Antibiotic Resistance Profile and Biofilm Formation in *Staphylococci* isolated from surfaces of Public Transport

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

“In the name of Allah, the Lord of Grace, the Ever Merciful.”

Dedication

This effort is Dedicated to our Holy Prophet Muhammad (Sallallaho Alaihi wa Aalihi wa-Sallam), beloved Parents, honorable Teachers and cooperative Friends.

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Fizza Raza

Certificate

This thesis submitted by Fizza Raza to the Department of Microbiology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan is accepted in its present form as satisfying the requirement of the degree of Master of Philosophy in Microbiology.

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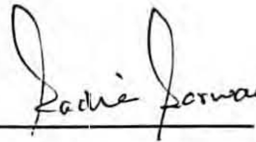

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List of Abbreviations

%	Percentage
°C	Degree Celsius/Centigrade
+ive	Positive
µ	Micro
AST	Antimicrobial Susceptibility Testing
AMEs	Aminoglycosides Modifying Enzymes
bp	Base Pair
BSI	Blood Stream Infections
C	Chloramphenicol
CA	Community Acquired
ccr	Cassette Chromosome Recombinases
CDC	Center for Disease Control and Prevention
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
CoNS	Coagulase Negative *Staphylococci*
CA-MRSA	Community-Acquired MRSA
CSF	Cerebrospinal Fluid
CVP	Central Venous Pressure
DA	Clindamycin
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTPs	Deoxynucleotide Triphosphate
DRs	Direct Repeats
E	Erythromycin
EDTA	Ethylene Diamine Tetra acetic Acid
ENT	Ear, Nose and Throat
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FD	Fusidic Acid
FOS	Fosfomycin

FOX	Cefoxitin
G	Gram
GTI	Genital Tract Infections
H_2O_2	Hydrogen Peroxide
HA	Hospital Acquired or Associated
HCl	Hydrochloric Acid
ICU	Intensive Care Unit
IRs	Inverted Repeats
IS	Insertion Sequence
ISS	Integration Site Sequence
-ive	Negative
IWG-SCC	International Working Group on Classification of *Staphylococcus* Cassette Chromosome Element
J	Joining or Junkyard
kDa	Kilo Dalton
l	Liter
LZD	Linezolid
M	Molar
MDR	Multidrug Resistance
$MgCl_2$	Magnesium Chloride
MGE	Mobile Genetic Element
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
ml	Milli Liter
MLS	Macrolide, Lincosamide and Streptogramin
MLST	Multilocus Sequence Typing
mM	Milli Molar
mm	Millimeter
M-PCR	Multiplex PCR
MSA	Mannitol Salt Agar
MRCONS	Methicillin Resistant Coagulase Negative *Staphylococci*
MSRA	Methicillin Resistant *Staphylococcus aureus*
N or n	Number

NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NI	Nosocomial Infections
NT	Non-Typeable
O_2	Oxygen
OPD	Out Patient Department
ORF	Open Reading Frame
orfX	Open Reading Frame of Unknown Function
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PIMS	Pakistan Institute of Medical Sciences
pmol	Pico-mole
PVL	Panton-Valentine Leukocidin
PWGF	Private Ward Ground Floor
QAU	Quaid-i-Azam University
QD	Quinupristin/Dalfopristin
RD	Rifampicin
rpm	Revolutions Per Minute
RTI	Respiratory Tract Infections
S. aureus	*Staphylococcus aureus*
SCCmec	Staphylococcal Cassette Chromosome *mec*
SDS	Sodium Dodecyl Sulphate
spa	Staphylococcal Protein A
SSI	Skin and Soft Tissue Infection
ST	Sequence Type
SXT or TMP/SXT	Trimethoprim-Sulfamethoxazole
TBE	Tris Borate EDTA
TE	Tetracycline
TE Buffer	Tris-EDTA Buffer
TGC	Tigecycline
UK	United Kingdom

USA	United States of America
UTIs	Urinary Tract Infections
UV	Ultraviolet
V	Volt
VA	Vancomycin
VISA	Vancomycin-Intermediate *Staphylococcus aureus*
VRSA	Vancomycin-Resistant *Staphylococcus aureus*
w/v	Weight by Volume
WBC	White Blood Cells
β	Beta

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Abstract

Abstract

An alarming number of fatalities occur annually due to the growing global problem of antibiotic resistance. Members of the Staphylococcus genus play a critical role among those responsible for the spread of antibiotic resistance. Staphylococcus species thrive in communal situations, multiplying within families, close-knit communities, and even using transportation systems as a means of transmission. This study examines the antibiotic resistance characteristics of 175 staphylococcal species isolates obtained from a variety of public transportation vehicles, including taxis, buses, and vans from Islamabad and Rawalpindi as well as passenger trains from Karachi. Through culture on Mannitol Salt Agar (MSA), morphological characteristics of the isolates were evaluated. Biochemical analyses included catalase and coagulase tests, which allowed the distinction between coagulase-positive and coagulase-negative staphylococcal species was done. The Kirby-Bauer disk diffusion technique was used for the Antimicrobial Susceptibility Testing (AST) and microtiter plate (MTP) test was performed to assess biofilm development capacity of isolates. The cefoxitin disk diffusion test was used to phenotypically identify MRSA (methicillin resistant *S. aureus*) isolates. Polymerase chain reaction (PCR)-based *mecA* gene amplification was also done for MRSA molecular identification. Of 175 isolates,141 were mannitol fermenter and 34 of the isolates were found to be non-mannitol fermenter. Whereas 150 were coagulase-positive staphylococci (CoPS) and 25 were found to be coagulase-negative staphylococci (CoNS) by biochemical identification. The study evaluated the trend of antibiotic resistance in *Staphylococcus* species. Notably, resistance was observed for penicillin (83%), clindamycin (57%), erythromycin (53%), cefoxitin (49%), quinupristin/dalfopristin (49%), linezolid (47%), rifampicin (40%), trimethoprim-sulfamethoxazole (32%), ciprofloxacin (20%), nitrofurantoin (19%), tetracycline (18%), gentamycin (7%), and chloramphenicol (5%). Using the cefoxitin disk-diffusion test, 43.6% of isolates were found to be methicillin-resistant Staphylococcus aureus (MRSA) bacteria. Significant robust in biofilm formation was observed

under shaking conditions. Of 175 isolates, 12 isolates were shown to be strong biofilm producers under stationary conditions whereas 21 isolates exhibited potential to form strong biofilm under shaking conditions. Out of the 72 methicillin-resistant isolates, 62 were MRSA, and 10 were methicillin-resistant coagulase-negative Staphylococci (MRCoNS). In addition, PCR-based amplification revealed that 38 out of 72 methicillin-resistant isolates carried the *mecA* gene. In this study penicillin had the highest resistance, whereas chloramphenicol exhibited the lowest resistance level and 79% of the isolates were found to be MDR (multi-drug resistant). The presence of MRSA in areas associated with public transportation acts as a possible reservoir for transmission. The findings underline the urgent need for enhanced antimicrobial stewardship and infection control measures.

Chapter 1

Introduction

Introduction

As their name implies, *Staphylococci* are gram-positive cocci with a length ranging from 0.5 and 1.0 µm. As they grow, they may form chains, pairs, or clusters. The clusters are the result of *Staphylococci* splitting in two planes. *Staphylococci* are frequently hemolytic and salt tolerant (Lorian, Zak, Suter, & Bruecher, 1985). They are non-motile, facultative anaerobes, and do not produce spores. All of the *Staphylococcal* species colonize mucus membrane and skin. However, they are also called opportunistic pathogens of animals. Although these species are animal colonizers but they can also colonize humans and have been isolated in many clinical samples(von Eiff, Arciola, Montanaro, Becker, & Campoccia, 2006).

According to taxonomic classification, the genus *Staphylococcus* is divided into 40 species some of which are further divided into sub-species. *Staphylococcal* species and sub-species are present in different environments. The most well-known species are *S. aureus*, *S. epidermidis, S. lugdunensis, S. haemolyticus, S. warneri, S. hominis subsp. hominis, S. chromogenes* and *S. capitis* (Becker, Heilmann, & Peters, 2014).

Typically, *Staphylococcal* species were separated into two classes based on how effectively they were able to cause blood plasma to clot i.e., based on the coagulase reaction. Both *S. intermedius* and *Staphylococcus aureus* have the ability to produce coagulase. The other *Staphylococci* lack this ability. The most harmful species of *Staphylococci* that produce coagulase i.e., *S. aureus*. There are already around 30 more species of coagulase-negative *staphylococci* (CoNS). The CoNS are typical skin commensals, yet some species can cause diseases. CoNS are further categorized in two groups based on their susceptibility to novobiocin. *S. haemolyticus, S. hominis, S. schleiferi, S. epidermidis* are the species which are susceptible to novobiocin whereas *S. xylosus* and *S. saprophyticus* are resistant to novobiocin (von Eiff, Peters, & Heilmann, 2002).

The catalase test is essential for differentiating catalase positive *Staphylococci* from catalase negative *streptococci*. The test is done by adding 2-3 drops of 3% H_2O_2 (hydrogen peroxide) to broth culture or agar slant. species that produce catalase immediately bubble. Blood will naturally form bubbles; hence blood agar should not be used for the test (Corrente et al., 2013).

S. aureus can endure extreme conditions such high salt concentrations of up to 15% sodium chloride (NaCl) and a wide pH range of between 4.8 and 9.4 These traits enable it to live in host mucosal and epidermal layer settings as well as on the surfaces of medical equipment (Berlanga, 2010; Somerville & Proctor, 2009). Numerous types of infections can be brought by *Staphylococci*. e.g. *S. aureus* generates localized abscesses, skin lesions like styes and boils, skin infections as well as underlying infections including endocarditis, furunculosis and osteomyelitis. *S. aureus* also is the main contributor to nosocomial infections like wound infections and while medical implant-related infections are caused by *S. epidermidis* (Lowy, 1998).

In addition to releasing superantigens in the blood that cause TSS (toxic-shock syndrome), *S. aureus* also produces enterotoxins into the food source that cause food poisoning. UTIs are brought by *S. saprophiticus*, particularly in females. Rare pathogens include the other *staphylococci* like *S. haemolyticus, S. lugdunensis, S. warneri, S. intermedius* and *S. schleiferi*. Several extracellular proteins and polysaccharides are synthesized by *S. aureus*, some of which are associated with pathogenicity (Diekema et al., 2001).

In the last 20 years, due to an enhanced use of medical implants and an increase in number of immunocompromised patients, infections due to pathogenic microorganisms have increased and coagulase-negative staphylococci play a very crucial role in this aspect. CoNS such as *Staphylococcus* epidermidis are present normally on the mucous membrane and human skin and they can easily adhere to medical devices and form biofilms and cause infection (von Eiff et al., 2002).

S. lugdunensis is a CoNS which causes risky infection of blood stream and is associated with prosthetic devices or materials which are used in cardiovascular surgeries e.g., pacemakers, artificial heart valves, catheters etc. It also causes endocarditis and orthopedic infections which are implant associated, peritonitis, UTI, and osteomyelitis. *S.saprophyticus* which is also CoNS is well known for its ability to cause UTI and also regarded as a uropathogen and is resistant to novobiocin. *S.hemolyticus* is also another well-known CoNS is a common cause of infection in neonates. It also causes bacteremia and community and hospital acquired UTI (Otto, 2013a).

Tens of thousands of fatalities each year occur due to antimicrobial resistance (AMR). It is predicted that if this problem is not solved, more than 10 million deaths will occur each year, much more than cancer deaths. If we look at AMR, then *Staphylococcal* species play a crucial role. Especially *Staphylococcus aureus* plays an important role in many clinical infections such as endocarditis, bacteremia, and numerous other illnesses linked to invasive medical equipment.(Rossi, Pereira, & Giambiagi-deMarval, 2020)

Penicillin was first used clinically to treat S. aureus infections in the 1940s. But soon after, S. aureus acquired a beta-lactamase plasmid and became resistant to penicillin . (Deurenberg et al., 2007)Methicillin was the medicine of choice in 1960 for treating infections brought on by S. aureus infections that were resistant to penicillin. A S. aureus isolate in England dramatically became methicillin resistant within a year as a result of obtaining and integrating a 2.1 kb *mecA* gene in the chromosome. MRSA was the designation given to this isolate.(Katayama, Ito, & Hiramatsu, 2000)

Methicillin resistance is present on MGEs such as "staphylococcal cassette chromosome mec" in *Staphylococci*. MRSA is MDR if its resistant to three or more of the following drugs erythromycin, ciprofloxacin, clindamycin, trimethoprim/sulfamethoxazole, gentamicin, vancomycin and linezolid (Styers, Sheehan, Hogan, & Sahm, 2006).

CA-MRSA has transmission cores where it spreads inside families, close-knit communities, or even through public transportation. In the population, healthy individuals who are asymptomatic are also being infected with CA-MRSA (like in public transport or within families). In many instances, CA-MRSA resettles the nasal cavity or cheek, as well as the axilla, groin and umbilicus. A small proportion of children have tested positive for MRSA colonization. In the context of public transportation (buses, trains, subways) in Japan, the MRSA detection rate stands at 2.5%. Notably, these transportation systems serve as a significant distribution pathway for MRSA (Yamamoto et al., 2010).

The *mecA* gene is a key factor in the establishment and persistence of antibiotic resistance in the context of bacterial infections. *MecA*, which is found in some strains of *Staphylococcus aureus* bacteria, codes for the synthesis of PBP2a, a protein that fundamentally changes how bacteria react to drugs. The methicillin-resistant *Staphylococcus aureus* (MRSA) strain, which is recognized for its resistance to beta-lactam drugs, is the result of this genetic mutation, which has

significant consequences (Ali Alghamdi et al., 2023; Timothy J. Foster, 2017; Guo, Song, Sun, Wang, & Wang, 2020). While MRSA has historically been prevalent in healthcare settings, a different variant known as CA-MRSA has gained notoriety outside of hospitals and clinics. Due to its tendency to infect apparently healthy people outside of the clinical context, CA-MRSA offers a serious problem (Snitser et al., 2020).

