-drug resistant (MDR) and Non- multi-drug resistant (Non-MDR) isolates

MDR		**Non-MDR**		**P. value**
No.	%	No.	%	
45	79	12	21	0.000

The chi-square test shows that the prevalence of MDR is significantly higher than Non-MDR in isolates. Previous studies reported a similar high prevalence of multiple antibiotic resistance in *S. aureus* (Onanuga *et al*.,2005; Jayaraman *et al*.,2008). Strains 111, 112, and 113 from the USA (microbial keratitis; isolated in 2004) and M43-01 from the Australian (microbial keratitis; isolated in 2018) group are extensively drug resistant (XDR) strains, which is defined as resistant to almost all antibiotics classes (Khan *et al*., 2020). The susceptibility of *S. aureus* ocular infection strains varied by origin of isolates and their prior exposure to antibiotics, with being more likely to be MRSA and multidrug resistant.

4.4. Detection of Biofilm Formation by *Staphylococcus aureus* Using the Tissue Culture Plate Method (TCP) Assay

In this study, 57 isolates are tested using the tissue culture plate method (TCP) assay, which Christensen *et al*. (1985) describe as a semi-quantitative microtiter plate test biofilm assay. The interpretation of biofilm formation is done according to the Stepanovic *et al.* (2007) criteria presented in table (3-7).

The results show that out of 57 isolates 43(75%) are produce biofilm, only 6 (10%) are strong positive in which that formed biofilm, moderate positive 22 (39%), weakly positive 15 (26%) and negative 14 (25 %) as shown in (appendix table 2) and figure (4.4). Długaszewska et al., (2016) found that biofilms protect against antimicrobial agents very well. Biofilm-forming ability has been increasingly recognized as an important virulence factor in Staphylococci, facilitating their persistence in the host, evading its defenses and allowing bacterial survival at high antimicrobial concentrations. Staphylococcus aureus remains a major pathogen. (Oliveira *et al*., 2006).

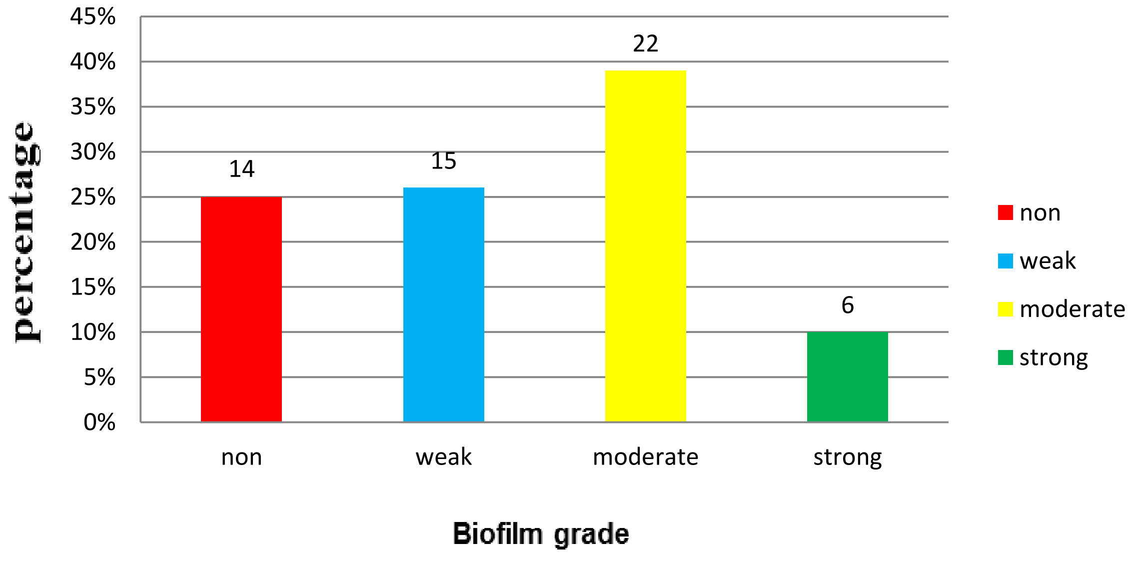


Figure (4.4): Biofilm formation grade in *S.aureus* isolated from ocular disease

The discrepancy in results between different studies may be attributed to many factors such as the different countries from which the specimens are collect, the number and the type of clinical specimens from which the isolates are obtain and also the differences in isolates capability to form biofilm. The primary number of cells that succeeded in adherence and the differences of quality and quantity of auto inducers (quorum sensing signaling molecules) that are produced from each isolate may also play an essential and an important role (Haji, 2018).

The present study shows that all biofilm grade producer isolates (moderate, strong) are multi-drug resistant (MDR) (100%), while only 47% and 71% is multi-drug resistant (MDR) for weak and non-biofilm producer isolates respectively as shown in table (4-9).

Table (4-9): correlation between biofilm formation and multi-drug resistant

Biofilm Grade	Percentage of Biofilm Grade		MDR	
	No	%	No	%
non	14	25%	10	71%
weak	15	26%	7	47%
moderate	22	39%	22	100%
strong	6	10%	6	100%

The TCP assay is described in the literature as a simple and rapid method to quantify biofilm formation of different bacterial strains. Crystal violet is a basic dye known to bind to negatively charged molecules on the cell surface as well as nucleic acids and polysaccharides, and therefore gives an overall measure of the whole biofilm. It has been used as a standard technique for rapidly accessing cell attachment and biofilm formation in a range of Gram positive and Gram-negative bacteria (Al-Dahmoshi, 2013).

This study shows significant difference ($p<0.05$) between biofilm grade and antibiotics resistance where the almost isolates are resistance to Cefoxitin, Oxacillin and Pencillin G in all biofilm grades, while the lower resistance were to the Linezolid and Nitrofurantoin, the isolate that produce biofilm in strong manner are resistance to almost antibiotics as shown in (appendix table 3).

There are two key reasons why the use of traditional antibiotic therapy makes biofilm bacteria hard to eliminate. Biofilm polysaccharide which is also referred to as slime, is a polymeric conglomeration generally composed of proteins and polysaccharides (Hall-Stoodley *et al.,* 2004). Biofilm formation provides bacteria with a means of persistently colonizing either living or inert surfaces within a human host (Murray *et al.,* 2010). Another reason is that the biofilm bacteria are either slow-growing or non-growing. Some antibiotics, like β-lactams, require rapid bacterial growth to kill cells (Kaplan *et al*., 2012).

Our studies also confirm that cells in the biofilm phase are several fold more resistant to antibiotics, a phenomenon associated with biofilm formation in bacteria, Earlier studies had indicated that several ocular bacteria including *S. epidermidis, S. aureus* and *Streptococcus spp*. form biofilms and majority of them are resistant to antibiotics, the MIC of the antibiotic in the biofilm phase is significantly greater than that required for killing the cells in the planktonic phase. This increase in MIC in the biofilm phase could be attributed to: inefficient penetration of the drug into the biofilm , inability of the drug to exerts its effect within the biofilm , transformation of the microorganisms in the biofilm into viable-but-nonculturable state , emergence of persisted cells which are resistant to drugs , ability to survive under nutrient and oxygen limitation conditions and up-regulation of drug resistance-associated genes, ability of extracellular polymeric substances (EPS) to limit diffusion of aminoglycosides (Konduri *et al*., 2021). Ability of EPS to inactivate antibiotics (Billings *et al*., 2015). Acquiring resistance to phagocytosis and induction of LPS modification genes (Wilton *et al*., 2016).

4.5. Detection of *Staphylococcus aureus* by 16S rRNA

The results of amplification of 16S rRNA of *Staphylococcus aureus* revealed that all 57 isolates give +ve result for this gene as shown in figure (4-5). This result is with (Al-Khafaji *et al*. 2019), demonstrated that the amplification of 16S rRNA of *Staphylococcus spp*. (in which all twenty-five isolates give +ve result for this gene).

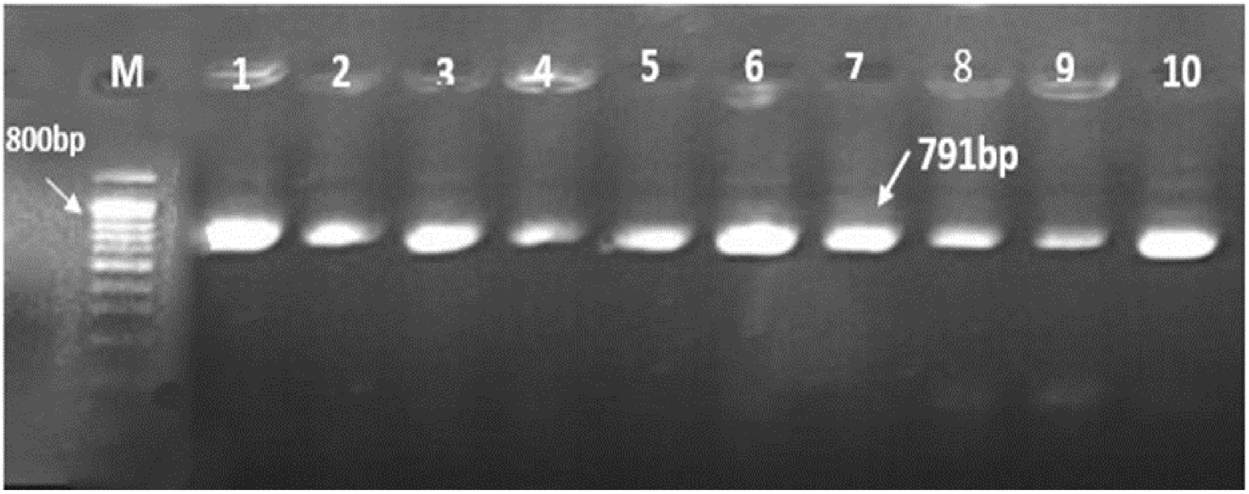


Figure (4.5): Sample of the 16S rRNA gene product of *S. aureus* (1.5%agarose gel electrophoresis at 72 volts for 80minutes in TBE of PCR products (791 bp) after amplification of 16S rRNA gene of *S. aureus*. M lane represent 100 bp DNA Marker while the rest lanes represents positive samples for gene).

4.6. Detection of Enterotoxin Genes of *Staphylococcus aureus* Isolates

Regarding to staphylococcal enterotoxin genes; PCR detecting sequences of the genes are positive, 24(42%) isolates carry *Sea* gene, 43 (75%) isolates carry *Seb* gene, 38 (67 %) isolates carry *Sec* gene, 35 (61 %) isolates carry *Sed* gene, 18 (32 %) isolates carry *See* gene as shown in figures (4.6 to 4.10) and table (4-10).

Table (4-10): percentage of *Sea, Seb, Sec, Sed* and *See* genes of enterotoxins in isolates of *S. aureus*

Genes	positive		negative		Total	P value
	No	%	No	%		
Sea	24	42	33	58	57(100%)	**0.000**
Seb	43	75	14	25	57(100%)	**0.000**
Sec	38	67	19	33	57(100%)	**0.000**
Sed	35	61	22	39	57(100%)	**0.000**
See	18	32	39	68	57(100%)	**0.000**

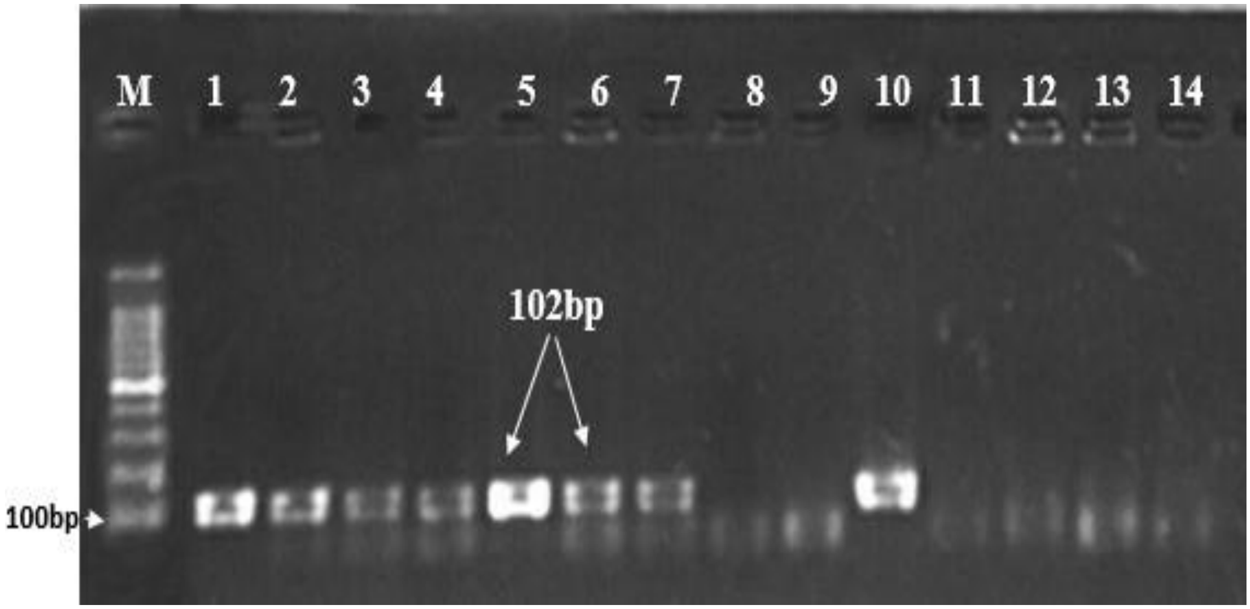


Figure (4.6): Sample of the *Sea* gene gene product of *S. aureus* (1.5% agarose in TBE of PCR products (102 bp) after amplification of *Sea* gene of *S. aureus*. M lane represent 100 bp DNA Marker, the (8-9-11-12-13-14) represents some of negative samples for this gene. While the rest lanes represent some of positive samples for this gene).