According to recent studies, *Staph. epidermiditis* contains genes which after horizontal transfer help *Staph. aureus* to survive in case of infection and resist antibiotics. These qualities are particularly noticeable in the case of MRSA. The most common cause of mortality in hospitalized patients is MRSA. *Staph. aureus* is a highly versatile bacterium that can infect individuals in a variety of ways and overcome immune system defenses. Many other *Staph.* species like *S. epidermidis* rarely cause disease and are typically harmless commensals (Vuong & Otto, 2002). S. aureus has several MGEs, like chromosomal cassettes, pathogenicity islands, plasmids, and transposons, that determine virulence and antibiotic resistance. Different strains of *S. aureus* can easily acquire or lost these MGEs, and this is the major cause of diversity of *S. aureus* strains (Otto, 2013a). Due to the horizontal transfer of antibiotic resistance genes between various staphylococcal species, their ability to form biofilms, and the lack of restricting, these species actively participate in the spread of resistance against various available drugs (Argemi, Hansmann, Prola, & Prévost, 2019).

The increased antimicrobial resistance in *Staphylococcus* species is because of their capability to produce biofilm. In bloodstream related infections, the most isolated fungal and bacterial pathogens are candida albicans and *Staph.* species. Almost 20% of cases of *C. albicans* blood stream infections have been coinfected with *S. aureus* and *S. epidermiditis*. The increased resistance of *Staphylococcus* species in biofilm is due to characteristics, such as the formation of ECM, the development of persister cells, and the overexpression of efflux pumps (Carolus, Van Dyck, & Van Dijck, 2019).

Furthermore, the main factors that determine how virulent or invasive a particular strain of *S. aureus* is its toxins. The superantigens of *S. aureus* like the toxic shock syndrome toxin which causes an aggravated immune response via a large amount of cytokine production and activation of T-cells. Additionally, *S. aureus* releases a wide range of toxins that disrupt practically all the human body's natural defense mechanisms. Other toxin-related illnesses such

as scalded-skin syndrome and TSS are caused by certain strains of *S. aureus*. The presence of toxin genes in strains is directly related to these disorders (e.g., genes coding for exfoliative toxins and toxic shock syndrome toxins). Toxin range can vary greatly between *Staphylococcus* species (Ahmad-Mansour et al., 2021).

Deoxyribonuclease (DNase), coagulase, protease, lipase, hemolysins (alpha, beta, and gamma toxins), and other pore-forming toxins like Panton-Valentine Leukocidin (PVL), among others, are some of the many virulence determinants of S. aureus(Sharaf, El-Sayed, & Abosaif, 2014; Somerville & Proctor, 2009).The fibrin coat that forms on the bacterial surface as a result of coagulase enzyme helps in the evasion of the immune response. This enzyme also helps in the clotting of blood. (Peetermans, Verhamme, & Vanassche, 2015) The toxins that damage membrane are produced by *S. aureus* and other *Staphylococcal* species are α-toxin, δ-toxin, β-toxin, leukocidins and γ-toxin (Cheung, Bae, & Otto, 2021).

Chapter 2

Literature Review

Literature review

2.1 Classification

Staphylococcus has been divided into six subgroups. According to Shaw et al. classification of the specie *Staphylococcus aureus*, organisms of the subgroup I secrete coagulase (1951). They can typically make acid both anaerobically and aerobically from mannitol, and they also make phosphatase. Despite being phosphatase-positive, representatives of subgroup 2 are distinguished from those in subgroup I by their inability to produce coagulase as well as acid using mannitol. These microorganisms are usually discovered in indoor dust as well as on surfaces of people and pigs. Additionally, according to Cavett, 1962, they are frequently found in bacon kept at 30'. Members of the phosphatase-negative subgroups V are strongly connected to subgroups 2. They inhabit environments that are similar to those of subgroup 2 organisms. Individuals of subgroup 3 have not recovered from home dust and human skin, however they are regularly detected on pig skin. In contrast to subgroup 2, this subgroup does not produce acetoin by using glucose or form acid using maltose, while its members are mannitol-negative and phosphatase-positive. Representatives of subgroup IV are phosphatase-negative and acetoin-positive and can only consume a minor variety of carbs. Pork and skin-like surfaces were the only sources of their isolation. Subgroup VI is home to those *Staphylococci* which cannot produce phosphate but can acid using mannitol, typically just aerobically, three varieties within this subgroup can be identified based on their tendency to target lactose and maltose (Baird-Parker, 1963).

2.2 Taxonomy

Many staphylococcal species are classified into 11 categories or groups according to the taxonomy, which is founded on sequences 16s rRNA. The twelfth group, *S. caseolyticus*, is now moved to a latest genus called *Macrococcus*, whose species are presently *Staphylococcus* closest. *Staphylococcus argenteus* and *Staph. schweitzeri*, two previously thought of as types of *S. aureus*, were both classified as species in 2015. *S.edaphicus*, a new species that lacks coagulase, has been discovered in Antarctica. This species likely belongs to the *S. saprophyticus* category. Three groups (B, A, and C) have been suggested as a result of an analysis of the content of orthologous genes (T. Takahashi, Satoh, & Kikuchi, 1999).

According to the comparison of oligonucleotide analysis of the 16S rRNA and DNA-rRNA hybridization, *staphylococci* constitute a cohesive group. This group is part of the larger Bacillus-Lactobacillus-Streptococcus complex, which is used to describe Gram-positive with low DNA G + C concentration. A minimum of 30 *staphylococci* species have been identified using biochemical testing, specifically DNA-DNA hybridization. Eleven out of them can be recovered from people and function commensally. The most pathogenic capability is shared by the common commensals *S. epidermidis* and *S. aureus*. UTIs are frequently brought on by *S. saprophyticus*. Infections in humans can also be brought on by *S. simulans, S. haemolyticus, S. cohnii,S. lugdunensis* and *S. warneri* (Baird-Parker, 1965).

2.3 Structure

Staphylococcus is a Gram-positive bacterium that belongs to the Staphylococcaceae family in the phylum Bacillales. These bacteria look like cocci i.e. spherical shaped when viewed under a microscope and tend to form grape-like bunch or cluster. *Staphylococcus* can grow both anaerobically and aerobically, making them facultative anaerobes. The name *Staphylococcus* was given by Alexander Ogston, a bacteriologist and Scottish-surgeon, in year 1880. He combined prefix "staphylo-" which means "bunch of grapes" in Ancient Greek with the suffix "coccus" which means "spherical bacterium" in Modern Latin (Harris, Foster, & Richards, 2002).

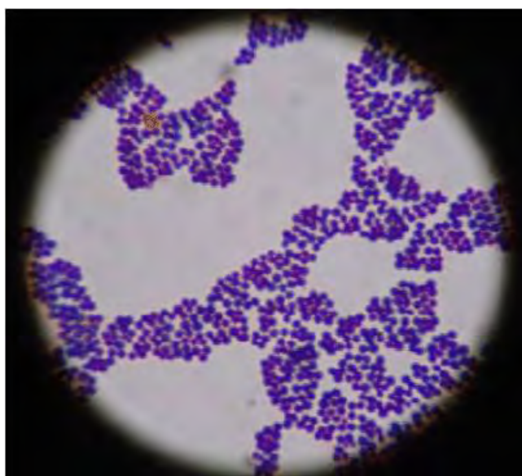


Figure 2. 1 Microscopic view of *Staphylococci*

The difficulty of treating *Staphylococcus* infections has increased as a result of antibiotic resistance, and bacteria are commonly found in hospitals where they can affect those most susceptible to infection. There are approximately 43 species of *Staphylococcus*, some of which do not cause infection or disease and are normally present on mucous-membranes and human beings' skin and on other animals. In addition, *Staphylococcus* species exist in nectar and soil microbiomes (Schleifer & Kroppenstedt, 1990).

2.4 Epidemiology

Establishing the links between isolates obtained while an epidemic is being examined is crucial since *S aureus* plays a substantial role in both CA- and HA-infections. Systems for typing must be reliable, discriminating, and easy to comprehend and use. The traditional method for categorizing *S aureus* is by phage typing. It is dependent on an unreliable phenotypic marker. A recent research at the CDC found that it only types 20% of isolates and requires the maintenance of several phage stocks and spreading strains, making it a process that can only be carried out by specialized facilities (Monaco, Pimentel de Araujo, Cruciani, Coccia, & Pantosti, 2017).

S aureus epidemiology, notably MRSA, has been studied using a variety of molecular typing approaches. Large-scale plasmid analysis is successful, but it has the disadvantage that plasmids are untrustworthy since they may be easily acquired or lost. Other methods like ribotyping and RFLP which uses rRNA genes and various gene probes are also not very successful in determining epidemiology. These methods need the restriction enzyme that is employed to destroy genomic DNA and the probe. Although any PCR primer may be used to differentiate between strains, *S aureus* still lacks a suitable primer. Pulsed field gel electrophoresis, which separates genomic DNA into large fragments of 50–700 kb using a restriction enzyme, is now recognized as the most reliable technique (Huh & Chung, 2016; Lakhundi & Zhang, 2018).

2.5 Isolation and Morphological Identification

Staphylococci would be the first thing seen in the lesion after looking at a clear Gram stain. Because there are only trace levels of bacteria in the blood, culture is necessary before microscopic examination. To isolate the organism, a sample is innoculated onto solid medium such as TSA, BA, or heart-infusion agar. The halo-tolerant *staphylococci* can thrive when samples that are susceptible to contamination with other bacteria are cultured on mannitol salt agar that contains sodium chloride (7.5 percent). Gram staining and other tests like coagulase and catalase production are done because they enable fast identification of the *S aureus* that is coagulase-positive (Majumdar & Gupta, 2020).

Another effective way to check for *S aureus* is to make thermostable deoxyribonuclease. In this method, fibrinogen- and immunoglobulin-coated latex particles attach to clumping factor and protein A on the surface of bacteria, respectively. and *S. aureus* colonies can be identified by agglutination when combined with latex particles. They are available commercially in market (like Staphayrex).(Stevens & Geary, 1989) Another new latex test like Pastaurex uses monoclonal antibodies against eight capsular polysaccharide and serotype 5 so that to decrease false negative results. Recently, such *S. aureus* clinical isolates have been discovered that don't produce clumping factor or coagulase which leads to difficulty in their identification (Furuhata et al., 2016).

The isolation of *Staph. epidermidis* and in less number remaining CONS from blood is crucial and not accidental contamination because they are linked with hospital - acquired infections linked to implanted medical devices, especially if subsequent blood cultures have been found positive. Today, marketed biotype detection kits like API Staph-Trac, API Staph Ident, Microscan Pos Combo and GPI Card are used to identify other *Staphylococcus* species and *S epidermidis*. These are prefabricated strips with test substrates in them (Almeida & Jorgensen, 1983).

2.6 Biochemical identification

A strain must meet certain criteria in order to be considered a part of the *Staphylococcus* genus, including being a coccus which is Gram-positive , clustering, having the proper structure of the cell walls (including the presence of teichoic acid and the peptidoglycan

type), and having DNA with a G + C composition of between 30 and 40 mol%.(Paul, Rahman, Salam, Khan, & Islam, 2021) Several quick assays can tell *Staphylococcus* species apart from other Gram-positive, facultatively anaerobic and aerobic cocci. The varieties of *Staphylococcus* seem to be facultative anaerobes (have ability to grow both anaerobically and aerobically). Bile salts promote the growth of all *Staphylococcus* species, It was formerly thought that all species of *S. aureus* produced coagulase, but this has since been demonstrated false. A fluid containing 6.5% NaCl can also support growth. On Baird Parker medium, *Staphylococcus* species grow fermentatively with a single exception of *Staph. saprophyticus*, that grows oxidatively. *Staphylococcus* is bacitracin resistant where concentration of disc is 0.04 U and resistance is equal to or less than 10 mm inhibition zone and is furazolidone susceptible i.e. concentration of disc is 100 µg and resistance is equal to or less than 15 mm zone. To determine down to the species level, additional biochemical testing is necessary (Matthews, Roberson, Gillespie, Luther, & Oliver, 1997).

2.7 Staphylococcal infections

The bacterium *S. aureus* commonly causes boils, impetigo, furuncles, and other superficial skin diseases in people. Furthermore, it might lead to more serious infections, especially in those who are already frail due to a chronic illness, burns, or immunodeficiency. Only a few of the ailments that are more commonly associated with hospitalized patients than with healthy persons in the community include meningitis, endocarditis, severe abscesses, mastitis, phlebitis, and osteomyelitis. Infections linked to implantable equipment, such as cardiovascular devices, joint prostheses, and artificial heart valves, are frequently caused by S epidermidis and S aureus (Chalmers & Wylam, 2020; Lowy, 1998).

In addition to *S. aureus*, other *Staphylococci* can also infect people. Infections in catheters and prosthetic devices are often caused by *S. epidermidis*, the most important CNS species. Additionally, CNS may result in endocarditis in patients with prosthetic heart valves and peritonitis in those on ongoing renal dialysis. These pathogens are frequently not acquired in hospitals. Occasionally occurring pathogens include *S. warneri, S. haemolyticus, S. hominis, S. intermedius, S. capitis, S. simulans and S. schleiferi.*(Becker et al., 2014) A recently discovered species is called *S. lugdunesis*. Because other infections and endocarditis instances have been documented, it is presumably more dangerous than other species of

CNS. It's likely that the incidence of illnesses caused by such organisms is overlooked because of difficulties in identification (Michalik et al., 2020).

It might be difficult to diagnose infections of the CNS. Most infections start slowly and exhibit few visible signs. This is so because *S. aureus* infections have more virulence factors and hazardous chemicals. Although *S. lugdunensis* is occasionally mistaken for *S aureus*, *S, epidermidis* is a common skin surface bacterium and one of the most common contaminants of samples sent to clinical laboratories. For the precise identification of CNS species, expensive diagnostic kits as API-Staph are necessary (Mulder, 1995).

2.8 Coagulase production

The ability of *staphylococci* to generate coagulase, an enzyme which results in the formation of blood clots, is one of the most significant phenotypical traits utilized for classifying *staphylococci*. *Staph. schleiferi* subspecie coagulans, *Staph. delphini, Staph. aureus, Staph. hyicus, Staph. lutrae,S. intermedius and Staph. pseudintermedius* are the seven species presently classified as coagulase producers. These species fall under two distinct groups which are the group *S. aureus* (which includes only one specie i.e., *S. aureus*) and group *S. hyicus* intermedius group (which includes 5 species). *Staphylococcus leei*, an eighth species that was isolated from patients with gastritis, is additionally identified (Jin, Rosario, Watler, & Calhoun, 2004).

Coagulase production is present in *S. aureus*, which is coagulase positive. Even though most *Staphylococcus aureus* strains produce coagulase, some might be abnormal or atypical if they are unable to produce. Catalase testing is helpful in differentiating *staphylococci* from streptococci and enterococci because *S. aureus* can produce enzymes called catalase and convert hydrogen peroxide to oxygen and water. *S. epidermidis,* which is coagulase-negative and lives on the epidermis, can infect people seriously if they have venous catheters or are immunosuppressed. S. saprophyticus, another coagulase-negative bacterium that is a normal member of the vaginal flora, is mostly to blame for UTIs in young, sexually active women. Additional *Staphylococcus* species, such as *S. caprae, S. schleiferi*, and *S. lugdunensis*, have lately been connected to human disorders. CoNS, CNST,

or CNS are frequent acronyms for *staphylococci* that are coagulase negative. The term "CoNS" refers to coagulase-negative *staph.* according to the ASM (Becker et al., 2014).

2.9 Adherence and its role in virulence

The pathogen enters the host and connects to tissues or cells of the host to cause an infection. *S. aureus* clings to proteins in the host. *S aureus* cells secrete proteins that help the host cells adhere to extracellular matrix proteins including fibronectin and laminin. Fibronectin can be found on the surfaces of endothelial and epithelial cells in addition to in blood clots. Additionally, the majority of strains produce the clumping factor i.e., a fibrin/fibrinogen binding protein that facilitates adhesion to injured tissue and blood clots. Fibrinogen binding proteins and fibronectin are expressed by most *S. aureus* strains. The strains responsible for septic arthritis and osteomyelitis are particularly linked to the receptor that encourages binding to collagen. In order to encourage bacterial adhesion to injured tissue when the bottom layers have been accessible, collagen interaction is also crucial (Dickinson & Bisno, 1989).

Experiments show that matrix-binding proteins of *Staphylococci* serve as virulence factors. In an animal experimental model of endocarditis, mutants with impaired binding to fibrinogen and fibronectin showed lower pathogenicity, indicating that fibrinogen and fibronectin facilitate bacterial adhesion to the sterile vegetations produced by endothelial surface damage in case of heart valve. In animal experimental model of septic arthritis, mutants deficient in the collagen-binding protein showed decreased pathogenicity (Heilmann, 2011).

As we know *S. epidermidis* and *S. aureus* can cause infections related to medical implants, e.g., infections related to catheters, implantable heart valves and prosthetic joints. The human body coats such implantable devices with a complex mix of platelets and host proteins very soon after it is implanted in body. Fibrinogen was demonstrated to be the predominant agent in one model organism including brief contact between biomaterial and blood and was predominantly in charge of *S. aureus* adhesion in following in vitro testing. Contrarily, fibrinogen is destroyed and no longer encourages bacterial adhesion in materials that have been inside the body for a prolonged amount of time, such as devices

such as catheters. Instead, intact fibronectin takes over as the primary protein encouraging attachment (Paulitsch-Fuchs et al., 2022; Sabaté Brescó et al., 2017).

Less is known about the pathophysiology of infections produced by *S. epidermidis* than *S. aureus*, in comparison. Adhering is undoubtedly a crucial first step in the growth of infections. The connection between the implant's plastic and S epidermidis has been extensively studied, and PS/A (polysaccharide adhesion) is observed. In an experimental model of an animal for studying medical implants associated infection, mutants missing polysaccharide adhesion are much less pathogenic or virulent, and vaccination with pure PS/A is safe. Interactions of bacteria with plastic most likely play a significant role in catheter colonization at the place of entrance. Yet proteins of host soon accumulate on implants. Although less strongly than that of *S. aureus*, most isolates of S epidermidis bind to fibronectin but not fibrinogen (Switalski et al., 1983). It is unknown, nevertheless, if the presence of a protein akin to *S. aureus*' fibronectin-binding protein is taking part.is at play. The formation of "slime" is a trait of S epidermidis isolates of clinical domain. It is a contentious subject. There is a belief that slime formation in vitro could be an indication of a microorganism's ability to create a biofilm in vivo, such as on implantable devices surface, and may serve as a virulence factor. In the laboratory, slime is generated as a biofilm on growth vessel's surface during broth growth. The slime's composition is likely affected by the type of growth medium used. A study using a defined medium demonstrated that the slime primarily contained teichoic acid, a polymer usually present in *Staphylococci*'s cell wall. Polysaccharides in bacterial slime from solid medium growth are often obtained from the agar (Hussain, Wilcox, & White, 1993; Vasileiou et al., 2018).

2.10 Overcome Host Defenses

The host defensive mechanisms may be hampered by a number of elements *Staph.* species express. Strong proof that these characteristics contribute to pathogenicity is missing.

2.10.1 Polysaccharide Capsule

Most clinical strains of *S. aureus* generate surface polysaccharides of serotype 8 or 5. In contrast to the countless capsules of other bacteria, which are all visible by light microscopic examination, this is why it is called a microcapsule since it can only be detected by electron microscopy after antibody tagging. *S. aureus* that has been obtained from lesions exhibits high levels of polysaccharide. What the capsule performs isn't entirely apparent. Although in vitro studies only provided evidence when complement was absent, it can make phagocytosis harder to perform. In contrast, experiments in an endocarditis experimental model comparing a mutant strain with a faulty capsule and a wild-type revealed that polysaccharide production may have prevented damaged heart valve colonization by hiding surface proteins (adhesins) (Kuipers et al., 2016; Tuchscherr, Löffler, Buzzola, & Sordelli, 2010).

2.10.2 Protein A

The early binding of bacterium to host cells is mediated by MSCRAMM adhesin proteins, that are called "microbial surface components-recognizing adhesive matrix-molecules." This is a crucial stage in the establishment of infection. *Staph. aureus*'s protein A, clumping factor A, SdrG , fibronectin binding protein A , *Staphylococcus* epidermidis' M protein, and protein G in other Streptococcus species are a few examples. Except for protein A, which attaches IgG, all other MSCRAMMs attach to fibrinogen. Other known MSCRAMM targets include fibronectin. The Fc portion of specific antibodies is where Protein M attaches. MSCRAMMs are potentially therapeutic and have primarily been investigated in Gram positive bacteria. Tefibazumab, a monoclonal antibody that has been studied in a phase II study, has been shown to target clumping factor A (T. J. Foster, 2019; Rivas, Speziale, Patti, & Höök, 2004).

Protein A, a surface protein that *S aureus* displays through its Fc region, is coupled to immunoglobulin G. By using this non-immune method, bacteria will incorrectly attach serum IgG. This tends to prevent opsonization and phagocytosis. Furthermore, investigations with mutants in infection models indicate that protein A increases pathogenicity. In vitro phagocytosis of *S aureus* mutants lacking protein A is also improved (Shi et al., 2021).

2.10.3 Leukocidin

The toxin that *S. aureus* can generate exclusively affects polymorphonuclear leukocytes. Given that phagocytosis is an essential defense mechanism against *Staphylococcal* infection, leukocidin should be a virulence factor (Ahmad-Mansour et al., 2021).

2.11 Damage to the Host

S. aureus can produce a wide range of protein toxins that are likely to be the cause of infection-related symptoms. Some can cause hemolysis by damaging the erythrocytes' membranes, but this is unlikely to be important in living things. Leukocytes' membrane is damaged by the leukocidin, but it is non-hemolytic. TSST-1 and Enterotoxins produce toxic shock, while systemically production of α-toxin produces septic shock (Ahmad-Mansour et al., 2021; Tam & Torres, 2019).

2.11.1 Toxins produced by *Staphylococcus*

Staphylococcus can produce toxins or penetrate through the skin and inflict a broad range of diseases on both people and animals. *Staphylococcal* toxins, which are made by bacteria trying to grow in unsafe food items, are a frequent reason for food poisoning. *Staphylococci* infections are the main bacterial cause of sialadenitis (Oliveira, Borges, & Simões, 2018).

2.11.1.1 α-toxin

The most effective and thoroughly studied *S. aureus* toxin that damages membranes is called α-toxin. It appears like a monomer that attaches to the cell membranes that are vulnerable. Following this, subunits oligomerize to create hexameric rings with a pore in between by which contents of cell leak. When the toxin binds to a specific receptor on the surface of sensitive cells, it forms microscopic holes that let monovalent cations flow through. The toxin's non-specific reaction with membrane lipids at greater doses results in wider pores that allow tiny molecules and divalent cations to pass. Yet, it is questionable if this applies under typical physiological circumstances. monocytes and platelets in individuals are especially vulnerable to α-toxin (Bhakdi & Tranum-Jensen, 1991). They have high affinity regions that enable toxin binding at physiologically acceptable levels. Cytokines and Eicosanoids are released as a result of a complicated chain of secondary events, which then spark the release of inflammatory mediators. Septic shock symptoms, which appear during serious infections brought on by S aureus, are brought on by these occurrences. Studies

using the pure toxin in organ culture and animals lend support to the idea that -toxin is a key component of *S. aureus*'s pathogenicity. Moreover, mutants deficient in α-toxin exhibit decreased virulence in a number of infection scenarios of animals (Freer & Arbuthnott, 1982).

2.11.1.2 β-toxin

A sphingomyelinase called β-toxin harms membranes that contain a large amount of this lipid. Lysis of RBCs of sheep is the conventional test for β-toxin. The most of *S. aureus* isolates from individuals lack β-toxin expression. In the toxin-encoding gene, a lysogenic bacteriophage is integrated. Negative phage conversion is the term for this process. The variable for both staphylokinase and an enterotoxin is carried by a few of the phages that inhibit the activity of the β -toxin gene. The most of specimens of bovine mastitis, in contrary, produce β-toxin, indicating that toxin is crucial in causing mastitis. It is confirmed by discovery that mutants lacking the β -toxin exhibit decreased pathogenicity in an animal experimental model of mastitis (Menestrina et al., 2003; Reyes-Robles & Torres, 2017).

2.11.1.3 δ-toxin

Several *S. aureus* strains produce the little peptide toxin known as " δ -toxin." *S. lugdunensis* and *S epidermidis* also synthesize it. It is unclear how δ-toxin contributes to disease (Su et al., 2020).

2.11.1.4 γ-toxin and leukocidin

Two-component toxins (protein) that harm the sensitive cells membranes include leukocidins and the γ-toxin. Although the proteins are synthesized independently, they work with each other to harm membranes. There's no proof that they multimerize before inserting themselves into membranes. Three proteins are synthesized by the locus of γ-toxin. While the components B and A are only mildly leukotoxic and hemolytic, the C and B components generate a leukotoxin with low hemolytic activity. The leukotoxin produced by the locus γ-toxin differs from the traditional PV leukocidin. It is not hemolytic, unlike γ-toxin, and has strong leukotoxicity. According to one study, just 2% of *S aureus* isolates produce PV leukocidin, but 90% of *S aureus* isolates recovered from necrotic lesions synthesize PV toxin. According to this, PV leukocidin may have a significant role in necrotizing infections of skin. Injecting PV-leukocidin under the skin in rabbits results in

dermo necrosis. In addition, the toxin generates inflammatory cytokines from individuals' neutrophils at low levels, resulting in degranulation. This may explain the histopathology of infections with dermonecrotic tissue (vasodilation, penetration (infiltration) and necrosis) (Conan et al., 2021; Supersac, Piémont, Kubina, Prévost, & Foster, 1998).

2.11.2 Superantigens: TSST-1 and enterotoxins

Enterotoxins, which come in six different serotypes (E, A, C, B, D, and G), and TSST-1 are two different forms of superantigen-active toxins that *S aureus* can produce. When consumed, enterotoxins produce vomiting and diarrhea and are the cause of food poisoning. Enterotoxins have the potential to manifest systemically and result in TSS; in fact, enterotoxins C and B are responsible for 50% of TSS (non-menstrual). TSST-1 lacks emetic function and has a very complicated relationship to enterotoxins. All cases of menstrual TSS, including 75% of all TSS, are caused by TSST-1. staphylococcal infection can result in TSS if an TSST-1 or enterotoxin is systemically produced, and the individual is deficient in the necessary neutralizing antibodies. TSS gained popularity after the development of highly absorbent tampons, and despite a sharp decline in the number of instances, they persist despite the removal of some brands from the market. (Schlievert & Davis, 2020) Superantigens excite T lymphocytes without the usual antigenic identification of the antigen. Just 1 in 10k T cells are stimulated after antigen presentation, whereas 1 in 5 T cells can be activated. The symptoms and manifestations of TSS are brought on by the large-scale release of cytokines. Outside of the typical antigen-binding region, superantigens directly bind to MHC-II of APCs. This complex solely detects the T - cells receptor's Vβ element. In contrast to how antigen specificity is often required for binding, any T - cell with the relevant Vβ element can thus be stimulated (Prechtl et al., 2000).

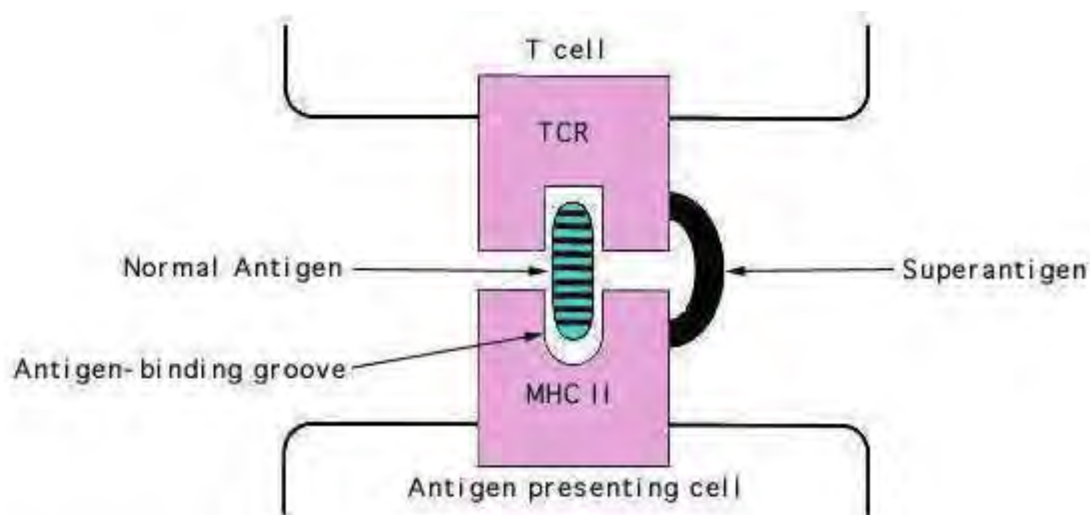


Figure 2. 2Superantigens and the non-specific T cells stimulation. (Abdulqader, Bakr, & Al-Hamdi, 2012)

2.11.3 Exfoliative or epidermolytic toxin (ET)

In newborns, this toxin results in scalded skin condition, which is characterized by extensive blistering and epidermal loss. The toxin comes in two antigenically different forms, ETB and ETA. These toxins appear to possess protease action. The three main crucial amino acids in the protease's active region are retained, and both toxins share a sequence resemblance with the serine protease of S aureus. Moreover, the toxin activity was fully abolished by switching the serine active site with glycine. Nevertheless, ETs have esterase activity but no detectable proteolytic activity. The esterase activity role in epidermal splitting is unclear. It's likely that toxins attack a very particular protein that plays a role in preserving the epidermis's integrity (Bailey, Lockhart, Redpath, & Smith, 1995).