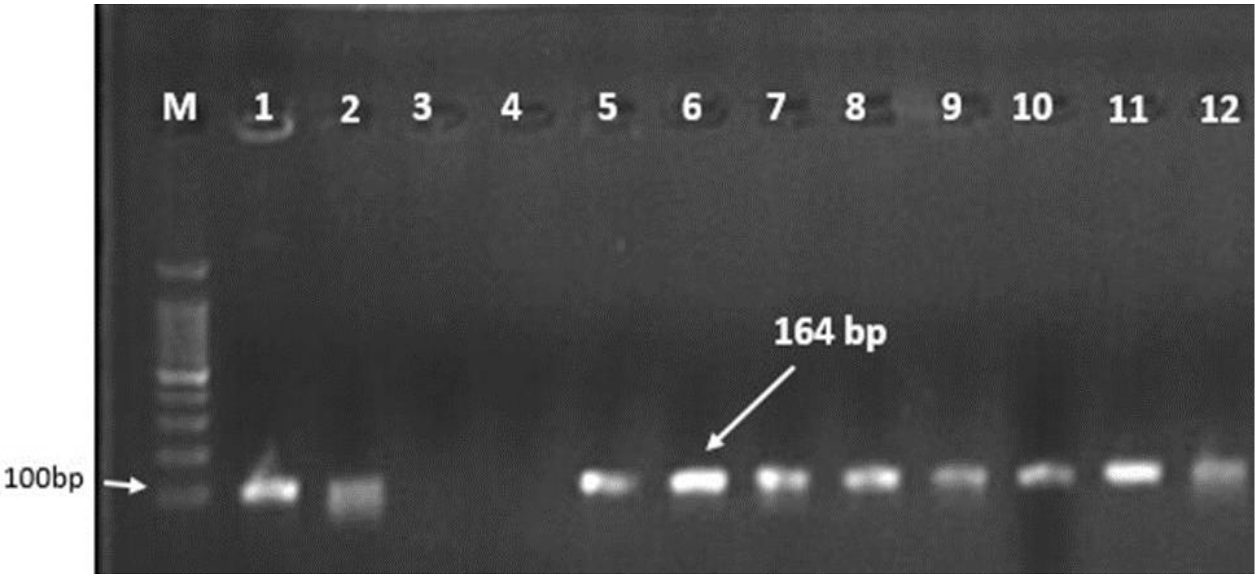


Figure (4.7): Sample of the *Seb*gene gene product of *S. aureus* (1. 5% agarose in TBE of PCR products (164 bp) after amplification of *Seb* gene of *S. aureus*. M lane represent 100 bp DNA Marker, the (3-4) represents some of negative samples for this gene. While the rest lanes represent some of positive samples for this gene).

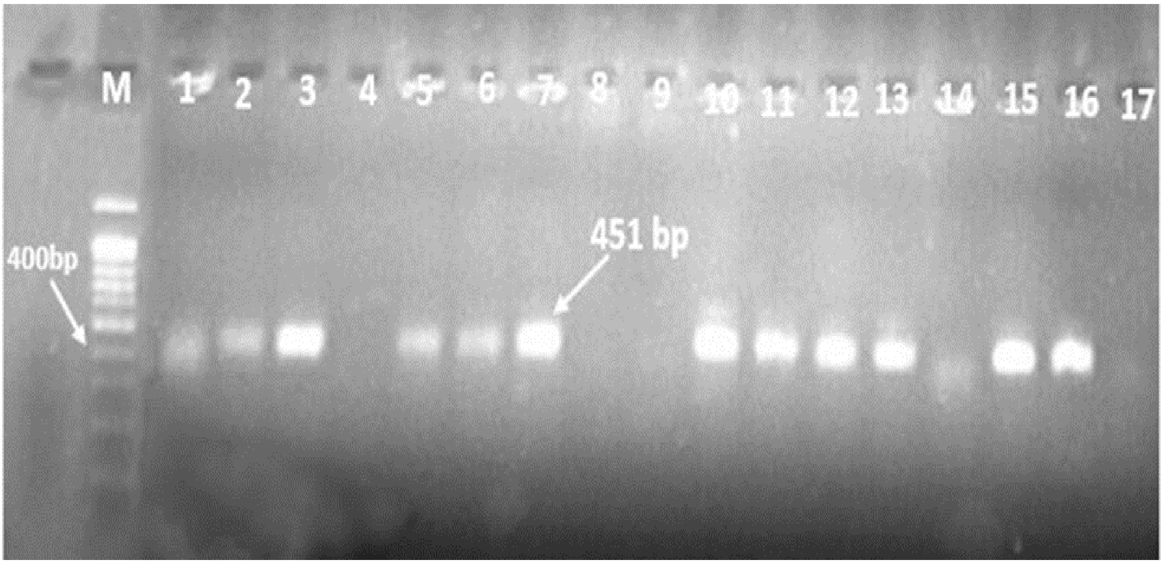


Figure (4.8): Sample of the *Sec* gene gene product of *S. aureus* 1.5% agarose in TBE of PCR products (451 bp) after amplification of *Sec* gene of *S. aureus*. M lane represent 100 bp DNA Marker, the (4-8-9-14-17) represents some of negative samples for this gene. While the rest lanes represent some of positive samples for this gene.

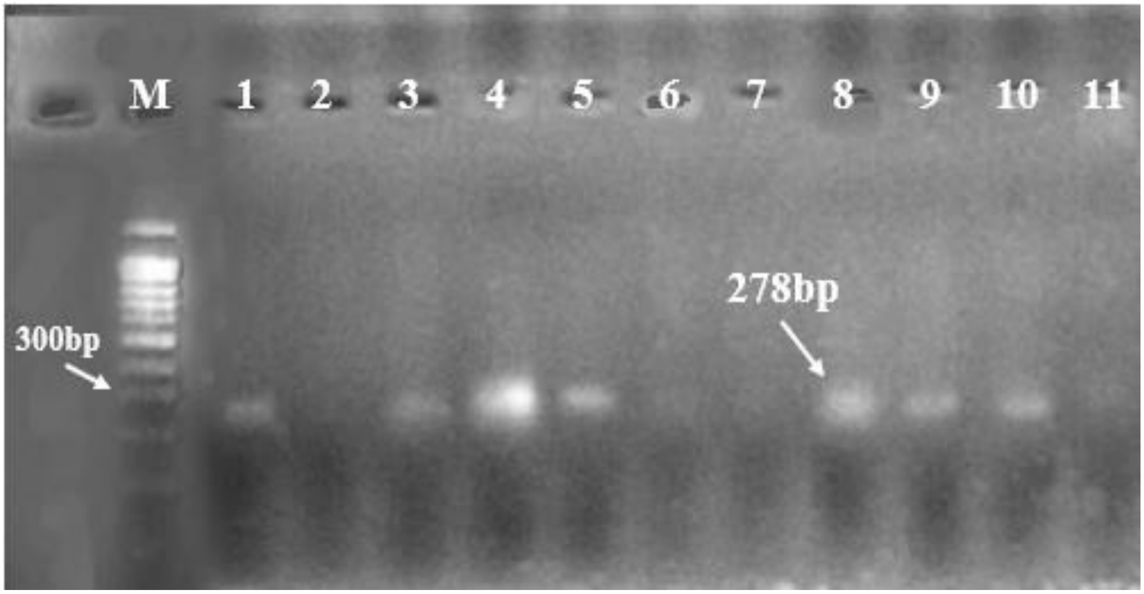


Figure (4.9): Sample of the *Sed* gene gene product of *S. aureus* (1.5% agarose in TBE of PCR products (278bp) after amplification of *Sed* gene of *S. aureus*. M lane represent 100 bp DNA Marker, the (2-6-7-11) represents some of negative samples for this gene. while the rest lanes represent some of positive samples for this gene).

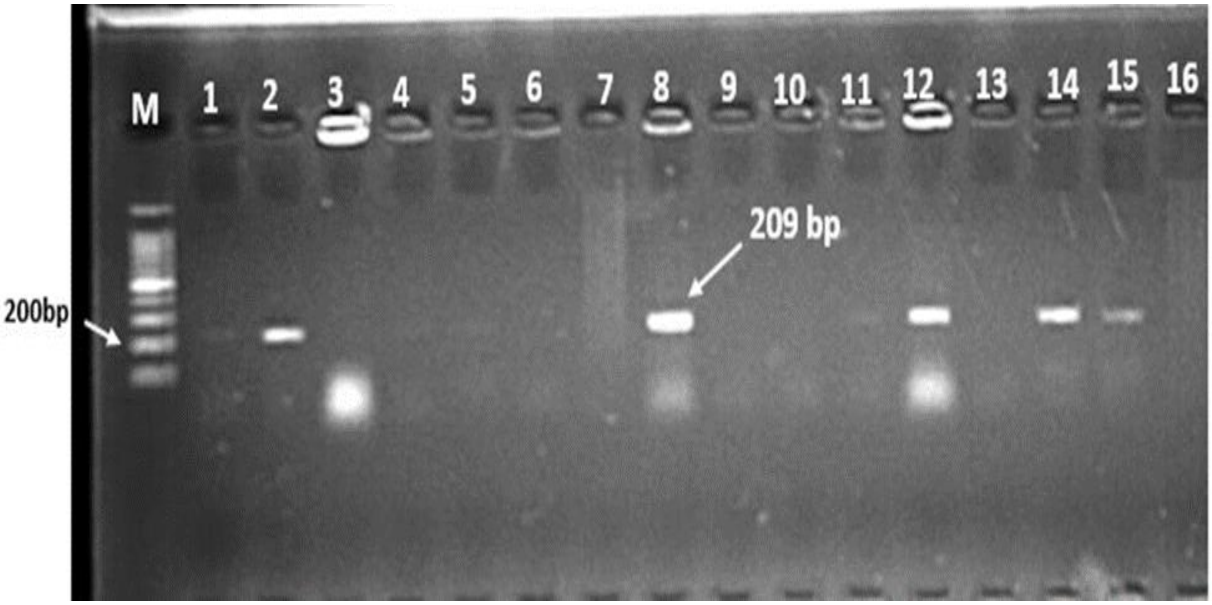


Figure (4.10): Sample of the *See* gene gene product of *S. aureus* (1.5% agarose in TBE of PCR products (209 bp) after amplification of See gene of *S. aureus*. M lane represent 100 bp DNA Marker while the (2-8-12-14-15) represents some of positive samples for this gene. while the rest lanes represent some of negative samples for this gene).

The 57 isolates of *S. aureus* tested, 50 (88%) are positive for one or more SEs genes, and 18 different genotypes are observed) as shown in table (4-11). Among the 57 *S. aureus* isolates, 6 isolates (10%) harbored only one enterotoxin gene (*Seb*, *Sec*, *Sed*), 7 isolates (12%) carried gene coding for two enterotoxins (*Sea*+*Seb* , *Seb*+*Sec* , *Seb* + *Sed* , *Sec*+*Sed* , *Sed*+*See*), 16 isolates (28%) carried gene coding for three enterotoxins (*Sea*+*Seb*+*Sec* , *Sea*+*Seb*+*Sed* , *Seb*+*Sec*+*Sed* , *Seb+Sec+See* ,*Sea+Sec+Sed,Seb+Sed+See*), 15 isolates (26%) carried gene coding for four enterotoxins(*Sea*+*Seb*+*Sec*+*Sed* , *Sea*+*Seb*+*Sec*+*See* , *Seb*+*Sec*+*Sed*+*See*). Genotypes encoding five enterotoxins (*Sea*+*Seb*+*Sec*+*Sed*+*See*) are detects in 6 isolates (11%). Genes encoding the enterotoxins Sea and See are not observed separately. Among the genes that code for classic enterotoxins (*Sea*-*See*), Seb is the most frequent, it is found in 43 isolates (75%) followed by *Sec* in 38 isolates (67 %) and *Sed* in 35 (61 %) isolate.