2.12 *Staphylococci* resistance to antimicrobial drugs

S. aureus strains obtained from hospitals have been found to be resistant to many antibiotics, including all clinically available drugs, except for teicoplanin and vancomycin. Methicillin resistance, known as MRSA, is prevalent among most methicillin-resistant strains and is often accompanied by multiple drug resistance. *Enterococci* have been found to carry plasmid for vancomycin resistance, which can be transferred to *S. aureus* in both laboratory and natural settings. S. epidermidis hospital-acquired isolates are also often resistant to

various antibiotics, such as methicillin. Along with antibiotic, *Staph. aureus* may also resist disinfectants and antiseptics like quaternary-ammonium compounds, which may help it survive in the environment of hospital (Lyon & Skurray, 1987).

Staphylococci have acquired antibiotic resistance through genetic means, including chromosomal gene mutations, extrachromosomal plasmid insertion, and the addition of new genetic material to the chromosome through transposons or other forms of DNA insertion. Recently, determinants from plasmids have been introduced into the chromosome at a location close to the determinant for methicillin resistance. Because they are more secure, chromosomal resistance determinants are beneficial to the organism. Antibiotic resistance in bacteria primarily occurs through the following mechanisms: (1) deactivation of the drug by enzymes; (2) modifications to the target or binding site of the drug to stop binding; (3) increased drug efflux to avoid harmful concentrations collecting in the cell; and (4) an escape mechanism in which a replaced target is expressed which is drug-resistant (Noble, 1997).

In 2019, *S. aureus* was recognized as the 2nd most frequent pathogen for fatalities linked to antimicrobial resistance. The production of penicillinase, a type of beta-lactamase that breaks down the β-lactam ring of molecule penicillin and renders drug inefficient, is the mechanism by which *staphylococci* develop tolerance to penicillin. Methicillin, oxacillin, dicloxacillin cloxacillin, flucloxacillin and nafcillin, are examples of β-lactam medicines which are penicillinase-resistant and can withstand breakdown penicillinase produced by *staphylococcus*. The mec operon, a component of SCCmec, enables methicillin resistance. The SCCmec family of MGEs have a significant role in the growth of *S. aureus*. The *mecA* gene, which produces a modified penicillin-binding protein (PBP2' or PBP2a) with a decreased ability to attach beta-lactams (cephalosporins, penicillins, and carbapenems), imparts resistance.. This makes all β-lactam drugs prone to resistance and prevents their therapeutic application in infections due to MRSA. According to studies, distinct MRSA strains do not share a common ancestor as MGEs were gained by various lineages in independent gene transfer processes. It's interesting to note that one research contends that MRSA compromises virulence, such as the ability to produce toxins and spread quickly, in order to survive and form biofilms (Jamrozy et al., 2017; Lakhundi & Zhang, 2018).

The action of aminoglycoside drugs, such as gentamicin, kanamycin and streptomycin, on staphylococcal infections is effected by protonated hydroxyl or amine association with the rRNA of microorganisms 30S subunit of the ribosome. Currently, it is generally accepted that there are three primary mechanisms of resistance against aminoglycosides i.e. enzymes for aminoglycoside modifications, mutations in ribosomes, and outpouring of drug from inside to outside. By covalently adding a nucleotide, acetyl , or phosphate component to alcohol or the amine functional group (or both) of the drug, AME inhibit the activity of aminoglycoside. This alters the antibiotic's charge and inhibits it, lowering its affinity for binding to ribosomes. In enterococci, the *vanA* gene comes from transposon Tn1546 present inside a plasmid and encodes for enzyme which develops an altered peptidoglycan so that vancomycin will just not attach to it, this is the mechanism by which glycopeptide resistance develops (Carter et al., 2000; Mlynarczyk-Bonikowska, Kowalewski, Krolak-Ulinska, & Marusza, 2022).

One of *S. epidermidis'* most potent pathogenic traits is its capacity to create biofilms on plastic items. Antibiotics like fluoroquinolones, rifamycin, gentamicin, tetracycline, sulfonamides and clindamycin are frequently ineffective against *S. epidermidis*. The prevalence of methicillin resistance is especially high, with 75–90 percent of medical samples exhibiting methicillin resistance. The intestine is where resistant microorganisms are most frequently located, but organisms that are present on the epidermis or on skin surface can also develop resistance from repeated interaction with antibiotics released in sweat (Michael Otto, 2009).

2.12.2 Cefoxitin disk diffusion test for MRSA identification

A diagnostic procedure used to identify methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria is the cefoxitin/oxacillin disk diffusion test. An agar plate that has been inoculated with the test bacteria (often *Staphylococcus aureus*) is used in the cefoxitin/oxacillin disk diffusion test. Antibiotic disks containing cefoxitin or oxacillin are then put on the plate. After 24 hours of incubation at 37° C, if the zone of inhibition surrounding the cefoxitin or oxacillin disks is less than or equal to 21 mm, the strain is thought to be sensitive to methicillin (MSSA). The strain is regarded as methicillin-resistant (MRSA) if the zone of inhibition surrounding the cefoxitin disks is less than 21 mm. More

conclusive testing, such as the identification of the *mecA* gene utilizing molecular techniques like PCR, can be used to further establish the presence of MRSA. Mostly cefoxitin is preferred over oxacillin for MRSA identification (Anand, Agrawal, Kumar, & Kapila, 2009a, 2009b; Bonjean et al., 2016).

2.12.3 Resistance mechanisms against commonly used drugs against *staphylococci*

Aminoglycosides are used in a synergistic manner, either with beta lactam or glycopeptides, to treat Staphylococcal infections. (Hu et al., 2015) The development of antibiotic-modifying enzymes, such as acetyltransferases and aminoglycoside-modifying enzymes (AMEs), can cause gentamicin resistance in *Staphylococci* (Dowding, 1977; Schmitz et al., 1999). The APH(2")/AAC(6') gene, which genes for a bifunctional enzyme with APH(2″) and AAC(6′) action, can also play a role in *staphylococci* resistance to gentamicin and other aminoglycosides. The Tn4001 composite transposon, which is found in both *Staph. aureus* and CNS, frequently harbors this gene (Schmitz et al., 1999).

Active efflux is caused when the *tetK and tetL* genes are acquired from a plasmid, which is one mechanism of tetracycline resistance in *Staphylococcus* species..(Emaneini et al., 2013; Schmitz et al., 2001; Trzcinski, Cooper, Hryniewicz, & Dowson, 2000) Tetracycline resistance can also result via ribosome protection, which is controlled by *tetM* or *tetO* genes located on chromosomes or in transposons. These genes create proteins that bind to the ribosome and prevent tetracycline from binding, protecting the bacterial cell from the antibiotic's inhibitory effects (Speer, Shoemaker, & Salyers, 1992; Trzcinski et al., 2000).

For nitrofurantoin resistance, lack of expression of UhpT transporter, which is in charge of bringing nitrofurantoin into the bacterial cell, is one of the prevalent processes. Another method is the existence of enzymes that can deactivate nitrofurantoin and are expressed by the gene fosA3 (Sorlozano-Puerto, Lopez-Machado, Albertuz-Crespo, Martinez-Gonzalez, & Gutierrez-Fernandez, 2020).

Penicillin resistance is a concern. Beta-lactamases are encoded by the *blaZ* gene, which is often located inside the blaI-blaR1-blaZ operon that is present in many plasmids and transposons. In staphylococcal isolates, the *blaZ* gene can confer penicillin resistance (Achek et al., 2018; Howden et al., 2023). Resistance to chloramphenicol can happen through the synthesis of the enzyme CAT (chloramphenicol acetyltransferase) , which

renders thiamphenicol and chloramphenicol inactive (Udo, Boswihi, Mathew, Noronha, & Verghese, 2021).

Staphylococcus aureus is highly resistant to SXT due to the dihydrofolate reductase gene, or *dfr* gene Dihydrofolate reductase's structure changes as a result of dfr gene mutations, rendering it less vulnerable to trimethoprim inhibition (Sato, Ito, Kawamura, & Fujimura, 2022). *Staphylococci* have been reported to have highly transferable resistance to trimethoprim (Eliopoulos & Huovinen, 2001).

Rifampicin resistance in *Staphylococcus aureus* is brought on by mutations in the RRDR (rifampicin resistance determining region), a highly stable region of *rpoB* gene (Wang et al., 2019) All of the rifampicin-resistant isolates were discovered to have mutations in the RRDR of the *rpoB* gene in a research on rifampicin-resistant. The *rpoB* gene's RRDR mutations can result in amino acid alterations that change how rifampicin binds to the RNA polymerase, decreasing the antibiotic's potency (Huang, 2021)

Numerous processes can lead to quinupristin/dalfopristin resistance. The *vgaA* gene, that produces an ABC transporter that effectively pumps the antibiotic out of the bacterial cell, is one of the common processes (Achek et al., 2018; Duran, Ozer, Duran, Onlen, & Demir, 2012). The existence of the erm genes, acts as another method (Abbas, Srivastava, & Nirwan, 2015; Uzun et al., 2014).

Resistance against MLSB group is due to erm genes, which encode methylases that change the 23S rRNA component of the bacterial ribosome, are the cause of the clindamycin, erythromycin and quinupristin/dalfopristin resistance. The erm genes exist on plasmids or transposons, which can move from one bacterium to another. The introduction of MGEs like transposons and plasmids may cause high clindamycin resistance in staphylococcal isolates from transportation (Yılmaz & Aslantaş, 2017) (Duran et al., 2012; Nicola, McDougal, Biddle, & Tenover, 1998).

2.12.1 Methicillin resistant staph aureus or MRSA

MRSA strains gain widespread recognition as serious hospital - acquired infections, which has a significant effect on patient management in hospital settings and raises cost of health care significantly. SCCmec, which contains the *mecA* gene, is inserted into a chromosome to

give *S. aureus* resistance to methicillin. This gene produces the changed penicillin-binding PBP-2a protein, that is unaffected by b-lactam antibiotics currently in use. The identification of MRSA can be done using a variety of antimicrobial susceptibility tests, such as cefoxitin or oxacillin disk diffusion technique, the oxacillin screening test, and the oxacillin MIC test. According to what appears to be a large number of reports, such conventional antimicrobial tests can yield MRSA identification results that are both mistakenly positive and incorrectly negative. Therefore, it's crucial to use more specialized and focused procedures, such as PCR, which is recognized as a DNA-based test. *MecA* is not present in MSSA (methicillin-sensitive *Staphylococcus aureus*) isolates, so finding this gene in either *S. aureus* isolates is a sign of MRSA. According to studies, *mecA*-positive *Staphylococci* exhibit a higher incidence of antimicrobial resistance than that Staphylococci that are *mecA*-negative. Additionally, research suggests MRSA isolates oftenly contain genes for resistance to other antibacterial drugs (Lakhundi & Zhang, 2018; Otto, 2013b).

2.13 Biofilm

A biofilm is a collective of microorganisms that cooperate, frequently adhering to a surface, and encasing themselves in a slimy extracellular matrix composed of EPS (extracellular polymeric substances). The matrix is made up of extracellular polysaccharides, DNA, proteins, and lipids, among other things. Due to their three-dimensional design and portrayal of a communal culture for microorganisms, they have been dubbed "cities for microbes" figuratively. The most extreme habitats can support the growth of biofilms, including frozen mountains and super-hot, briny hot springs with waters that range in pH from quite acidic to quite alkaline. many gram-positive bacteria including *staphylococcus* species form biofilm (Hall-Stoodley, Costerton, & Stoodley, 2004; López, Vlamakis, & Kolter, 2010).

Biofilms can form on non-living or living surfaces in hospital, industrial, and natural settings, and may be a part of a larger microbiome. In reaction to a variety of stimuli, including nutritional stimuli, the stimulation of cells to less or sub-inhibitory antibiotic concentrations, and cellular detection of particular or non-specific sites of attachment on the surface, microbes can develop biofilms. This allows for subpopulations of cells to perform specific activities such as sporulation, matrix synthesis, and motility, all of which contribute to the biofilm's overall efficacy (Aggarwal, Stewart, & Hozalski, 2015).

In biofilms, which are more than just bacterial slime films, the bacteria arrange into a well-organized functional community. To support the biofilm's general success, subgroups of cells in it carry out various tasks for motion, sporulation, and matrix production. In addition to being able to exchange nutrients, bacteria residing in biofilms are also shielded from environmental dangers including dryness, antibiotics, and the host body's immune system. Normally, when a free bacteria sticks to a surface, a biofilm begins to develop (Momeni, 2018).

2.13.1 Basis of biofilm

The emergence of biofilms is believed to have occurred as a survival strategy for prokaryotes in primitive Earth due to the harsh environmental conditions. These structures have been present in the Earth's oldest fossil for over 3.25 billion years, and they provide homeostasis to prokaryotic cells and promote complex cell interactions within the biofilm (Hall-Stoodley et al., 2004).

2.13.2 Development of biofilm

Free-swimming bacteria connect to a surface using hydrophobic properties and weak van der Waals forces to create the biofilm. These early colonist bacteria can create a more lasting anchor using structures like pili if they are not quickly detached from the surface. The capability of bacteria to make biofilms can be influenced by their hydrophobicity. Bacteria that are more hydrophobic have less repulsion between their cells and the surface they attach to, allowing them to anchor themselves more easily. However, non-motile bacteria have difficulty recognizing surfaces and aggregating, making it challenging for them to colonize on their own (Briandet, Herry, & Bellon-Fontaine, 2001).

During the colonization of the surface, bacteria cells can communicate through QS products (N-acyl homoserine lactone). As a result of cell division and recruitment, the biofilm expands and it becomes enclosed by a polysaccharide matrix. Within the biofilm, the matrix can entrap QS autoinducers, providing a defense mechanism against predators and promoting bacterial survival. The biofilm matrix may also include materials from the surroundings such as soil particles, minerals, fibrin and erythrocytes. The last phase of biofilm formation, dispersion, is when the biofilm reaches its final size and shape. Biofilm formation can enhance the resistance or susceptibility of bacterial colonies to antibiotics.

Quorum sensing, or cell-to-cell communication, is crucial for the development of biofilms across various bacterial species. The end result of a microbial growth process is a biofilm (Monroe, 2007; H. Takahashi, Suda, Tanaka, & Kimura, 2010). The diagram below illustrates the five main phases of bacterial growth, which serve as a summary of the process.

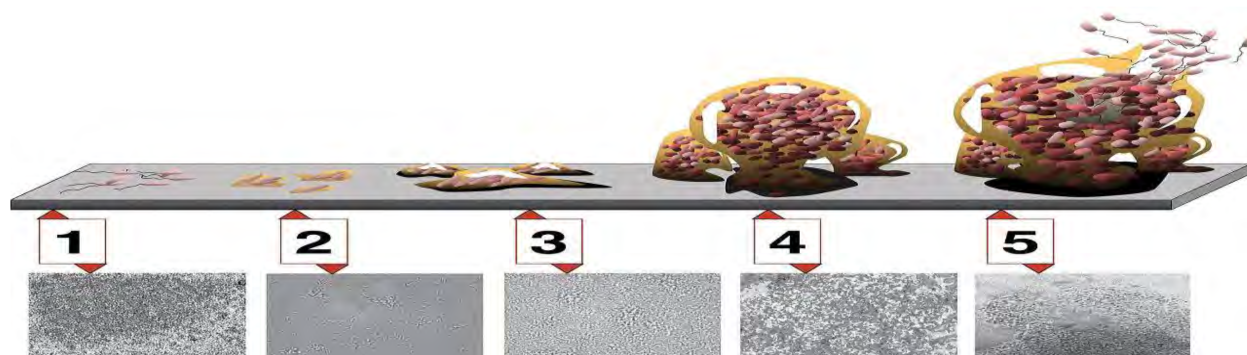


Figure 2. 3 Five phases of biofilm development i.e. (1)Initial attachment, (2)Irreversible- attachment, (3)Maturation I, (4)Maturation II, and (5) Dispersion (Monroe, 2007).

2.13.3 Developed biofilm structure

An environment with a diversity of subpopulations and a heterogeneous composition defines biofilm. A biofilm structure is made up of metabolically inactive cells (alive but uncultivable cells and persisters), active cells (tolerant and resistant) and also a molecular matrix made up of extracellular DNA, polysaccharides, and proteins. Due to the compact and packed structure, the development of biofilm is linked to an enhanced degree of mutations and HGT (horizontal gene transfer). Quorum sensing, a method of communication used by bacteria in biofilms, activates genes that contribute to the development of virulence factors (Hall & Mah, 2017; Rapacka-Zdonczyk, Wozniak, Nakonieczna, & Grinholc, 2021).

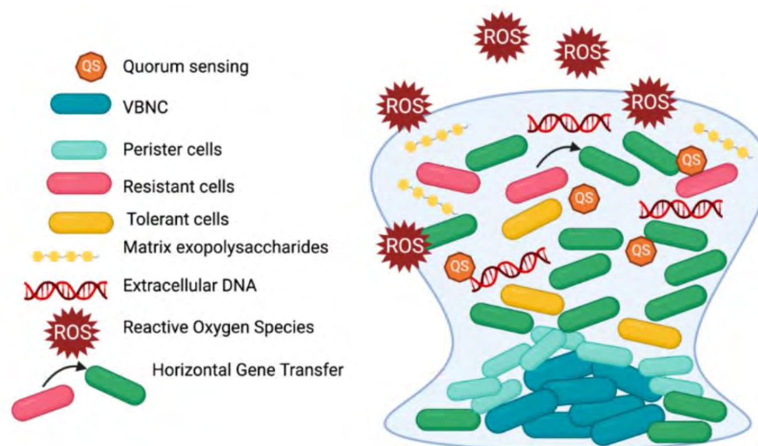


Figure 2. 4 Structure of mature or developed biofilm(Rapacka-Zdonczyk et al., 2021)

2.13.4 Extracellular matrix of biofilm

EPS matrix is made up of proteins, exopolysaccharides, and nucleic acids, with some components being hydrophobic while others are hydrated.(Branda, Chu, Kearns, Losick, & Kolter, 2006; Danese, Pratt, & Kolter, 2000) One example of a hydrophobic component is cellulose, that is made by various microorganisms. This matrix serves as a protective casing for the cells and enables them to communicate through gene transfer and biochemical signals. The matrix essentially functions as an external digestive process and permits steady, beneficial microconsortia of various species. Certain biofilms have water channels which can assist in distribution of signaling molecules and nutrients. The EPS matrix is so powerful to fossilize biofilms under specific conditions (Flemming et al., 2016).

Bacteria living in biofilms show distinct characteristics from free living bacteria of the same group, as the dense surrounding allows for cooperation and interaction among them. This setting also provides greater resistance to antibiotics and detergents, with the external layer of cells and the extracellular matrix protecting the interior community (Pandey, Mishra, & Shrestha, 2021; Stewart & Costerton, 2001). Antibiotic resistance in some instances can rise up to 5,000 times (Del Pozo, Rouse, & Patel, 2008). In both archaeal and bacterial biofilms, lateral transfer of genes is frequently promoted, which results in a more steady biofilm structure. A significant structural element of many diverse microbial biofilms is extracellular DNA. The biofilm structure may be weakened by the enzymatic breakdown of extracellular

DNA, allowing microbial cells to be released from the surface. But biofilms aren't always more resistant to medicines. The existence of persister cells may be the cause of this antibiotic resistance in both biofilms and stationary-stage cells (Spoering & Lewis, 2001).

2.13.5 Infectious diseases due to biofilm

Approximately 80% of all bacteria-related infections, including less serious conditions like endocarditis and fibrosis, and diseases of long-term implants like intervertebral discs, heart valves, and joint replacements, are thought to be caused by biofilms. These infections include common issues like urinary tract infections, bacterial vaginosis, ear infections, dental plaque formation, catheter-associated infections (Capoor et al., 2017; Lewis, 2001; Parsek & Singh, 2003). The formation of biofilm in *Staph. aureus* has been linked to the use of sub-therapeutic dosages of beta-lactam drugs, according to research. This sub-therapeutic level of antibiotics may be a result of the use of antibiotics in farming to promote growth or during the regular course of antibiotic therapy. DNase inhibited the development of biofilms brought on by low concentrations of methicillin, indicating that extracellular DNA release is also brought on by subtherapeutic concentrations of the antibiotic (Kaplan et al., 2012).

The pathogen *Staphylococcus aureus* can affect the lungs and epidermis, causing pneumonia and skin infections. Furthermore, *S. aureus*'s network of biofilm infections is crucial in stopping immune cells like macrophages from eradicating and killing bacterial cells (Thurlow et al., 2011). Additionally, bacteria like *S. aureus* that form biofilms become internally resistant to antimicrobial peptides (AMPs) and acquire resistance to antibiotics as a result of their biofilm formation, which prevents the pathogen from being inhibited and ensures their survival (Craft, Nguyen, Berg, & Townsend, 2019).

2.13.6 Impacts of Biofilm in medicine

Approximately two thirds of infections caused by bacteria are thought to contain biofilms (Del Pozo et al., 2008). Infections linked to biofilm development are typically difficult to get rid of. This is primarily caused by the antimicrobial tolerance and evasions of immune response that developed biofilms exhibit. On the inactive surfaces of implanted devices like intrauterine devices, artificial cardiac valves, and catheters, biofilms frequently develop. Infections brought on as a result of medical implants are some of the hardest to cure. The

quickly growing global market for tissue engineering-related items and biomedical devices currently stands at $180 billion annually, but microbial proliferation is still a problem in this sector. All medical gadgets and tissue engineering products are susceptible to microbial infections, regardless of their expertise. This leads to 2 million instances each year in the US, costing the healthcare system an additional $5 billion. Placement of a biomedical device is linked to 60–70% of HA-illnesses (Bryers, 2008).

In comparison to bacteria that do not form biofilms, the degree of antibiotic resistance in biofilms can be up to 5,000 times higher (Del Pozo et al., 2008; Vuotto, Longo, Balice, Donelli, & Varaldo, 2014). One of the main elements that can hinder drug penetration in a biofilm and increase antibiotic resistance is the extracellular matrix. Furthermore, studies have demonstrated that the biofilm lifestyle may have an impact on the development of antibiotic resistance (Santos-Lopez, Marshall, Scribner, Snyder, & Cooper, 2019).

2.13.7 Horizontal gene transfer in biofilm

Horizontal gene transfer is the term for the lateral passage of genetic material across organisms. Prokaryotes often experience it, whereas eukaryotes experience it less frequently. HGT in bacteria can occur via transduction (the intake of DNA by viruses), transformation (the intake of free DNA in the surroundings), or conjugation (the transmission of DNA between pili of two nearby bacteria). Other methods, like membrane vesicle transport or gene delivery agents, have also been discovered recently. Horizontal gene transmission is encouraged by biofilms in many ways (C. M. Thomas & Nielsen, 2005).

Because many biofilms are highly heterogeneous, they frequently support cross-species transport events. Biofilms encourage conjugation. A polysaccharide matrix also structurally confines biofilms, giving the close area conditions necessary for conjugation. Biofilms commonly show signs of transformation. Autolysis of bacteria is a crucial mechanism for controlling the structure of biofilms and offers a rich supply of competent DNA that is ready for transformative take - up. The efficacy of movable DNA may in some cases be improved by inter-biofilm QS, further encouraging transformation. Observations of *Stx* gene transmission via bacteriophage as carriers within biofilms imply that biofilms are also an appropriate habitat for transduction. When expelled membrane vesicles that contain genetic material combine with receiver bacteria, membrane vesicles horizontal gene transfer results,

expelling genetic material into the cytoplasm of the recipient bacteria (C. M. Thomas & Nielsen, 2005). New studies have shown that membrane vesicle horizontal gene transfer can encourage the development of single-strain biofilms, but it is still unclear how membrane vesicle horizontal gene transfer affects the development of multistrain biofilms. Proteins linked with biofilms, such as *PrgB, PtgA*, or *PrgC*, which encourage cell attachment, may be encoded by conjugative plasmids (needed for initial biofilm formation) (Luo, Wang, Sun, Liu, & Xin, 2021; V. C. Thomas & Hancock, 2009).

2.14 Molecular biology and genomics

Mu50 and N315 were the initial genomes of *Staphylococcus aureus* which get sequenced in 2001 (Ohta et al., 2004). Of all the widely sequenced bacteria is *S. aureus*, which has a large number of additional complete genomes that have been added to the database. Genomic information is also now frequently used and offers a great resource for scientists studying *S. aureus*. Microarrays and other whole genome approaches have revealed a huge diversity of *S. aureus* populations. Each strain includes unique mixtures of toxins and surface proteins. One of the main fields of study for staphylococcal organisms is how this knowledge relates to pathogenic behavior. The advancement of molecular typing techniques has made it possible to monitor various *S. aureus* strains. This might result in improved outbreak strain control. Finding current outbreak strains is becoming easier with a deeper insight into how *staphylococci* develop and evolves, particularly as a result of the accumulation MGEs coding genes for virulence and resistance. This knowledge may even help to stop the emergence of new outbreak strains (Chan, Beiko, & Ragan, 2011).

This information could possibly assist in preventing the establishment of fresh epidemic strains. The widespread prevalence of drug resistance in various strains of *Staphylococcus aureus* or across different *Staphylococcus* species is attributed to HGT that encode virulence and antibiotic resistance. According to recent research, HGT among *Staphylococcus* species occurs to a much larger extent than was previously thought and includes genes which functions other than virulence and antibiotic resistance as well as genes found outside of MGEs. *Staphylococcus* strains of all kinds are accessible from biological research facilities (Cafini et al., 2017; Chan et al., 2011).

Aim and objectives:

Aim:

The aim of the current study was to determine the antibiotic resistance in *staphylococci* isolated from transportation samples, as well as to examine their biofilm forming capacity.

Objectives:

- To identify different species of *Staphylococci* both morphologically and biochemically and differentiate them into CoPS and CoNS.
- To assess the antibiograms of various *Staphylococci* species for antibiotic resistance.
- To recognize MRSA phenotypically.
- To evaluate the *Staphylococci* species' biofilm forming ability and look into how the conditions (static or shaking) affect that ability .
- Molecular confirmation of *mecA* gene through PCR in methicillin resistant isolates

Chapter 3

Materials and Methodology

Materials and Methods

The current study was carried out to assess the antimicrobial susceptibility and biofilm forming capacity of several *Staphylococci* species isolated from transport vehicles of twin cities. The research investigation was performed at department of Microbiology, Quaid-i-Azam University Islamabad, Pakistan.

3.1 Study Isolates

In this research, bacteria that had previously been isolated from public transportation systems—such as wagons, taxis, public busses like metro buses, green- and blue-line buses, Quaid-i-Azam university buses, etc. at various locations, including Rawalpindi and Islamabad and also from passenger trains in Karachi from August 2022 to January 2023 were further examined for *Staphylococci*.

The strains were grown on Mannitol salt agar (MSA) plates from a glycerol stock. The plates were then incubated at 37°C for 24 hours. After that, the growth of *Staphylococci* on the plates was checked.

3.2 Sample size

In this study, 175 samples in total were studied and analyzed.