The result is consistent with the study that found that out of 52 *S. aureus* isolates tested, 42 (80.7%) are positive for one or more *Ses* genes, and 12 different genotypes are observed, but it certainly agrees with that finding that Sea is the most common, found in 16 isolates (30.7%) followed by *Seb* in 14 isolates (26.9%) and *Sed* in 8 isolates (15.37%) (Nazari *et al*., 2014). Another study is conducted by Nasaj *et al*., (2020) found. The *Sec* gene is the most frequent, which is detect in 48.4% of the isolates, followed by *Seb* in 27.5%, *See* in 12.1%, *Sed* in 3.3%, *Sea* in 2.2%. The *Sec* gene is the most common classic enterotoxin-encoding gene among all enterotoxin genes.

Table (4-11): Pattern of the gene (*Sea, Seb, Sec, Sed,* and *See*) groups of *S. aureus* isolates.

genotype	No.	total of genotypes	%
Seb	3	6	10
Sec	1		
Sed	2		
Sea,Seb	1	7	12
Seb,Sec	2		
Seb , Sed	1		
Sec, Sed	2		
Sed , See	1		
Sea,Seb,Sec	4	16	28
Sea,Seb,Sed	3		
Sea,Sec,Sed	1		
Seb,Sec, Sed	4		
Seb,Sec ,See	2		
Seb,Sed,See	1		
Sea,Seb,Sec, Sed	7	15	26
Sea,Seb,Sec, See	1		
Seb,Sec, Sed,See	6		
Sea,Seb,Sec, Sed, and *See*	6	6	11

The eyelid, tear duct, and conjunctiva are in contact with the tear film that contains multiple soluble factors able to protect against bacterial infection, but *S. aureus* infections of these sites are commonly encountered among the general population. It is known that *Staphylococcus aureus* encode a sizable and diverse arsenal of virulence factors that enable this organism to exploit a variety of environmental niches. Enterotoxin: are considered immunomodulators/ superantigens (SAgs) due to their ability to bind major histocompatibility complex class II (MHCII) molecules leading to widespread, non-specific T-cell activation and subsequent massive cytokine release (Spaulding *et al*., 2013).

immune response and the action of bacterial toxins can cause considerable tissue damage resulting in scarring that reduces visual acuity. Likewise, infections of the inner eye involve a potent host response that together with bacterial toxins can damage tissues critical to vision, especially the retina. The cornea is well protected from surface infections and its immune response is designed to provide an even greater degree of protection. Bacteria escape the bulk of immune defenses, allowing a variety of secreted toxins to directly damage tissues and stimulate a harmful immune response. The inflammatory process is also driven by innate and adaptive immunity directed toward eliminating the infectious agent. Such an immune process can prolong ocular inflammation and cause tissue damage as noted in tuberculous uveitis (Booth *et al*., 1997).

the role of enterotoxins in promoting *S. aureus* infections may be variable, depending on the specific disease setting, while enterotoxins are clearly not essential for ocular tissue infections, given their disproportionate prevalence among ocular isolates, they may provide a competitive advantage. Given the location of these genes on mobile genetic elements, horizontal gene transfer could facilitate the acquisition of enterotoxins (Fujishima *et al*., 2012).

The toxins of *S. aureus* appear to have robust cytotoxic actions on ocular tissues by altering the permeability of cell membranes, toxins can kill or manipulate the functions of the immune cells, and disrupt epithelial barriers to promote bacterial growth and spreading. By interacting with various cell surface proteins, toxins are able to target diverse cell types—mostly cells of the immune system which only express those receptors and which increases the severity of the disease Additionally, Enterotoxin B (Seb) has been shown to be toxic to corneal epithelial cells as well as induce changes in cytokine expression in an in vitro cell culture model. Furthermore, treatment with Staphylococcal Enterotoxin B has been used to suppress immune rejection during murine corneal transplantation potentially due to its effects on T-cell depletion and host non-specific tolerance (Hassenstein and Meyer, 2009).

The low prevalence of some *Ses* genes including *See* in this study could be due to the theory that indicated enterotoxin-like (SEl) toxin genes are more abundant among commensal strains as compared to pathogenic ones (Rahimi and Shokoohizadeh, 2018). Other studies also showed significantly higher rates of the occurrence of *Sea*, *Seb*, *Sec*, *Sed*, *See*, *Seg*, *Seh*, *Sei* and *Sej* genes in strains of *Staphylococcus* coagulase-positive (CoPS) and negative isolated from coalho cheese, and detected the presence of the following genes: *Seh* (53.2%) in CoPS strains and *Sec* (46.8%) in CoNS strains (Andrade *et al*.,2019).

In order to further understand the distribution of these enterotoxin across the ocular isolate, we sought to determine if there is an association between this virulence factors that may promote infection, thereby deepening our understanding of this important human pathogen, capable of causing damage to tissues and organs, as well as modulating the immune response to these infections. Unfortunately, in the context of ocular infections, this can mean blindness for the patient

A high prevalence of *Ses* genes is observed in the present study (88%), the most common *Ses* genes are *Seb*, *Sec* and *Sed* which accounted for (75%; 43/57), (67%; 38/57), and (61%; 35/57), respectively. It is dissimilar to another study that showed a high prevalence of Ses. genes (52%), the most common *Ses* genes are *Sed*, *Sec*, and *Sea* which accounted for (20%; 15/75), (16%; 12/75), and (16%; 12/75), respectively. (Goudarzi *et al*., 2020). Other Turkish study also indicated a high prevalence of *Ses* genes (62.6%) and the *Sec* and *sed* genes are the most abundant toxin genes in clinical *S. aureus* isolates (Aydin *et al*., 2011).

Similarly, *Seb*, *Sec*, and *Sed* are the predominant *Ses* gene detected in the current study. Similar to the previous reports from different countries (Monecke *et al*., 2011). Other Chinese study, indicated that *Ses* genes detect in MSSA strains are including *Sea* (31%), *Sec* (21.1%), *Seb and Sed* (9.9%). (Yu *et al*., 2015). A total of 87 *S. aureus* isolates are collect from the external body surface and the internal gut content. Sixty-two isolates (71%) of *S. aureus* produced staphylococcal enterotoxins (SE). Majority of the enterotoxin producers are of *Seb* (45%). The result indicated that 26%, 5%, 8%, 10% and 6% of isolates are *Sea*, *Sec*, *Sed*, *Sea* + *Seb*, and *Sea* + *Sec* producer respectively (Rashki Ghalehnoo, 2015).

This study reveals that there is a harmony between results of antibiotic resistance and positive molecular detection of these genes. The highest resistance rate to Cefoxitin, Oxacillin, Pencillin G and Erythromycin is observed in the isolates of *Sea*,*Seb*,*Sec*,*Sed* and *See* genes, whereas the low resistance rate to Linezolid, Nitrofurantoin is observed in the isolates of *Sea*,*Seb and Sec* genes, A total of 50(88%) enterotoxigenic *S. aureus* and their antimicrobial resistance pattern tested analysed isolate showed antimicrobial resistance properties at least at one of antibiotics tested. The results provided evidence that the presence of enterotoxigenic and antimicrobial resistant strains of *S. aureus* has become remarkably widespread.

Most isolates carried *Sea*, *Seb*, and *Sec* genes, is the most frequent enterotoxin profile and exhibited resistance to at least one antibiotic. High resistance to Cefoxitin, Oxacillin and penicillin G; low resistance to Chloramphenicol, rifampin, and Gatifloxacin; and very low resistance to Linezolid, Nitrofurantoin, and Vancomycin are exhibited by *S. aureus*. In addition, some antibiotic resistance exhibited a strong correlation with enterotoxigenicity in *S. aureus*. as shown in table (4.12).

Table (4-12): Pattern of enterotoxin genes and antibiotics resistances of *S. aureus*

antibiotics	*Sea*		*Seb*		*Sec*		*Sed*		*See*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Cefoxitin	24	100	43	100	38	100	35	100	18	100
Clindamycin	3	13	4	9	4	11	2	6	1	6
Ciprofloxacin	6	25	7	16	7	18	5	14	3	17
Chloramphenicol	3	13	4	9	4	11	2	6	0	0
Doxycycline	3	13	5	12	5	13	4	11	1	6
Erythromycin	30	83	26	60	26	68	10	29	18	100
Gatifloxacin	2	8	4	9	4	11	3	9	1	6
Gentamicin	6	25	12	28	10	26	10	29	5	28
Levofloxacin	7	29	9	21	8	21	9	26	5	28
Linezolid	1	4	1	2	1	3	0	0	0	0
Nitrofurantoin	1	4	1	2	1	3	0	0	0	0
Norfloxacin	8	33	9	21	9	24	6	17	2	12
Oxacillin	21	88	41	95	34	89	33	94	18	100
Ofloxacin	7	29	10	23	9	24	5	14	3	17
Pencillin G	22	92	41	95	36	95	35	100	18	100
Rifampin	2	8	4	9	4	11	3	9	1	6
Trimethoprim	13	54	19	44	16	42	14	40	6	33
Vancomycin	1	4	2	5	2	5	2	6	1	6

Food is an important factor for the transfer of antibiotic resistances. Such transfer can occur by means of antibiotic residues in food, through the transfer of resistant food-borne pathogens or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms (Pesavento *et al*., 2007).*S. aureus* strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within microabscesses, which limit the action of drugs (Gündoğan *et al*., 2006).

Sila *et al*. (2009) found 7 genes more frequently detected in MRSA isolates: *Sea, Seb, Sed, Seg, Sei, Sej* and *Eta*, coding for the production of enterotoxins A, B, D, G, I, J and the exfoliative toxin A. On the other hand, the *Pvl*, *Tst* and *Sec* genes for Panton-Valentine leukocidin, TSST-1 and enterotoxin C were most frequent in MSSA. The characterization of different *S. aureus* isolates collected from different food origins showed that 38% of the studied strains are MRSA, and 19% of the tested isolates are both enterotoxigenic and oxacillin positives (Pereira *et al*.,2009).

4.7. Detection of Virulence Genes (*tet M*, *erm B* and *Wzm*) of *S. aureus* Isolates

Regarding to staphylococcal virulence genes; PCR detecting sequences of the genes are positive, 39 (68%) isolates carry *tet M* gene, 11 (19%) isolates carry *erm B* gene and 39 (68%) isolates carry *Wzm* gene as shown in figures (4.11 to 4.13) and table (4-13).

Table (4-13): percentage of *tet M*, *erm B* and Wzm in isolate of *S. aureus*

Genes	positive		negative		Total	P value
	No	%	No	%		
tet M	39	68	18	32	57(100%)	**0.000**
erm B	11	19	46	81	57(100%)	**0.000**
Wzm	39	68	18	32	57(100%)	**0.000**

Other study found that incidence of *ermA* resistance genes are 50%. Incidence *ermB* (25%)are lower than other identified resistance genes. Arithmetical important difference is seen between the incidence of *ermA* and *ermB* (P <0.05) and *tetK* and *tetM* (P <0.05) antibiotic resistance genes (Rahi *et al*.,2020).