3.3 Identification of isolates

Isolates were grown on MSA for the purposes of confirming *Staphylococci* species and determining which bacteria ferment lactose and which do not. MSA is a media that is both selective and differential. The high salt concentration (7.5%) in MSA benefits the *Staphylococcus* genus since they can resist high salt levels. MSA also includes mannitol (a sugar) and phenol red (a pH indicator). Mannitol can be fermented by an organism, which results in the formation of an acidic residue that turns the phenol red inside the agar yellow. *Staphylococcus aureus* and other pathogenic *staphylococci* typically ferment mannitol and turn the agar yellow. For additional *staphylococci* species confirmation, various biochemical assays were used to differentiate them into CoPS and CoNS.

3.4 Catalase test

Principle

Catalase, an enzyme, breaks down hydrogen peroxide into water and oxygen. It is obvious that the enzyme is present when a little suspension of a bacterial isolate is put to hydrogen peroxide and the rapid generation of oxygen bubbles occurs. The lack of catalase is indicated by the absence of bubbles or their feeble production. To distinguish between *Staphylococcus* species that are catalase positive and *Streptococcus* species that are catalase negative, catalase testing is frequently employed.

3.4.1 Preparation of 3% Hydrogen peroxide

From 35% H_2O_2, 3 percent H_2O_2 was prepared. For that 11 parts of water were added to 1 part of 35 percent hydrogen peroxide. The falcon tube 15mL was used for making this dilution.

Procedure

used a toothpick to transfer a little amount of colony development onto the top of a glass slide that was dry and clean. Then, 3% H2O2 was added in a small amount to the glass plate. and then observed the development of oxygen bubbles. If numerous bubbles are produced and active bubbling was seen, then considered positive. The production of no or few bubbles were interpreted negative.

3.5 Coagulase test

Principle

The coagulase test is used to differentiate between coagulase-positive *Staphylococci*, such as *Staphylococcus aureus*, and coagulase-negative *Staphylococci*, such as *S. saprophyticus* and *S. epidermis*. Coagulase, a protein that changes fibrinogen into fibrin, causes plasma to clot.

3.5.1 Plasma extraction

Human blood plasma was used for the coagulase test. Therefore, blood was drawn from a healthy individual and placed in an EDTA tube. The tube was then rotated at 4000 rpm for five minutes. Blood cells settled, and the plasma, which was the supernatant, was collected and used for the coagulase test in a sterile Eppendorf.

Procedure

Slide coagulase test was performed by putting one drop of saline to each end of the slide. A part of isolated colony was then emulsified within both drops using the toothpick in order to make two suspensions. Gently mixed one of the suspensions with one drop of human plasma. In 10 seconds, observed for clumping. For the other suspension, no plasma was mixed in to distinguish between the two and look for actual coagulase clumping. Clumping showed coagulase positive result whereas no clumping showed coagulase negative results.

3.6 Antibiotic susceptibility testing

Following CLSI recommendations, isolates were examined for antimicrobial susceptibility testing against thirteen antibiotics which include penicillin, clindamycin, erythromycin, cefoxitin, quinupristin/dalfopristin, linezolid, rifampicin, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin, tetracycline, gentamycin, and chloramphenicol using the Kirby Baur disk diffusion method.

Materials

- MHA (Mueller Hinton Agar)
- Sterile plates or petri plates
- Cotton swabs
- McFarland, 0.5 Turbidity
- Normal saline
- Antibiotic discs
- Antibiotic disc dispenser
- Forceps
- Measuring scale or ruler

Procedure

1. Firstly, MHA plates were prepared by using MHA agar (Oxoid) following manufacturer's instructions.
2. Then inoculum was prepared by picking 2-3 colonies from the fresh culture plates and suspending them directly into 1ml normal saline in Eppendorf.
3. Then turbidity of prepared colony suspension was matched with 0.5 McFarland standard and turbidity was adjusted.
4. Excess solution was then wiped off the Eppendorf walls using a cotton swab soaked in the suspension. MHA plates must be dried before swabbing.
5. Then swabbed entire surface of the plate uniformly by rotating the plate to make lawn culture. Swabbing must be done in 15 minutes after preparation of colony suspension.

6. Then by using antibiotic disc dispenser, antibiotic discs are placed onto the agar plates. Sterile forceps were used to adjust the antibiotic discs if needed and ensured their proper contact with the agar surface by pressing them down onto the agar. Discs were placed at approximately 24mm or more.
7. Then the plates were incubated at 37ºC in the incubator for the next 18-24 hrs.
8. After 24 hrs of incubation, zone size was measured carefully with the help of scale and noted and compared with zone size interpretative chart given in CLSI guidelines. The antibiotics used in the test are given in the table below.

Table 3. 1 **Class, abbreviation and potency of antibiotics used.**

S. No	Antimicrobial agent	Class of antibiotic	Abbreviation	Disc potency
1	Linezolid	Oxazolidinones	LZD	30µg
2	Rifampicin	Ansamycin	RD	5µg
3	Cefoxitin	Penicillin derivatives	FOX	30µg
4	Ciprofloxacin	Fluoroquinolones	CIP	5µg
5	Gentamycin	Aminoglycosides	CN	10µg
6	Erythromycin	Macrolide	E	15µg
7	Penicillin	Beta-lactam antibiotics	P	10µg
8	Chloramphenicol	Phenicols	C	30µg
9	Trimethoprim/sulfamethoxazole	Folate pathway antagonists	SXT	25µg
10	Tetracycline	Tetracyclines	TE	30µg
11	Quinupristin/dalfopristin	Group B streptogramins	QD	15µg
12	Clindamycin	Lincosamide	DA	2µg
13	Nitrofurantoin	Nitrofurantoins	F	300µg

3.8 Microtiter plate biofilm assay

Microtiter plates were utilized for the quantification and assessment of biofilm forming ability of *staphylococci* spp. Microtiter plate contains 96 wells that permit the biofilm to develop around the walls and bottom of the well.

3.8.1 Material Required

- Tryptic soya broth media or TSB
- Microtiter plate
- Crystal Violet or CV
- Multichannel pipette
- Glacial acetic acid 33 %
- Wash bottle
- Phosphate buffer saline or PBS

3.8.2 Procedure

The microtiter plate test is a frequently used technique to measure *Staphylococcus* spp. biofilm development. The basic procedures for carrying out a microtiter plate assay for staph species is as follows:

1. Firstly, isolates were streaked on MSA and incubated overnight.
2. Next day 2-3 Staph colonies were picked with the help of a sterile toothpick and added to 500µl of TSB in labeled Eppendorf and then placed in a shaking incubator for overnight at 37°C.
3. Transfer 20µl of overnight culture to each well of a clean, flat-bottomed 96-well microtiter plate that was already filled with 230µl sterile TSB.
4. Each isolate was inoculated in triplicates onto a microtiter plate. i.e., one isolate per three wells. The plate's six corner wells were reserved for control media. The controls were also examined in triplicates. Uninoculated TSB was taken as negative control.
5. The dish was then covered with its lid and tapped and kept in incubator at 37°C to promote biofilm formation for 24 hours.

6. Then Inverted the dish and pressed it lightly on a paper towel to empty the culture medium from the wells.
7. With PBS, washed the wells three times.
8. Plates were inverted on paper towel.
9. After the plates were dried, 250 µL methanol was poured in the wells of both plates.
10. The plates were allowed to be fixed for 20-25 minutes, then methanol was discarded, and plates were turned upside down to be dried.
11. Added 200 µL of 2% CV to every single well and incubated for 5 minutes at ambient temperature to stain biofilm.
12. The wells were gently rinsed with distilled water to get rid of extra stain.
13. Added 150 microliter of 33% glacial acetic acid to all well to dissolve the stain.
14. Utilized a Multiscan to determine each well's optical density (OD) at 570 nm.
15. Measured the OD of a well that hasn't been inoculated, then deducted that value from experimental wells' OD to consider for background absorbance.
16. To describe the formation of the biofilm as a percentage of the control, compute the average optical density of experimental wells.

    ODc = Average of OD of negative control+ 3(Average of square of standard deviation of negative control)

Table 3. 2 Characterization of biofilms based on OD

Biofilm strength	Optical density
Strong biofilm producers	$OD \geq 4ODC$
Moderate biofilm producers	$OD \leq 4ODC$ while $>2\ ODC$
Weak biofilm producers	$OD \leq 2ODC$
Non biofilm producers	$OD \leq ODC$

3.9 DNA Extraction

The DNA of staph species was isolated utilizing TENT (Tris-HCl, EDTA, NaCl, Triton X-100) protocol which was regarded as a common protocol for genomic DNA isolation of gram-positive bacteria.

3.9.1 Materials Required

- Bacterial culture
- TENT buffer (Tris-HCl, EDTA, NaCl, Triton X-100) - with the following concentrations:
    - Tris-HCl: 100 mM, pH 8.0
    - EDTA: 1 mM, pH 8.0
    - Triton X-100:1 %
- Microcentrifuge tubes

3.9.2 Procedure

1. First, 100ml of lysis buffer was prepared according to above mentioned concentrations. 100ml of lysis buffer contains:
    - 5mLs of 20% triton X-100
    - 1ml of 1M Tris-HCL
    - 0.2mls of 0.5 M EDTA

2. Overnight culture of bacteria was grown in a suitable media such as TSB.
3. Then bacterial cells were collected with centrifugation at 10,000 x g for 5 minutes.
4. Cells were washed with 1ml of lysis buffer in the next step.
5. Pellet with centrifugation at 13,000 x g for 5 mins.
6. Then the pellet was suspended in 100µl of lysis buffer.
7. Next boiled for 30 minutes at 95 °C in water bath.
8. After boiling, cell debris was removed with centrifugation at 13,000 x g for 5 mins.
9. Then supernatant containing DNA was transferred in a new tube.

3.9.3. DNA Extraction Verification

For DNA verification, 1% w/v agarose gel was prepared in 1X TBE. For this purpose, 2 g of agarose was dissolved in 20ml of 10X TBE and 180ml distilled water to make 200 ml of 1% w/v agarose gel. After it, the gel was heated in the oven until it was completely dissolved, and then ethidium bromide was added for DNA visualization. The combs were adjusted in a gel tray accordingly to make wells for DNA loading. The liquid gel was then poured into the gel tray and allowed to solidify for 30 minutes. The DNA that was extracted was loaded into the wells along with a loading dye. 1X TBE was added to the electrophoresis tank until the gel was completely submerged. Then the proper voltage was applied to run the gel, usually 110 volts for almost 50 minutes. UV light was used to illuminate the gel while it was placed on a transilluminator to reveal the DNA bands. DNA fragments were shown up as bands of various sizes.

Table 3. 3 **Composition of 10X TBE buffer**

S. No.	Reagents	Quantity
1.	Tris-base	108 g
2.	Boric acid	55 g
3.	0.5 M EDTA (pH 8.0)	40 ml
4.	Distilled water	1 L

Table 3. 4 **Composition of 0.5 M EDTA**

S. No	Reagents	Quantity
1.	EDTA	186.1 g
2.	Distilled water	1 L
3.	Sodium Hydroxide pellets (for pH adjustment to 8.0)	18-20 g

3.10 Primer for detection of *mecA*

The primers designed by Hiramatsu et al. were used to identify the *mecA* genes. (Hiramatsu, 1992). The oligonucleotide primer of *mecA* is shown in table 3.5.

Table 3. 5 Oligonucleotide primers used for *mecA*

Primer Name	Nucleotide Sequence (5'-3')	Primer Specificity	Amplicon Size	Reference
MecA	**F: TGCTATCCACCCTCAAACAGG** **R: AACGTTGTAACCACCCCAAGA**	***mecA***	**285 bp**	**(Hiramatsu, 1992)**

3.11 Polymerase Chain Reaction

In this research, the PCR was carried out using the 2X PCR Master Mix (FERMENTAS). that contains all the chemicals needed for PCR, including magnesium chloride (MgCl2), buffer, Taq polymerase, and deoxynucleotide triphosphate (dNTP). Primer, template DNA and PCR/Nuclease free water was mixed with thw above mentioned master mix and a simplex PCR was run to identify the *mecA* gene A thermocycler (Labnet, USA) was used to carry out PCR.

3.11.1. PCR for *MecA* Gene Detection

The presence of the *mecA* gene provided genotypic confirmation for the MRSA that had been phenotypically identified using cefoxitin disc screening. All 72 phenotypic MRSA included in the current investigation underwent a simplex PCR. The reaction mixture, which consisted of the PCR Master Mix (2X), nuclease-free water, and both the forward and reverse primers, was limited to a total reaction volume of 10 µl. Table 3.5 provides the PCR methodology utilized to create the PCR reaction mixture for *mecA* detection.

Amplification of *mecA* gene was done by using the particular thermocycler conditions: predenaturation at 94 ℃ for 5 mins, then 33 cycles of denaturation at 94 ℃, followed by annealing at 59.4 ℃ and then extension at 72 ℃ for a period of thirty seconds each, then followed by a single cycle of post extension at 72 ℃ for 10 mins and then hold at 4 ℃ for an indefinite period until the products of PCR in the PCR tubes were taken out and stored at -20 ℃.

Table 3. 6 Total PCR reaction volume for detecting *mecA*

PCR Reaction Components	Volume per Reaction
PCR Master Mix	6 µl
Forward Primer	0.3 µl
Reverse Primer	0.3 µl
Nuclease Free Water	2.9 µl
Template DNA	2.5 µl
Total PCR Reaction Volume	12 µl

3.12 Gel electrophoresis for PCR product identification

The PCR products were distinguished by electrophoresis utilizing 1.5% (w/v) agarose gel in 1X TBE-buffer after the PCR reaction process was finished. 1.5 Agarose Gel by Invitrogen, USA was made for this purpose by dissolving 3 g of agarose in 200 ml of 1X TBE-solution and heated in the oven until the agarose dissolved completely and solution turned transparent. and then ethidium bromide was added for DNA visualization. The combs were adjusted in a gel tray accordingly to make wells. The liquid gel was then poured into the gel tray and allowed to solidify for 30 minutes.

For each reaction, 5 µl of PCR products were loaded in a 1.5% gel with a DNA marker (New England BioLabs' 100 bp DNA Ladder). The gel was then run in a gel electrophoresis tank (Wealtec Corp., USA) for 80 minutes at 110 V in 1X TBE buffer. Gel was examined under ultraviolet (UV) trans-illuminator to visualize the PCR bands after adjusted time interval. Additionally, bands were also captured using Bio-Rad's Gel Documentation System.

3.13 Statistical Analysis

To calculate the p-value, a statistical test was performed to determine if there was a significant difference between the proportions of biofilm producers under the stationary and shaking phases. This was done through chi-square analysis utilizing the software Statistical Package for the Social Sciences (SPSS) to estimate statistical significance. One commonly used test for this scenario was the chi-square test of independence.

Firstly, the null and the alternative hypotheses were:

3.13.1 Null hypothesis (H_0): The proportions of biofilm producers are the same under the stationary and shaking phases.

3.13.2 Alternative hypothesis (H_1): The proportions of biofilm producers are different under the stationary and shaking phases.

Next, contingency table was constructed with the observed frequencies:

Chapter 4

Results

Results

The bacterial isolates used in the current study were previously isolated from various transport settings, including the doors, holding handles, and various surfaces of various buses, taxis, wagons, and trains from various bus stations, metro stations, and the green line and blue line buses, taxis, and wagons at various locations in Islamabad and Rawalpindi. From September 2022 to March 2023, MPhil student Ms. Alizay isolated them. At the Department of Microbiology Zahra Lab Quaid-i-Azam University, Islamabad, Pakistan, 150 isolates in total were investigated.

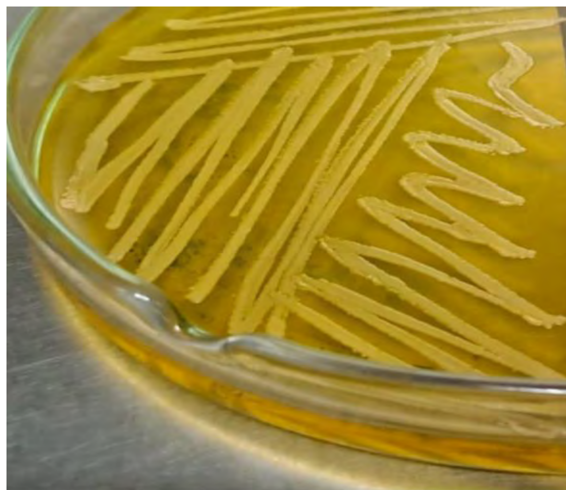
4.1 Morphological Identification

4.1.1 Growth on MSA

The isolates were cultured on MSA, a selective and differentiating medium. *Staphylococci* spp. that ferment mannitol as well as those that do not had been isolated. In contrast to non-fermenters, mannitol fermenters caused the media to turn yellow whereas non-fermenters did not change the color as depicted in figure 4.1.

Figure 4. 1MSA plate displaying growth of both the mannitol fermenters (right) and non-fermenters (left) *Staph.* species.

On MSA some isolates showed resemblance while others had entirely different morphology. Colonies' sizes ranged from small, punctiform to regular sized whereas some colonies also have filamentous structures as in the case of atypical *staphylococci*. Most colonies are yellow or pale yellow in the case of mannitol fermenters and small pink or white in the case of mannitol non-fermenters. Some lactose fermentation colonies are mucoid whereas most are not.



(A) **(B)**

Figure 4. 2 Golden yellow colonies of *Staph. aureus* grown on plate (A). Filamentou structure atypical *staphylococci* hrown on plate (B).

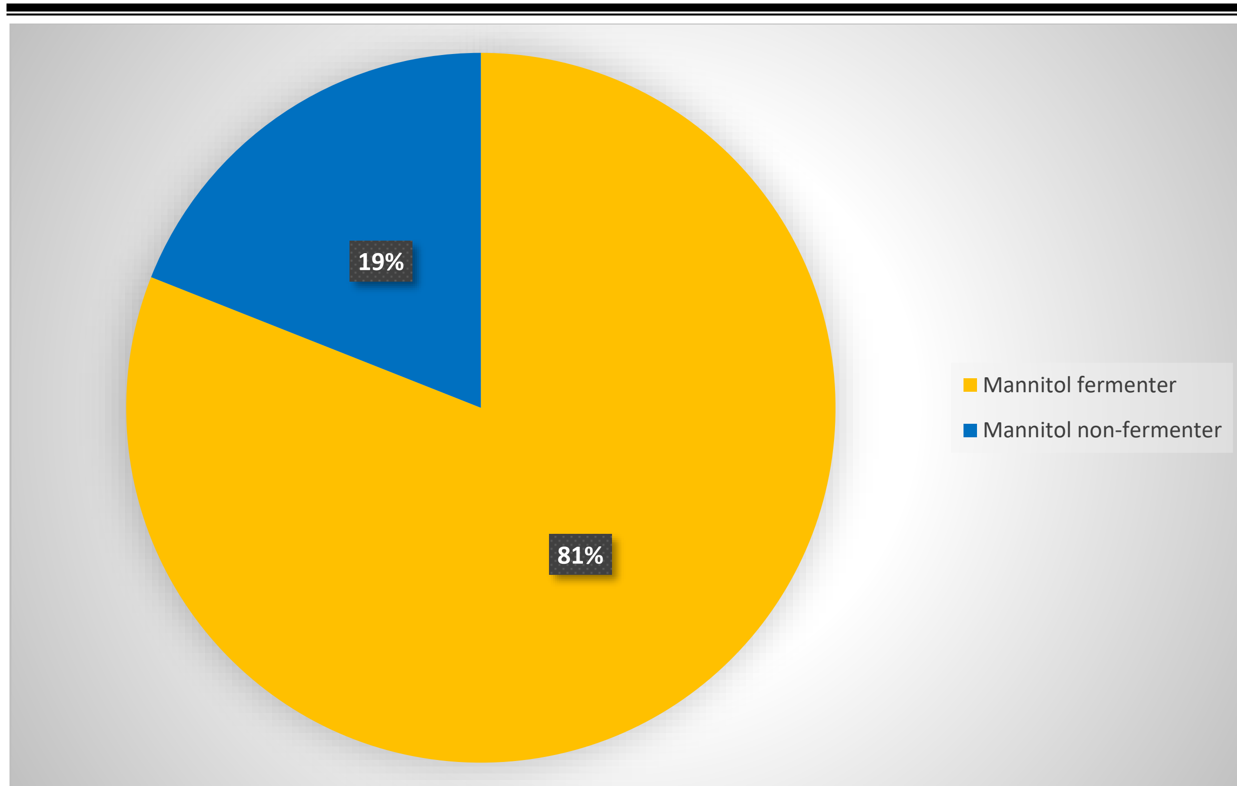


Figure 4. 3 Pie chart showing the percentage of Mannitol fermenter and non-fermenter *Staphylococci* spp.

4.3 Catalase test

All of the samples were subjected to the catalase test, which confirmed *staphylococci* in all cases and yielded 100% positive results. Figure 4.4 displays the glass slide image of the catalase test.

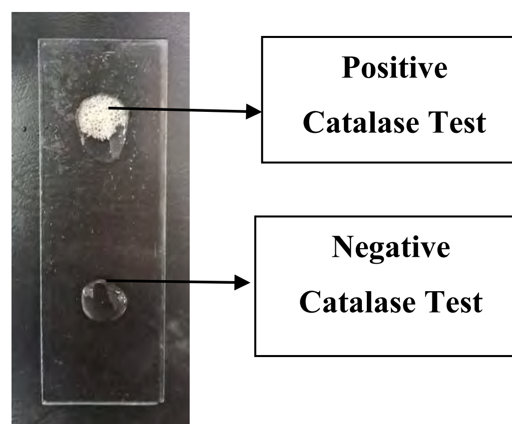
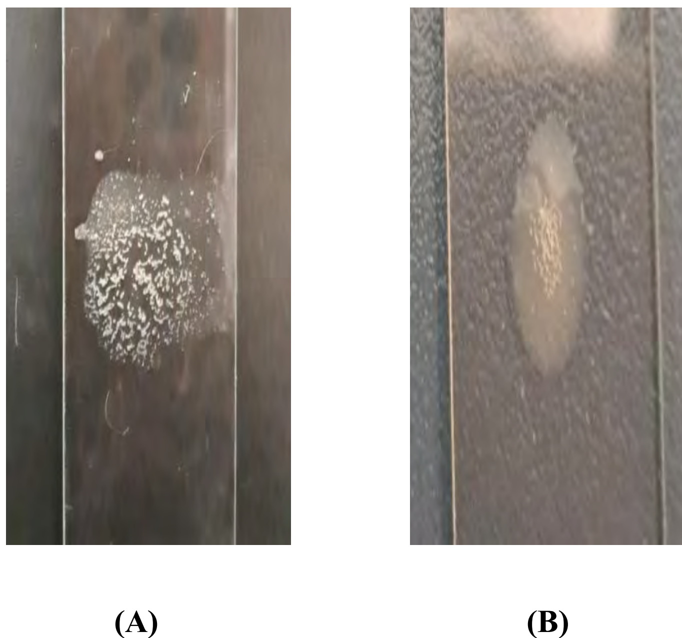


Figure 4. 4 Glass slide representing catalase positive test (bubble formation) and negative catalase test (no bubble formation)

4.4 Coagulase test

For each isolate, a slide coagulase test was run. Table 4.1 displays the prevalence of coagulase-negative and coagulase-positive staphylococci species. Figure 4.5 displays slide coagulase test pictures. Of the 175 isolates, 150 were positive for coagulase, whereas 25 tested coagulase negative.



(A) **(B)**

Figure 4. 5 (A) shows the positive coagulase test (clumping) whereas figure 4.5 (B) displays the negative coagulase test (no clumping).

4.6 Antibiotic susceptibility testing or AST

As depicted in figure 4.7, the Kirby-Bauer disk-diffusion technique was used to conduct the AST. The CLSI 2023 recommendations were implemented in order to interpret the findings. Table 4.2 discusses the AST findings.

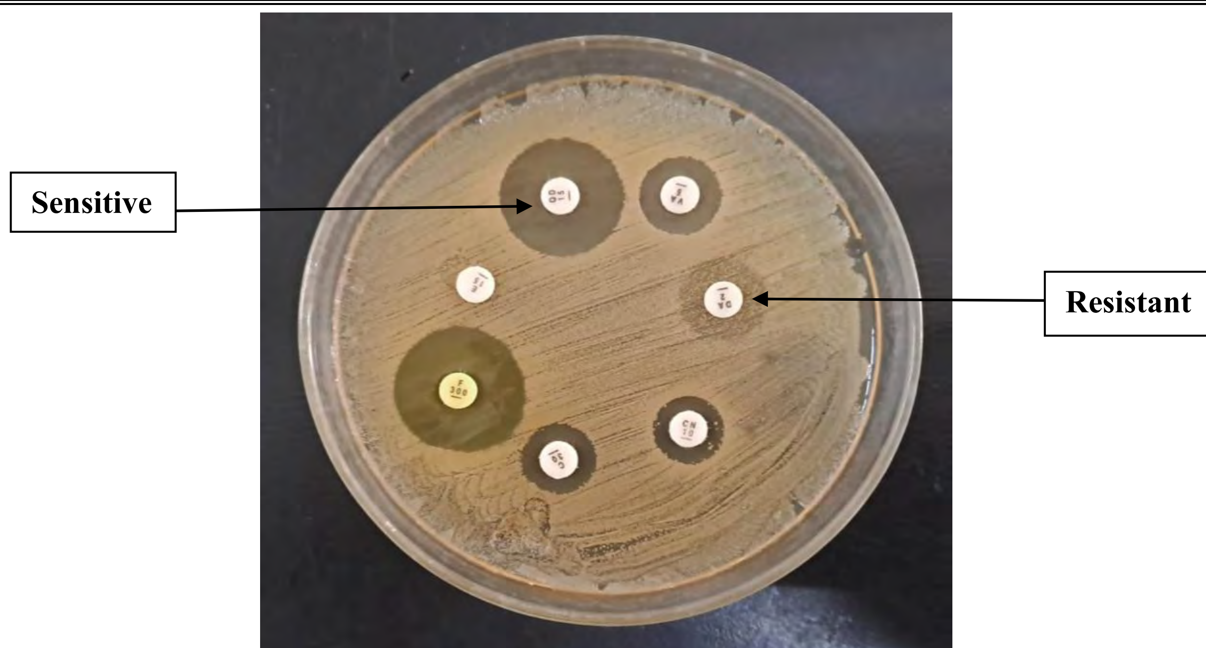


Figure 4. 6Antibiotic susceptibility testing plate showing zone of inhibition.

Table 4. 1 Table demonstrating AST results.