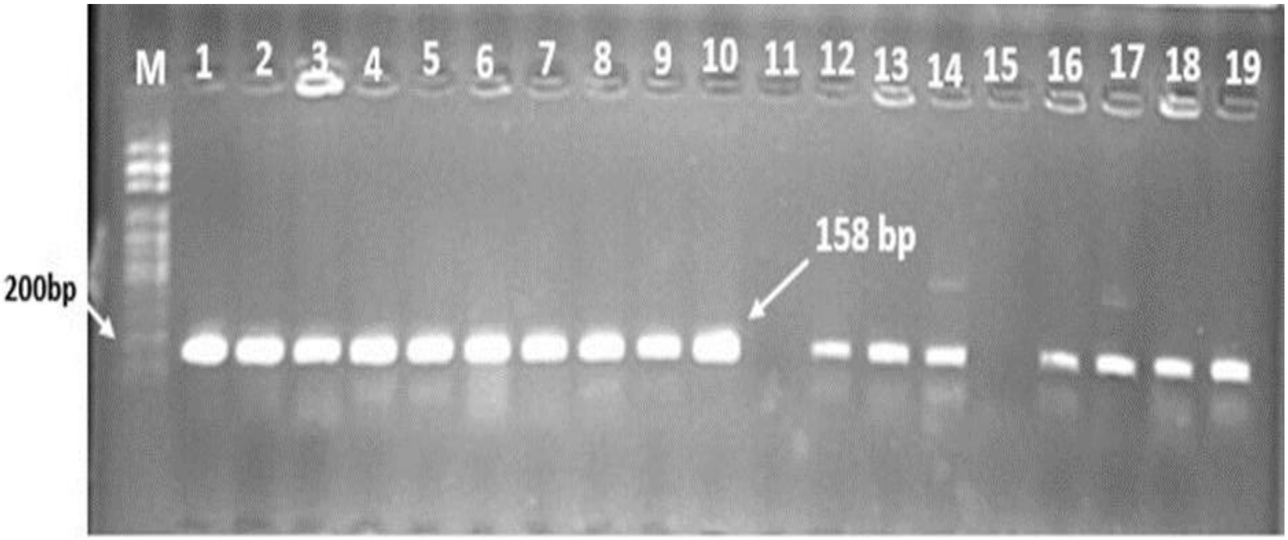


Figure (4.11): Sample of the *tet M* gene gene product of *S. aureus* (electrophoretic graph of PCR product to using agarose gel electrophoresis (1.5 %) at 72 volts for 80 minutes of process PCR to *tet M* gene amplicon (158bp) of *S. aureus*. M lane represent 100 bp DNA Marker, the (11-15) represents some of negative samples for this gene. While the rest lanes represent positive some of samples for this gene).

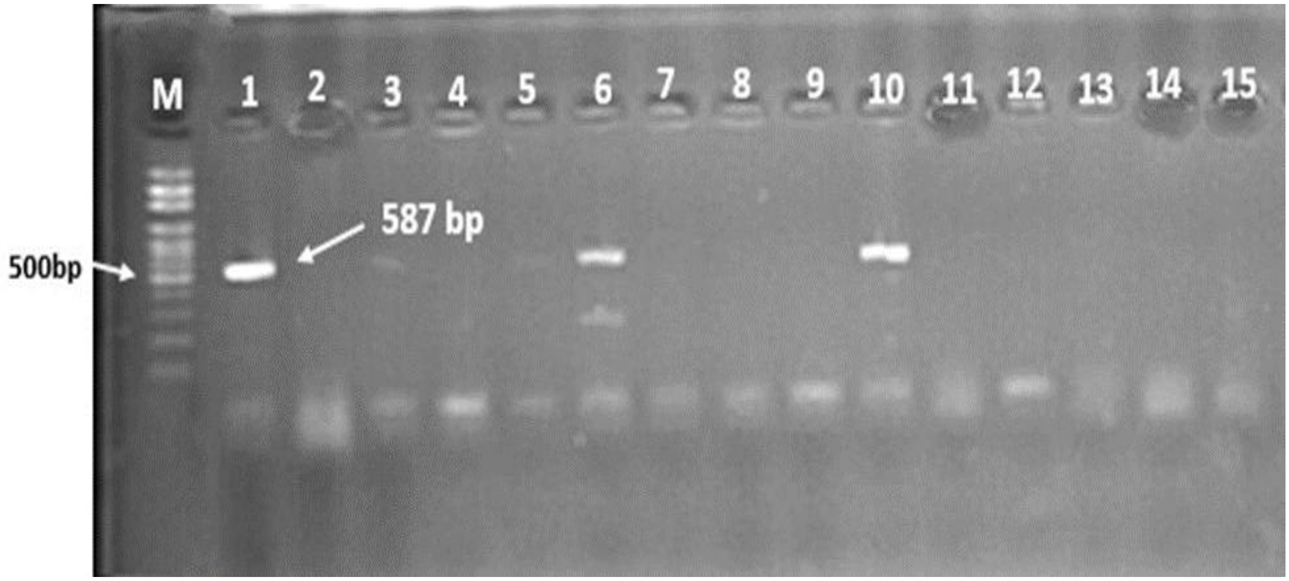


Figure (4.12): Sample of the *ermB* gene gene product of *S. aureus* (electrophoretic graph of PCR product to using agarose gel electrophoresis (1.5 %) at 72 volts for 80 minutes of process PCR to *ermB* gene amplicon (587bp) of *S. aureus* isolates. M lane represent 100 bp DNA Marker, the (1-6-10) represents some of positive samples for this gene. While the rest lanes represent some of negative samples for this gene).

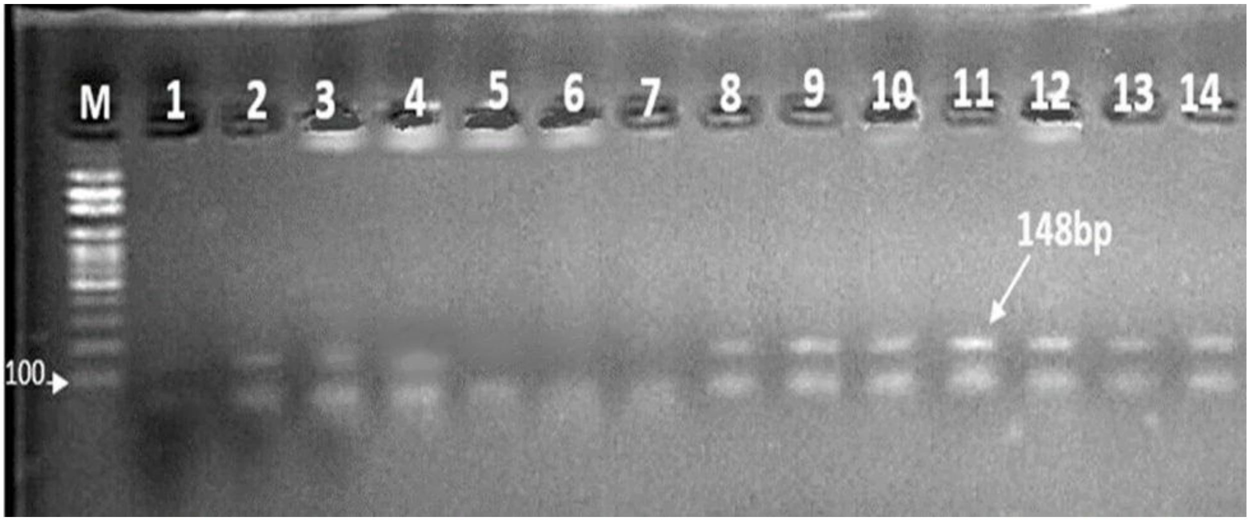


Figure (4.13): Sample of the *Wzm* gene gene product of *S. aureus* (1.5 %) Agarose gel electrophoresis at 72 volts for 80 minutes of PCR to *Wzm* gene amplicon (148 bp) of *S. aureus* isolates. M lane represent 100 bp DNA Marker, the (1-5-6-7) represents some of negative samples for this gene. While the rest lanes represent some of positive samples for this gene.

This study reveals that there is a harmony between results of antibiotic resistance and positive molecular detection of these genes. The highest resistance rate to Cefoxitin, Oxacillin is observed in the isolates of *tet M* and *erm B* genes, whereas the low resistance rate to Linezolid, Nitrofurantoin and Vancomycin is observed in the isolates of *tet M* and *erm B*, as shown in table (4-14).

Table (4-14): Pattern of *tet M and erm B* of antibiotics of *S.aureus*

antibiotics	*tet M*		*erm B*	
	No.	%	No.	%
Cefoxitin	39	100	11	100
Clindamycin	4	10	2	18
Ciprofloxacin	7	18	0	0
Chloramphenicol	4	10	1	9
Doxycycline	5	13	0	0
Erythromycin	30	77	8	73
Gatifloxacin	3	8	1	9
Gentamicin	13	33	2	18
Levofloxacin	8	21	2	18
Linezolid	1	3	1	9
Nitrofurantoin	1	3	1	9
Norfloxacin	9	23	1	9
Oxacillin	37	95	11	100
Ofloxacin	10	26	1	9
Pencillin G	38	97	11	100
Rifampin	6	15	2	18
Trimethoprim	19	49	4	36
Vancomycin	2	5	0	0

In the vast majority of *S. aureus* isolates, resistance to macrolides is due to N6-dimethylation of the adenine at position 2058 of 23S rRNA. Recent clinical isolates possess the *erm(A)* and/or the *erm(C)* gene coding for rRNA methylases; hospital strains carrying *erm*(B) are rather infrequent (Schmitz *et al*., 2000). Tetracycline resistance in *S. aureus* is either based on modification of the ribosome encoded by the widely disseminated *tetM* gene or mediated by *tetK* encoded efflux of the variety of different tet genes coding for efflux mechanisms, *tetK* is most often found in *S. aureus* (Schmitz *et al*., 2001; Trzcinski *et al*., 2000). Tetracycline-resistant MRSA isolates (up to 50% of MRSA isolates (Schmitz *et al*., 2001), carrying the *tetK* gene are susceptible to minocycline, whereas minocycline-resistant strains harbor *tetM* or both *tetK* and *tetM* (Trzcinski *et al*., 2000.)

Other study found that *Staphylococcus aureus* isolates that have *tetM* gene is resistance to penicillin; oxacillin; gentamicin; erythromycin; clindamycin; oxytetracyline; ciprofloxacin; rifampin. (Strommenger *et al*.,2003). The *ermB* gene is present in 7 (32%; 7/22) of Erythromycin-resistant isolates, while *tetM* and *tetA* are recorded in 89% (8/9) and 22% (2/9), respectively, for Tetracycline-resistant strains (Fri *et al*., 2020).

Boost incidence of the genes encode resistance toward penicillins (*blaZ*), aminoglycosides (*aacA-D*), tetracyclines (*tetK* and *tetM*), macrolides (*ermA, ermB, msrA, msrB* and *mefA*), fluoroquinolones (*gyrA* and *grlA*), lincosamides (*linA*), folate inhibitors (*dfrA1*), phenicols (*cfr*), and ansamycins (*rpoB*) is one of the chief ways for occurrence of severe antibiotic resistance (Hasanpour Dehkordi *et al*., 2017; Safarpoor Dehkordi *et al*., 2017)

Akanbi *et al*., (2017) reported that *blaZ, mecA, rpoB, ermB* and *tetM* are the most generally identified antibiotic resistance genes amongst the *S. aureus* bacteria recovered from food samples in South Africa which is relatively similar to our findings. Another Iranian investigation showed that oxacillin, gentamicin, penicillin, tetracycline and erythromycin resistant *S. aureus* bacteria recovered from milk and dairy products carried considerable incidence of *blaZ, aacA-aphD, mecA, tetK* and *tetM, ermB, ermA, ermT, ermC, msrB* and *msrA* antibiotic resistance markers likewise to our survey (Jamali *et al*., 2015).

This study reveals that there is a harmony between results of biofilm formation and positive molecular detection of *Wzm* gene ($p< 0.05$). all isolates (100%) that produce biofilm (moderate and strong) are have *Wzm* gene as shown in table (4-15).

Table (4-15): Distribution of *Wzm* gene and biofilm formation of *S. aureus*

gene	Biofilm grade								P. value
	Weak		Moderate		Strong		none		
	No	%	No	%	No	%	No	%	
Wzm	11	73	21	95	5	83	2	14	0.024

Other study found that 100 % of isolates are produce biofilm gene (*Wzm*) (Jassim and Kadhim,2021). LPS transport (*wzm*) seem involved in *K. pneumoniae* biofilm (Vuotto *et al*., 2017). *S. aureus* is an important pathogen that causes a variety of infections. The formation of biofilms on indwelling medical devices enables *S. aureus* to evade host immune responses and establish chronic infections. The ability to develop biofilms varies among different *S. aureus* isolates. Multiple environmental factors, including nutrients, antibacterial agents, pH, shearing force, temperature, and so on are able to induce stress responses and can profoundly affect the life cycle stages of biofilm formation, including initial attachment, maturation, and detachment (Liu *et al*., 2020).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The following conclusions is obtained from the present study:

1. This study concludes that *Staphylococcus aureus* is prevalent in ocular infection and are more likely to be MRSA, which is now one of the most common multidrug-resistant pathogens that infects people and causing infections.
2. *Staphylococcus aureus* isolates showed many virulence factors which could lead to chronic and severe cases ocular infection.
3. In this study, isolates of *S. aureus* showed a high rate of resistance to various antibiotics (MDR). Nitrofurantoin, Linezolid, and Vancomycin are the most effective antibiotics, and our study also showed that the drug used to treat eye infections such as Gentamycin, Ciprofloxacin and Chloramphenicol is still effective, but with the continued increase in antibiotic resistance it will no longer be effective as an empirical treatment for *S. aureus* infections.
4. Relatively high prevalence of enterotoxins more than 88% of the *staphylococcal* isolates carried one or more of *SEs* genes and *Seb* gene is discovered to be closely linked to ocular isolates.
5. There is a significant relationship between virulence genes and antibiotic resistance patterns. Genetic investigations could provide a global and comprehensive aspect of risk prediction.
6. Conjunctivitis caused by *S. aureus* is often recurrent and associated with chronic blepharitis. It was most common among isolates and there was statistically significant difference in types of Infection (P = 0.00).
7. the present study revealed that the *Wzm* gene consider a target for biofilm detection of *S. aureus,* the result showed the effectiveness of using genetic determinants for biofilm formation by the *Wzm* gene among isolates.

Recommendations:

1. Study of other virulence genes of *Staphylococcus aureus* isolated from eye infections and their prevalence.
2. Comparing of *Staphylococcus aureus* isolated from eye infections with isolates collected from other diseases and finding out the differences in the spread of virulence genes.
3. More study needed regarding how MRSA stains, spread and control in the community and in Hospitals as MRSA is prevalent worldwide.
4. The identification of the responsible bacteria and their antimicrobial susceptibility patterns is essential in establishing an accurate antibiotic therapy for the treatment of ocular infections.

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APPENDIX

QUESTIONNAIRE

PATIENT QUESTIONNAIRE

General Data

SpecimenID		***Name***		
Gender	***Male***: ☐		***Female:*** ☐	
Age				
Residence	***Urban***: ☐		***Rural***: ☐	
Date of Sampling		***DD***	***MM***	***YYYY***
Site of Specimen				
Type of Infection	***Conjunctivitis***	***Blepharitis(Eyelids Infection)***	***Keratitis***	***Other Infection***
Date of Infection		***DD***	***MM***	***YYYY***
Other Chronic Diseases				
Antibacterial Treatment				

Figure 1: Patient Questionnaire

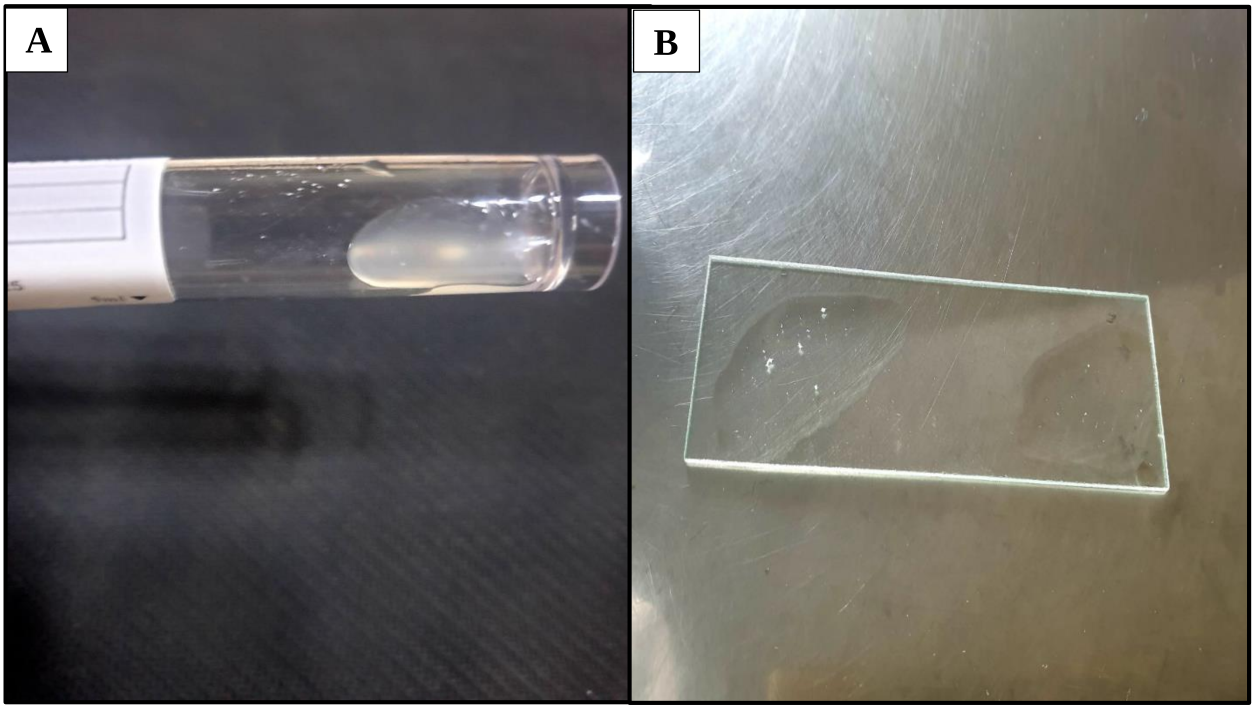


Figure 2: coagulase test for *S. aureus*(A) Tube coagulase test (free coagulase) ***(B) Slide agglutination test (bound coagulase or clumping factor)***

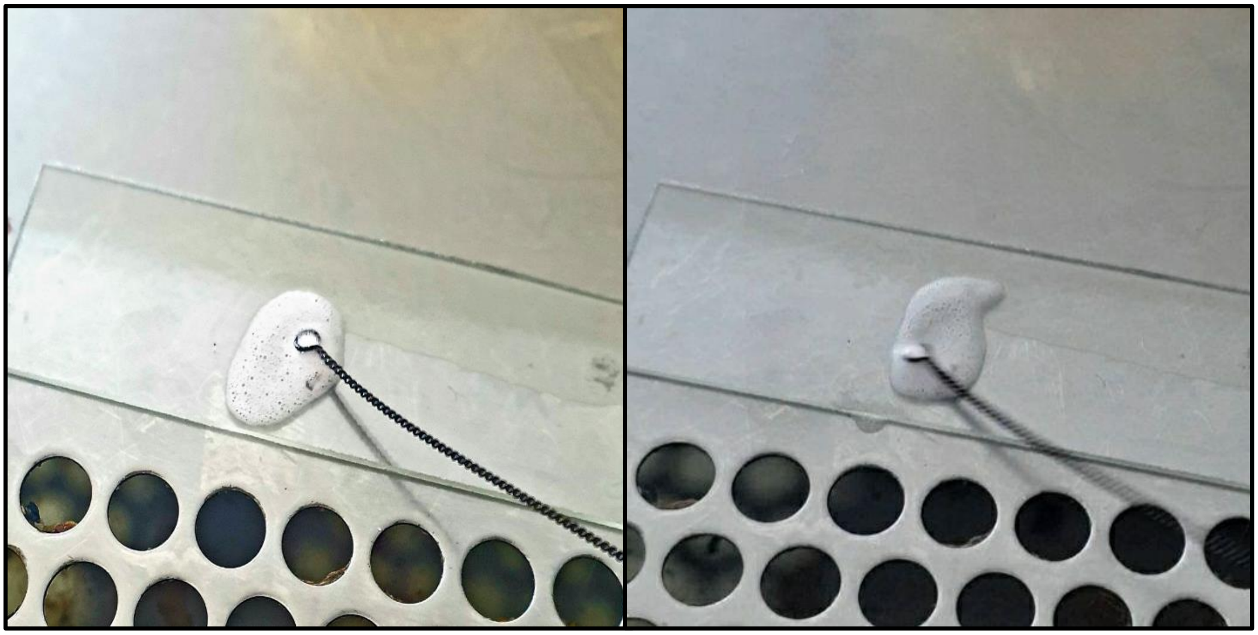


Figure 3: catalase test for *S. aureus*

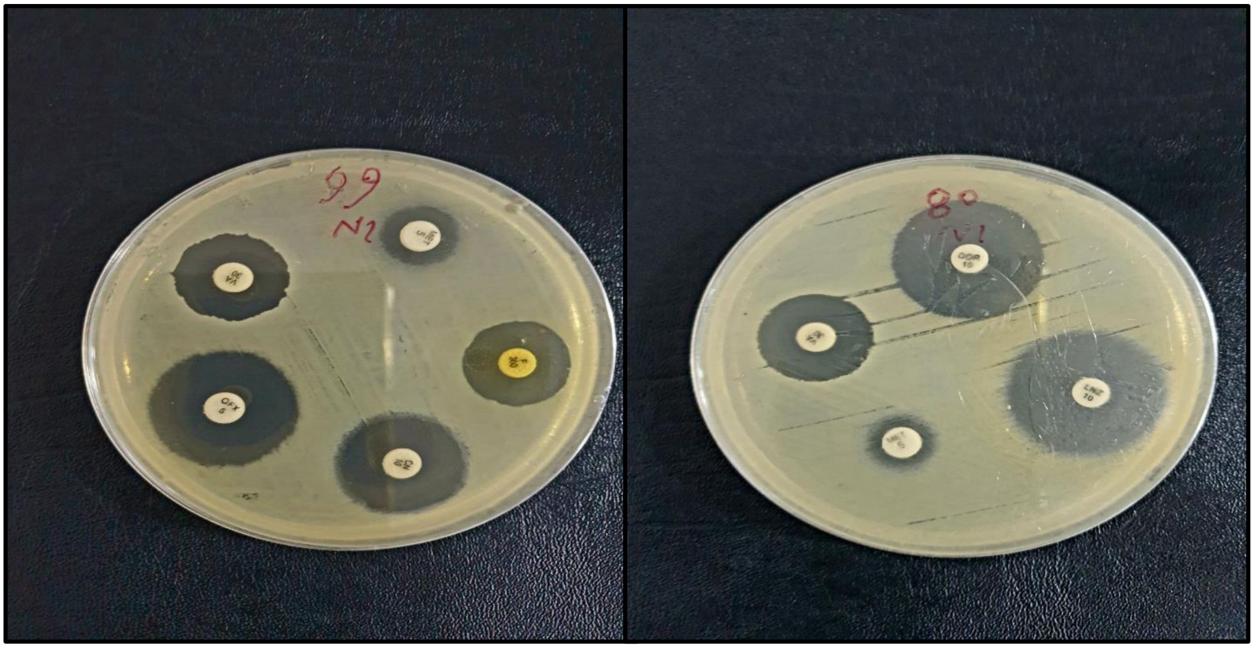


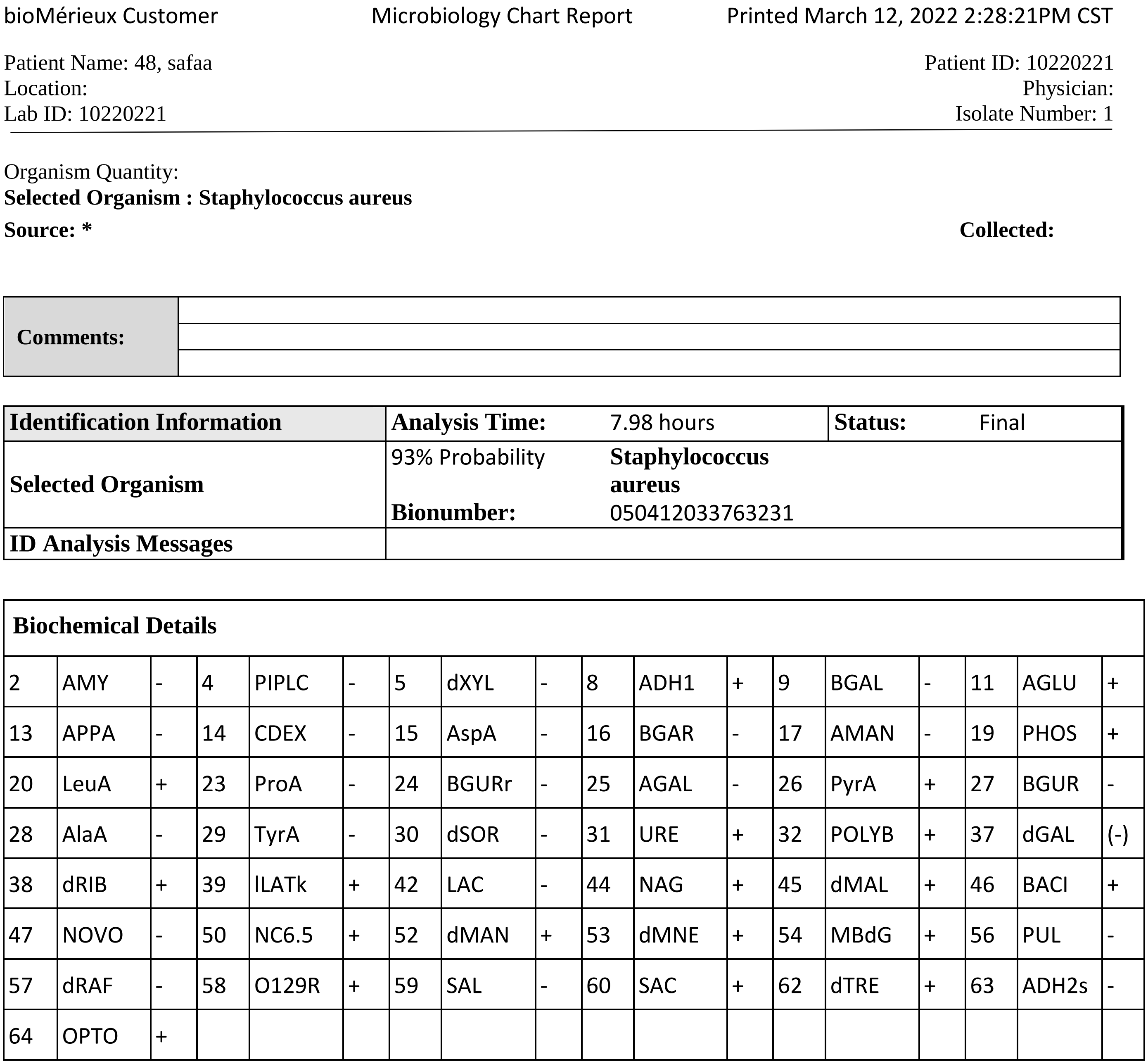
Figure 4: *S. aureus* bacteria sensitivity test

Table (1): Phenotypic of antibiotic susceptibility of *S. auraus* isolates

isolates	CX	CD	CIP	C	DXT	E	GAT	CN	LEV	LZN	F	NX	OX	OFX	P	RD	TMP	VA
1	R	S	S	S	S	R	S	S	S	S	S	S	R	S	R	S	S	S
2	R	R	S	S	IN	R	S	S	S	S	S	S	R	S	R	S	S	S
3	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
4	R	S	S	S	R	R	S	S	S	S	S	S	R	S	R	S	R	S
5	R	S	S	R	S	R	S	S	S	R	S	S	R	S	R	S	S	S
6	R	R	S	S	S	R	S	S	S	S	S	S	R	S	R	S	S	S
7	R	S	S	S	S	R	S	R	S	S	S	S	R	S	R	S	R	S
8	R	R	R	R	R	R	8	S	S	S	S	S	R	R	R	S	R	S
9	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
10	R	S	S	S	S	R	S	R	S	S	S	R	R	R	R	S	R	S
11	R	S	S	S	IN	R	S	R	S	S	S	S	R	S	R	S	R	S
12	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S
13	R	S	S	S	IN	R	S	S	S	S	S	S	R	S	R	S	R	S
14	R	S	S	S	S	S	R	R	S	S	S	S	R	R	S	S	S	S
15	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
16	R	S	S	S	IN	R	S	R	S	S	S	S	R	S	R	S	R	S
17	R	S	S	S	S	R	S	S	R	S	S	S	R	IN	R	S	R	S
18	R	R	S	R	S	R	S	IN	S	R	R	R	R	R	R	R	R	S
19	R	S	S	S	S	R	S	IN	S	S	S	S	R	S	R	S	R	S
20	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
21	R	S	S	S	R	R	S	S	S	S	S	S	R	S	R	S	S	S
22	R	S	R	S	IN	R	S	R	R	S	S	R	R	R	R	S	R	S
23	R	R	S	S	S	R	S	R	S	S	S	S	R	R	R	R	R	S
24	R	S	S	S	R	R	S	S	S	S	S	S	R	S	R	S	S	S
25	R	S	R	S	IN	R	S	R	S	S	S	R	R	R	R	R	R	S
26	R	S	R	R	R	R	S	R	R	S	S	R	R	R	R	S	R	R
27	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
28	R	S	S	S	S	R	S	S	S	S	S	S	R	S	R	S	S	S
29	R	S	S	S	S	S	S	S	R	S	S	S	R	S	R	S	S	S
30	R	S	S	S	S	R	R	S	S	S	S	S	R	S	R	S	S	S
31	R	S	S	S	S	R	S	S	S	S	S	S	R	R	R	S	S	S
32	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S

isolates	CX	CD	CIP	C	DXT	E	GAT	CN	LEV	LZN	F	NX	OX	OFX	P	RD	TMP	VA
33	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
34	R	S	S	S	S	R	S	R	S	S	S	S	R	S	R	S	R	S
35	R	S	S	S	IN	R	S	S	S	S	S	S	R	S	R	S	S	S
36	R	S	S	S	IN	R	S	R	S	S	S	S	R	S	R	S	S	S
37	R	S	S	S	S	S	S	S	R	S	S	S	R	S	R	S	S	S
38	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
39	R	S	R	S	IN	R	R	S	S	S	S	R	R	R	R	S	R	S
40	R	S	R	S	IN	R	S	IN	R	S	S	R	R	S	R	S	R	S
41	R	S	S	S	S	R	S	S	S	S	S	S	R	S	R	S	R	S
42	R	R	S	S	S	R	S	IN	S	S	S	S	R	S	R	S	S	S
43	R	S	S	S	S	R	S	R	S	S	S	R	S	S	R	S	R	S
44	R	S	S	S	S	R	S	S	S	S	S	S	R	S	R	S	S	S
45	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
46	R	S	IN	S	S	R	S	R	S	S	S	S	R	S	R	S	R	S
47	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
48	R	S	S	S	S	R	R	S	S	S	S	R	R	S	R	R	S	S
49	R	S	S	S	R	R	S	R	R	S	S	S	R	IN	R	S	S	S
50	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	R
51	R	S	S	S	R	R	S	S	S	S	S	S	R	S	R	S	S	S
52	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
53	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
54	R	S	R	S	IN	R	S	S	R	S	S	S	R	R	R	S	R	S
55	R	S	S	R	S	R	S	R	S	S	S	S	R	S	R	R	R	S
56	R	S	IN	S	S	R	S	R	S	S	S	S	R	S	R	S	R	S
57	R	S	S	S	S	R	S	IN	S	S	S	S	R	S	R	S	S	S

Abbreviations: [Cefoxitin (CX), Clindamycin (CD), Ciprofloxacin (CIP), Chloramphenicol (C), Doxycycline (DXT), Erythromycin (E), Gatifloxacin (GAT), Gentamicin (CN), levofloxacin (LEV), Linezolid (LZN), Nitrofurantoin (F), Norfloxacin (NX), Oxacillin (OX), Ofloxacin(OFX), Pencillin G (P), Rifampin (RD), Trimethoprim (TMP) and Vancomycin (VA)].



bioMérieux Customer Microbiology Chart Report Printed March 12, 2022 2:28:21PM CST

Patient Name: 48, safaa
Location:
Lab ID: 10220221

Patient ID: 10220221
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus
Source: * **Collected:**

Comments:	

Identification Information	**Analysis Time:**	7.98 hours	**Status:**	Final
Selected Organism	93% Probability **Bionumber:**	**Staphylococcus aureus** 050412033763231		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	(-)
38	dRIB	+	39	lLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 5: *S. aureus* bacteria identification Vitek

bioMérieux Customer Microbiology Chart Report Printed March 12, 2022 2:28:22PM CST

Patient Name: 12, safaa
Location:
Lab ID: 10220222

Patient ID: 10220222
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus
Source: * **Collected:**

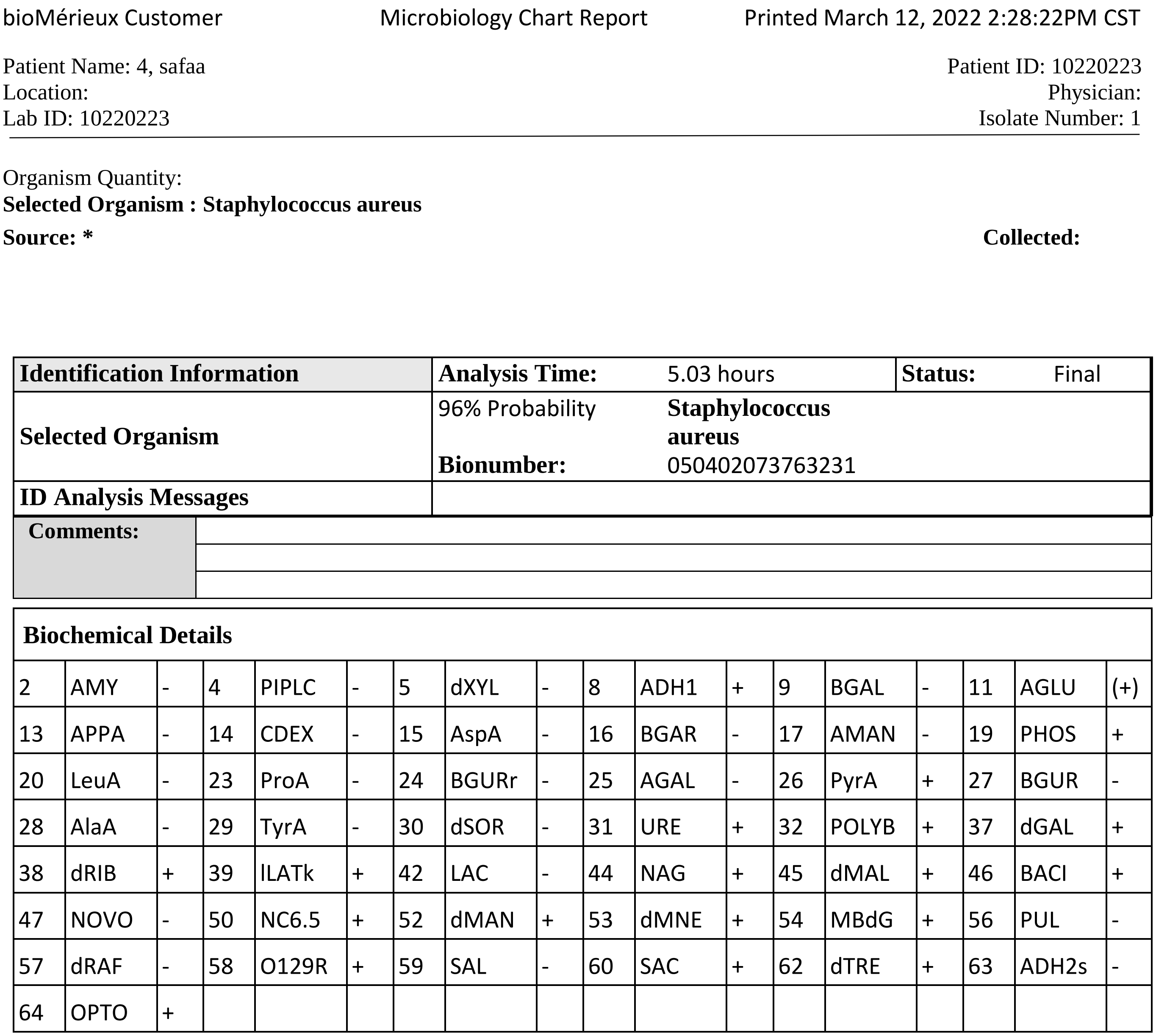
Comments:	

Identification Information	**Analysis Time:**	6.03 hours	**Status:**	Final
Selected Organism	95% Probability	**Staphylococcus aureus**		
	Bionumber:	050402033763231		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	(-)	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	-
38	dRIB	+	39	lLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 6: *S. aureus* bacteria identification Vitek



bioMérieux Customer Microbiology Chart Report Printed March 12, 2022 2:28:22PM CST

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Location:
Lab ID: 10220223

Patient ID: 10220223
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus
Source: * **Collected:**

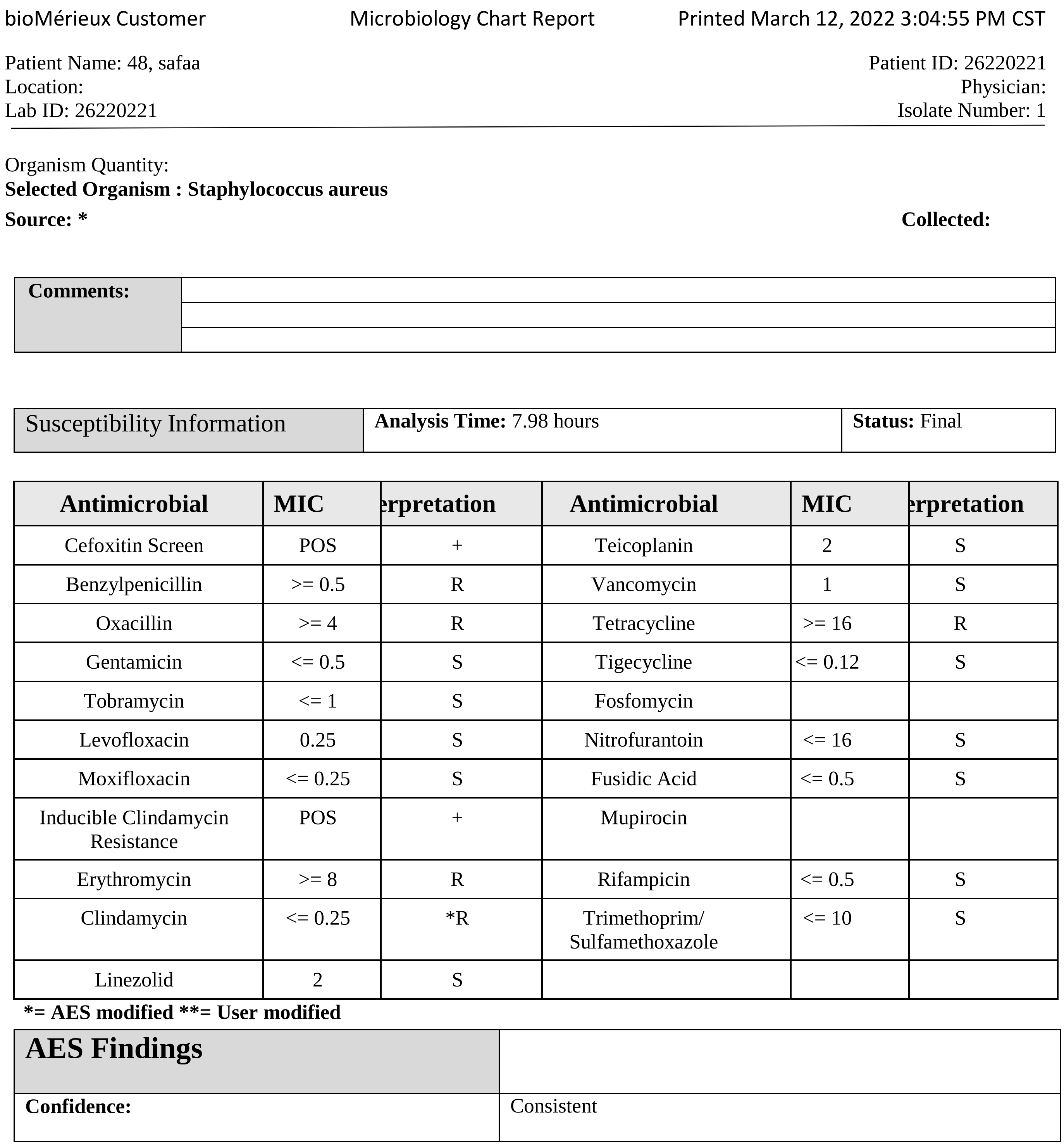
Identification Information	**Analysis Time:**	5.03 hours	**Status:**	Final
Selected Organism	96% Probability **Bionumber:**	**Staphylococcus aureus** 050402073763231		
ID Analysis Messages				

Comments:	

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	(+)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	lLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 7: *S. aureus* bacteria identification Vitek



bioMérieux Customer   Microbiology Chart Report   Printed March 12, 2022 3:04:55 PM CST

Patient Name: 48, safaa
Location:
Lab ID: 26220221

Patient ID: 26220221
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus
Source: *
Collected:

Comments:	

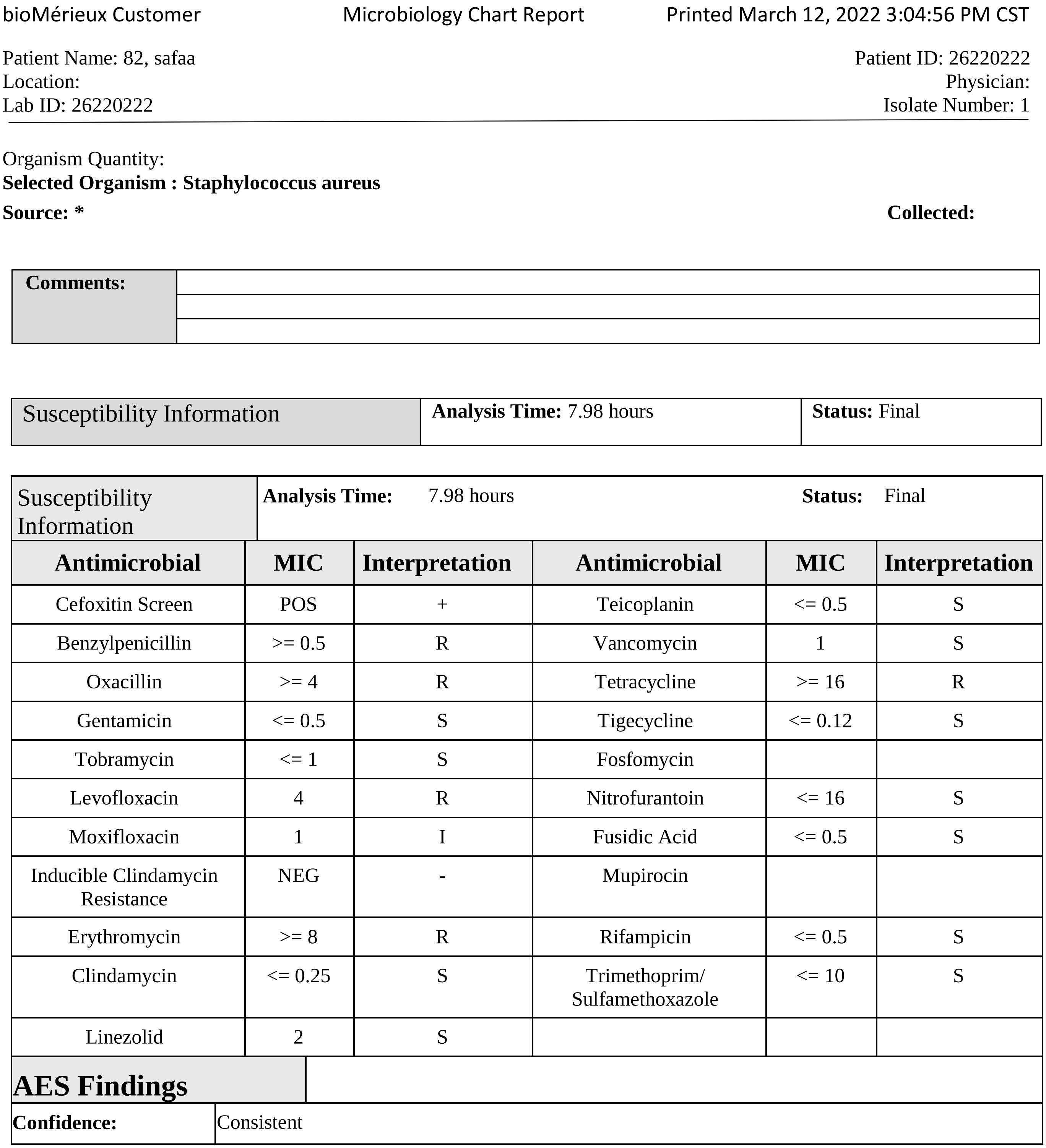
Susceptibility Information	**Analysis Time:** 7.98 hours	**Status:** Final

Antimicrobial	MIC	erpretation	Antimicrobial	MIC	erpretation
Cefoxitin Screen	POS	+	Teicoplanin	2	S
Benzylpenicillin	>= 0.5	R	Vancomycin	1	S
Oxacillin	>= 4	R	Tetracycline	>= 16	R
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
Tobramycin	<= 1	S	Fosfomycin		
Levofloxacin	0.25	S	Nitrofurantoin	<= 16	S
Moxifloxacin	<= 0.25	S	Fusidic Acid	<= 0.5	S
Inducible Clindamycin Resistance	POS	+	Mupirocin		
Erythromycin	>= 8	R	Rifampicin	<= 0.5	S
Clindamycin	<= 0.25	*R	Trimethoprim/ Sulfamethoxazole	<= 10	S
Linezolid	2	S			

***= AES modified **= User modified**

AES Findings	
Confidence:	Consistent

Figure 8: *S. aureus* bacteria susceptibility Vitek



bioMérieux Customer　　Microbiology Chart Report　　Printed March 12, 2022 3:04:56 PM CST

Patient Name: 82, safaa　　Patient ID: 26220222
Location:　　Physician:
Lab ID: 26220222　　Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus
Source: *　　**Collected:**

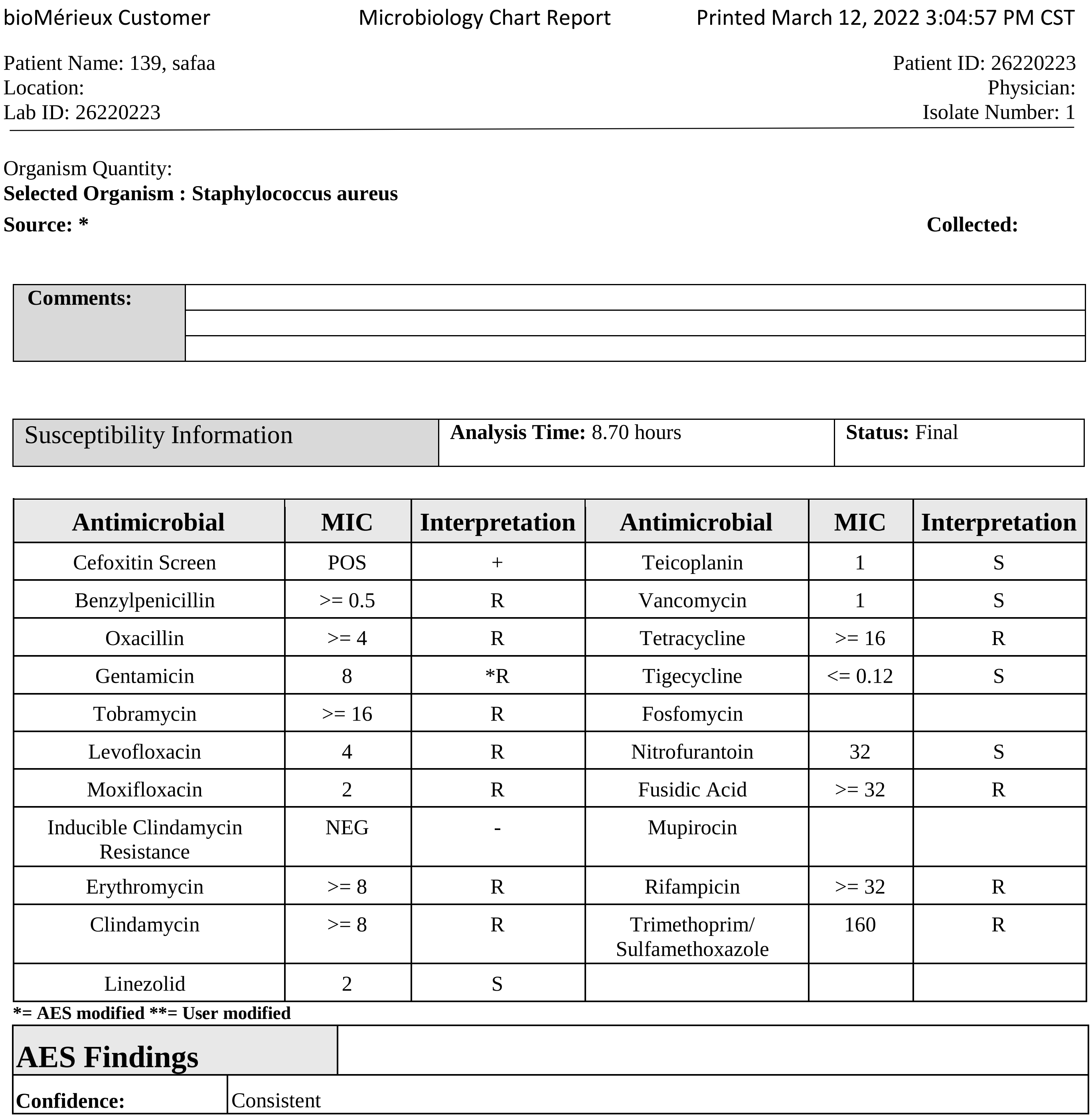
Comments:	

Susceptibility Information	**Analysis Time:** 7.98 hours	**Status:** Final

Susceptibility Information	**Analysis Time:** 7.98 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Cefoxitin Screen	POS	+	Teicoplanin	<= 0.5	S
Benzylpenicillin	>= 0.5	R	Vancomycin	1	S
Oxacillin	>= 4	R	Tetracycline	>= 16	R
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
Tobramycin	<= 1	S	Fosfomycin		
Levofloxacin	4	R	Nitrofurantoin	<= 16	S
Moxifloxacin	1	I	Fusidic Acid	<= 0.5	S
Inducible Clindamycin Resistance	NEG	-	Mupirocin		
Erythromycin	>= 8	R	Rifampicin	<= 0.5	S
Clindamycin	<= 0.25	S	Trimethoprim/ Sulfamethoxazole	<= 10	S
Linezolid	2	S			

AES Findings	
Confidence:	Consistent

Figure 9: ***S. aureus*** **bacteria susceptibility Vitek**



bioMérieux Customer Microbiology Chart Report Printed March 12, 2022 3:04:57 PM CST

Patient Name: 139, safaa
Location:
Lab ID: 26220223

Patient ID: 26220223
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus
Source: * **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 8.70 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Teicoplanin	1	S
Benzylpenicillin	>= 0.5	R	Vancomycin	1	S
Oxacillin	>= 4	R	Tetracycline	>= 16	R
Gentamicin	8	*R	Tigecycline	<= 0.12	S
Tobramycin	>= 16	R	Fosfomycin		
Levofloxacin	4	R	Nitrofurantoin	32	S
Moxifloxacin	2	R	Fusidic Acid	>= 32	R
Inducible Clindamycin Resistance	NEG	-	Mupirocin		
Erythromycin	>= 8	R	Rifampicin	>= 32	R
Clindamycin	>= 8	R	Trimethoprim/ Sulfamethoxazole	160	R
Linezolid	2	S			

***= AES modified **= User modified**

AES Findings	
Confidence:	Consistent

Figure 10: ***S. aureus*** **bacteria susceptibility Vitek**

Table (2): Biofilm forming capacity of *S. aureus*

NO. isolate	OD	biofilm producer	NO. isolate	OD	biofilm producer	NO. isolate	OD	biofilm producer
1.	0.252	moderate	20.	0.201	weak	39.	0.388	moderate
2.	0.107	non	21.	0.394	moderate	40.	0.241	moderate
3.	0.098	non	22.	0.482	strong	41.	0.093	non
4.	0.095	non	23.	0.377	moderate	42.	0.079	non
5.	0.094	non	24.	0.284	moderate	43.	0.088	non
6.	0.099	non	25.	0.482	strong	44.	0.384	moderate
7.	0.125	weak	26.	0.557	strong	45.	0.189	weak
8.	0.248	moderate	27.	0.102	non	46.	0.493	strong
9.	0.184	weak	28.	0.084	non	47.	0.175	weak
10.	0.195	weak	29.	0.165	weak	48.	0.291	moderate
11.	0.133	weak	30.	0.396	moderate	49.	0.296	moderate
12.	0.237	moderate	31.	0.282	moderate	50.	0.389	moderate
13.	0.243	moderate	32.	0.192	weak	51.	0.283	moderate
14.	0.288	moderate	33.	0.194	weak	52.	0.089	non
15.	0.085	non	34.	0.285	moderate	53.	0.134	weak
16.	0.278	moderate	35.	0.124	weak	54.	0.581	strong
17.	0.281	moderate	36.	0.383	moderate	55.	0.109	non
18.	0.293	moderate	37.	0.679	strong	56.	0.191	weak
19.	0.165	weak	38.	0.179	weak	57.	0.097	non

Table (3): Correlation between biofilm grade and antibiotic resistance.

Antibiotic	**Biofilm grade**								***P*. value**
	Weak		**Moderate**		**Strong**		**none**		
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	
Cefoxitin	15	100	22	100	6	100	14	100	**0.000**
Clindamycin	0	0	3	14	0	0	3	21	**0.000**
Ciprofloxacin	0	0	3	14	4	67	0	0	**0.000**
Chloramphenicol	0	0	2	9	1	17	2	14	**0.000**
Doxycycline	0	0	5	23	1	17	1	7	**0.000**
Erythromycin	6	40	19	86	1	17	10	71	**0.000**
Gatifloxacin	0	0	4	18	0	0	0	0	**0.000**
Gentamicin	4	27	6	27	4	67	2	14	**0.000**
Levofloxacin	2	13	3	14	4	67	0	0	**0.000**
Linezolid	0	0	1	5	0	0	1	7	**0.000**
Nitrofurantoin	0	0	1	5	0	0	0	0	**0.000**
Norfloxacin	1	7	4	18	3	50	1	7	**0.000**
Oxacillin	14	93	22	100	6	100	12	86	**0.000**
Ofloxacin	1	7	7	32	4	67	0	0	**0.000**
Pencillin G	14	93	21	95	6	100	14	100	**0.000**
Rifampin	0	0	3	14	1	17	1	7	**0.000**
Trimethoprim	5	33	9	41	5	83	4	29	**0.000**
Vancomycin	0	0	1	5	1	17	0	0	**0.000**

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل / كلية العلوم
قسم علوم الحياة

تقييم جينات الضراوة في المكورات العنقودية الذهبية المعزولة من المرضى المصابين بالتهاب العيون

رسالة مقدمة الى

مجلس كلية العلوم – جامعة بابل كجزء من متطلبات نيل درجة الماجستير في العلوم / علوم حياة

من قبل

صفاء جاسم فليفل محيسن

بكالوريوس علوم حياة / جامعة الانبار / 2001

بإشراف

ا. م. فرح طارق عبد الرضا ا. انوار كاظم حسين الصفار

١٤٤٤هـ 2022 م

الخلاصة

تعد المكورات العنقودية الذهبية من أكثر مسببات الأمراض شيوعًا التي تسبب العدوى البكتيرية للعين. يمكن أن تسبب تلف التركيب التشريحي للعين على مستويات متعددة. يشمل (الملتحمة والقرنية والجفون والجهاز الدمعي والصلبة والشبكية) مع احتمال العمى. حيث انها قادرة على إنتاج العديد من عوامل الضراوة التي تساعد على الاصابة عن طريق الالتصاق وغزو الأنسجة والتهرب من الاستجابة المناعية للمضيف. الهدف الرئيسي لهذه الدراسة هو عزل وتشخيص المكورات العنقودية الذهبية من التهابات العيون البكتيرية والتحقق من وجود بعض جينات الضراوة جزيئا وعلاقتها بالإصابة.

أذ تضمنت الدراسة جمع مسحات العين من (160) مريض من المرضى المراجعين لاستشارية العيون في المستشفيات، بما في ذلك مستشفى ابن الهيثم التعليمي للعيون/ في محافظة بغداد ومستشفى الامام الصادق ومستشفى الحلة التعليمي / في محافظة بابل بعد تشخيصهم سريريا من قبل الطبيب الاستشاري والذين كانوا يعانون من إصابات بكتيرية للأجزاء الخارجية في العين (الملتحمة والجفون وكيس الدمع) تتراوح أعمارهم بين (4-74 سنة) لكلا الجنسين للفترة (من تشرين الثاني 2021 الى اذار 2022) ، بينت نتائج هذه الدراسة ارتفاع نسبة الإصابة لدى الذكور أذ بلغت 71% عنه في الاناث حيث كانت نسبة الإصابة 29% ، كما ظهر ان معدل التهاب ملتحمة العين هو الأعلى (75%) تلاه معدل التهاب الاجفان (20%)، التهاب القرنية (4%) واخيرا جاء معدل التهاب كيس الدمع (1%).

زرعت جميع العينات على أوساط زرعيه مختلفة، حضنت هوائيًا عند درجة حرارة 37درجة مئوية لـ (24-48) ساعة. ولقد أظهرت نتائج الزرع أن 139(87%) عينة أعطت نمو بكتيري إيجابي، بينما 21(13%) عينة لم يكن لها نمو بكتيري.

ومن مجموع 139عينة زرع موجبة فقط 57عزلة كانت تعود لبكتريا المكورات العنقودية الذهبية بنسبة (41.07%) وقد تم تشخيصها حسب الطرق القياسية لتشخيص البكتريا مظهريا ومجهريا وكيموحيويا وكذلك باستخدام نظام Vitek 2 compact system.

استخدم وسط MRSA Chromogenic Agar Base للكشف عن المكورات العنقودية الذهبية المقاومة للميثيسيلين. تم تأكيد نتائج العزلات البكتيرية عن طريق اختبار حساسية المضاد الحيوي سيفوكسيتين،

والذي كان بنسبة 100% مقاومة كاملة للسيفوكسيتين لتحديد مقاومة الميثيسيلين. أظهرت نتائج دراستنا أن جميع العزلات كانت مقاومة للميثيسيلين. كذلك فحصت حساسية البكتريا للمضادات الحيوية بواسطة طريقة كيربي بور على طبق مولر هنتون حيث استخدم 18مضادا حيويا (سيفوكستين و كليندامايسين و سيبروفلوكساسين و الكلورامفينيكول و دوكسيسيكلين و الاريثروميسين و جاتيفلوكساسين و ليفوفلوكساسين و لينزوليد و نتروفورانتوين و نورفلوكساسين و أوكساسيلين و أوفلوكساسين و بنسيلين جي و ريفامبين و تريميثوبريم و فانكومايسين) أظهرت نتائج فحص الحساسية للمضادات الحيوية أعلى مقاومة بين المضادات الحيوية عند معدل المقاومة (100%) الى سيفوكستين ،(96.49%) إلى بنسيلين جي ،(94.73%) إلى أوكساسيلين بينما توجد أقل معدلات مقاومة مع نتروفورانتوين بنسبة (1.75%)، (3.5%) الى كل من فانكومايسين و لينزوليد ،(8.77%) للريفامبين والكلورامفينيكول و جاتيفلوكساسين، أظهرت النسبة المئوية لمقاومة المضادات الحيوية للعزلات ان 45 (79%) عزلة متعدد المقاومة للأدوية والتي عادة ما ترتبط بالمكورات العنقودية المقاومة للميثيسيلين.

كما تضمنت الدراسة الكشف عن اختبار تكوين الأغشية الحيوية باستخدام طريقة لوحة زراعة الأنسجة tissue culture plate method (TCP)، وأظهرت النتائج أن 43عزلة (75%) لديها القدرة على إنتاج الأغشية الحيوية. تم استخدام تقنية تفاعل البلمرة المتسلسل (PCR)لتأكيد العزلات عن طريق تضخيم البرايمر المحدد لجين *16SrRNA*الخاص بالمكورات العنقودية الذهبية، جميع العزلات أعطت نتيجة موجبة لهذا الجين. أيضا تم استخدام تقنية تفاعل البلمرة المتسلسل(PCR) لتحديد بعض الجينات المسؤولة عن ضراوة البكتيريا المعزولة.

تم الكشف عن قابلية البكتريا لإنتاج السموم المعوية وفقًا لخمسة جينات معوية كلاسيكية *Sea, Seb,* *Sed* (*See* ،*Sec,*) وجد أن 50عزلة من المكورات العنقودية الذهبية (88%) تحتوي على جين أو أكثر من الجينات المعوية. كان جين *Seb* هو الجين الأكثر شيوعًا لترميز الذيفان المعوي من بين الجينات الأخرى التي تم اختبارها بنسبة (75%) من الجينات المعوية المكتشفة يليه الجين Sec الذي شكل (67%)، ثم الجين *Sed* بنسبة (61%)وجين *Sea*بنسبة (42%) بينما كان *See* بنسبة(32%) .

أظهرت النتائج المتحصل عليها من هذه الدراسة أن 6(10%) من عزلات المكورات العنقودية تحتوي على جين واحد فقط للسموم المعوية، في حين أن 7 (12%) من العزلات تمتلك جينين معظمهما *Seb*و16 عزلة من المكورات العنقودية (28%) تحتوي على ثلاثة جينات مشفرة للسموم، 15عزلة (26%) تحتوي

على أربعة جينات مشفرة للسموم، و 6عزلات من المكورات العنقودية (11%) تحتوي على خمسة جينات مشفرة للسموم.

أستخدم نوعين من الجينات للكشف عن مقاومة البكتريا للمضادات الحيوية وهما جين *erm B أحد الجينات المسؤولة عن مقاومة البكتريا لمضاد الارثرومبيسين* وجين *tet M* المشفر لمقاومة مضادات التتراسايكلين *وأظهرت* النتائج أن نسبة الجينات في العزلات (68%) لـ *erm B* و (19%) لـ *tet M*

بينما ظهرت نتيجة الكشف عن النمط الجيني في بكتريا المكورات الذهبية لتشكل الغشاء الحيوي بنسبة عالية وفقا لنتائج تفاعل البلمرة المتسلسل لـ 57عزلة لوحظ وجود جين *Wzm* في 39عزلة بنسبة (68%) وكانت النتيجة مقاربة لنتيجة اختبار تكوين الأغشية الحيوية باستخدام طريقة لوحة زراعة الأنسجة (TCP).