S. No.	Antibiotics	Susceptible	Intermediate	Resistant
1.	Penicillin	17%	0%	83%
2.	Clindamycin	22%	21%	57%
3.	Erythromycin	2%	26%	53%
4.	Cefoxitin	51%	0%	49%
5.	Quinupristin/Dalfopristin	41%	10 %	49%
6.	Linezolid	53%	0%	47%
7.	Rifampicin	43%	17%	40%
8.	Trimethoprim-Sulfamethoxazole	59%	9%	32%
9.	Ciprofloxacin	78%	2%	20%
10.	Nitrofurantoin	65%	16%	19%
11.	Tetracycline	71%	11%	18%
12.	Gentamicin	91%	2%	7%
13.	Chloramphenicol	89%	6%	5%

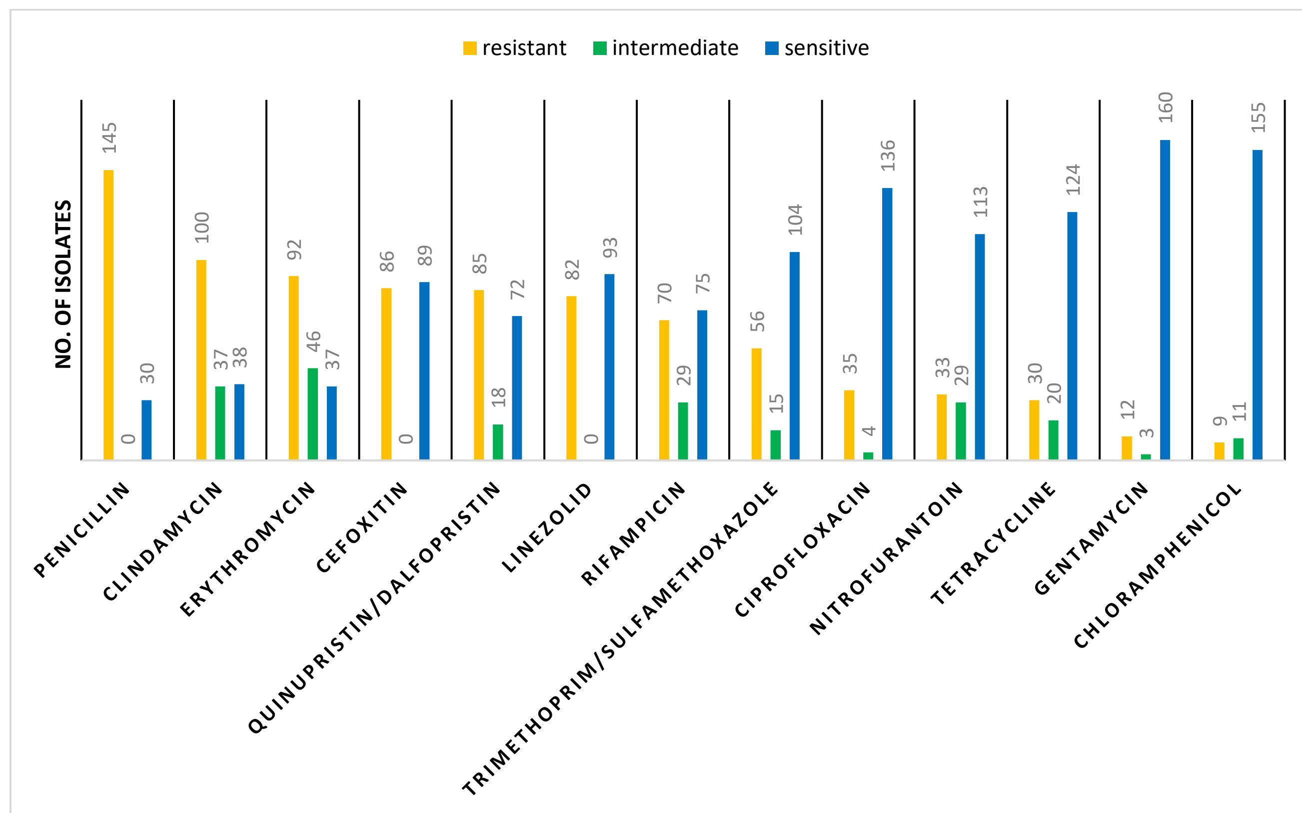


Figure 4. 7 Antibiogram of *staphylococci* species

4.7 Prevalence of multi-drug resistant MDR *staphylococci*

Those bacteria that are resistant to three or more classes of antibiotics are considered as MDR bacteria. Figure 4.9 shows the frequencyof MDR isolates.

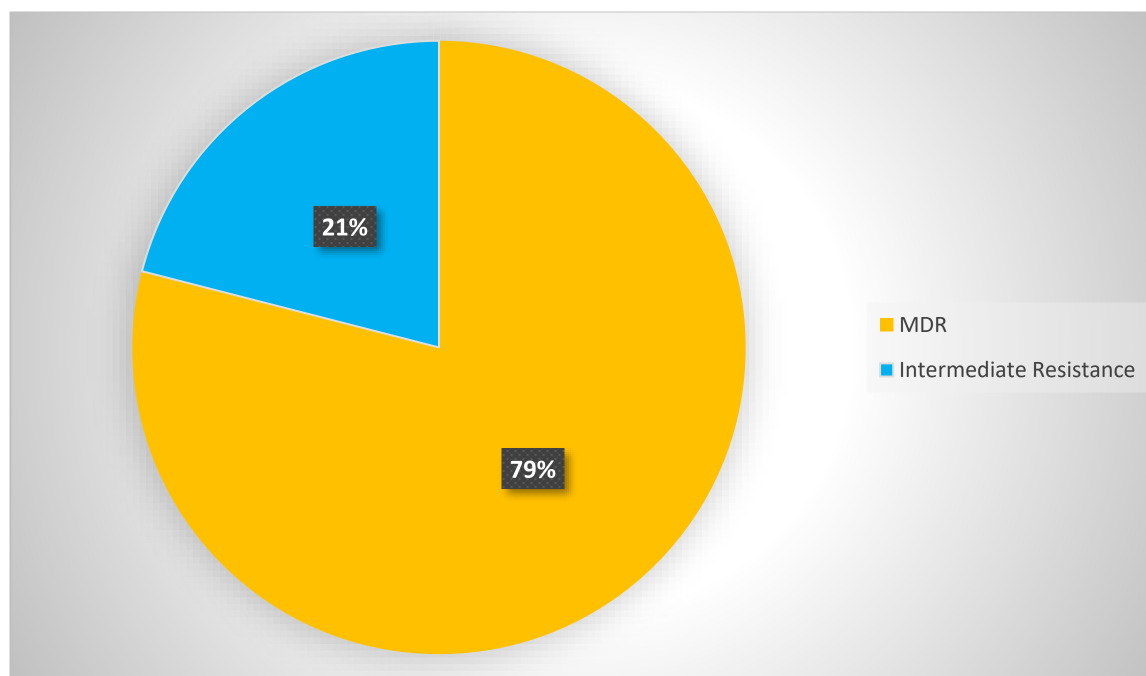


Figure 4. 8 Frequency of MDR isolates

4.8 Biofilm formation assay

Biofilm formation assay was done by microtitre plate method. For biofilm formation, isolates were allowed to grow in MTP as illustrated in figure 4.10. After the incubation period of 24 hrs, plates were washed and stained with crystal violet. Figure 4.11 shows the stained plate before measuring OD. Biofilm formation was stidied under both stationary and shaking conditions. To determine variation in formation of biofilm under both static and shaking conditions, the chi-squre test was performed. p value is less than 0.05 which indicates there is a statistically significant difference between the proportions of biofilm producers during the stationary and shaking phases.

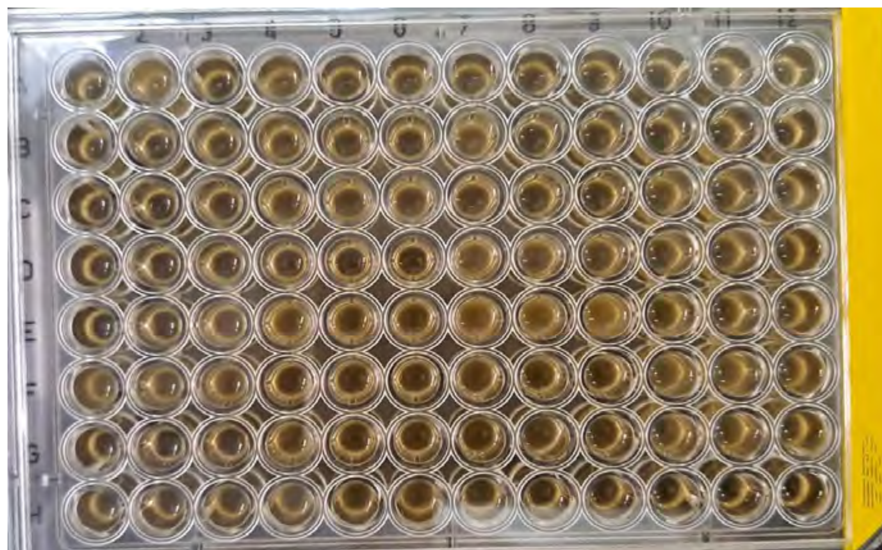


Figure 4. 9 Growth of *staphylococci* spp. in MTP

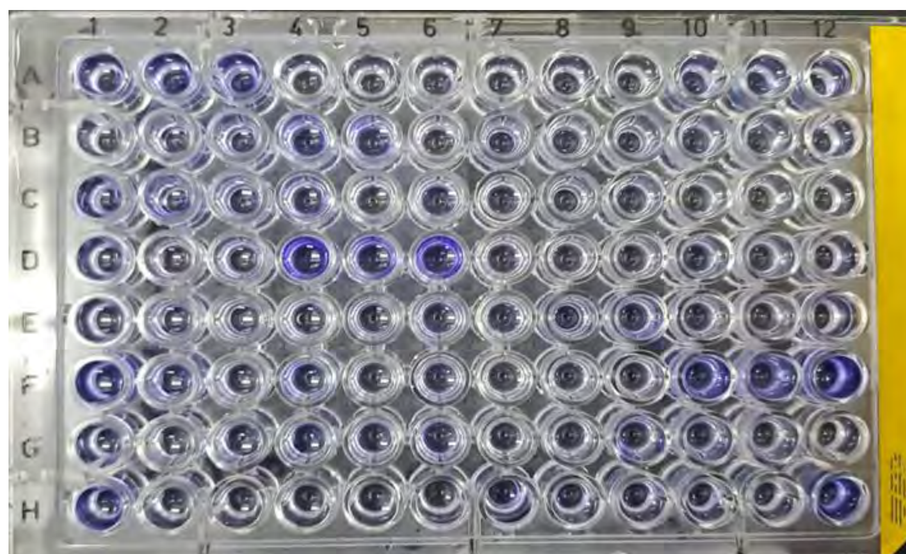


Figure 4. 10 Stained MTP before measuring OD

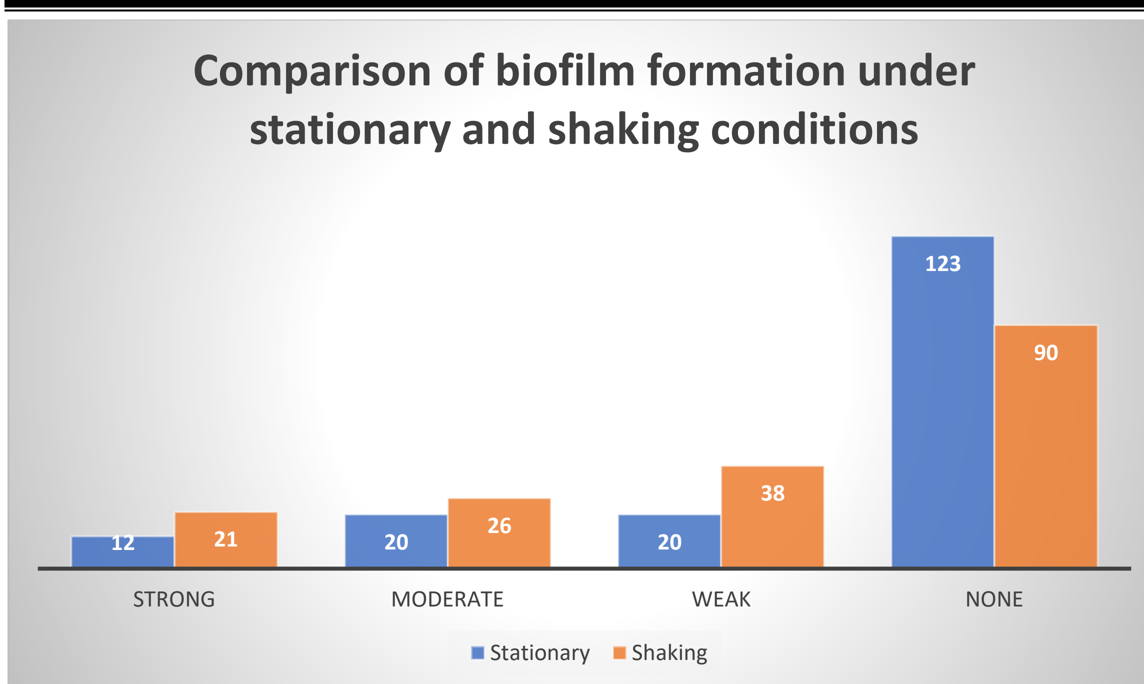


Figure 4. 11 Comparison of strong, moderate, weak, and non- biofilm producers under stationary and shaking phase.

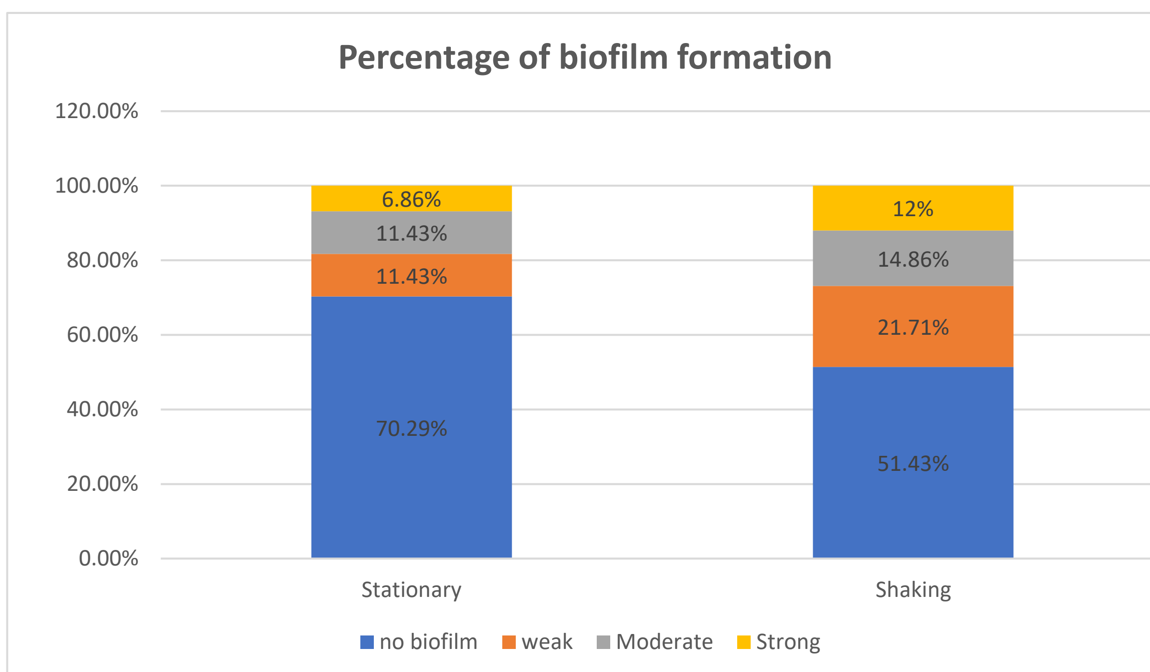


Figure 4. 12Percentage of biofilm producers under different conditions

4.8.1 Biofilm formation potentials in COPS and CONS

Figure 4.14 qnd 4.15 illustrates the biofilm forming potential of 25 CONS and 150COPS under different conditions.

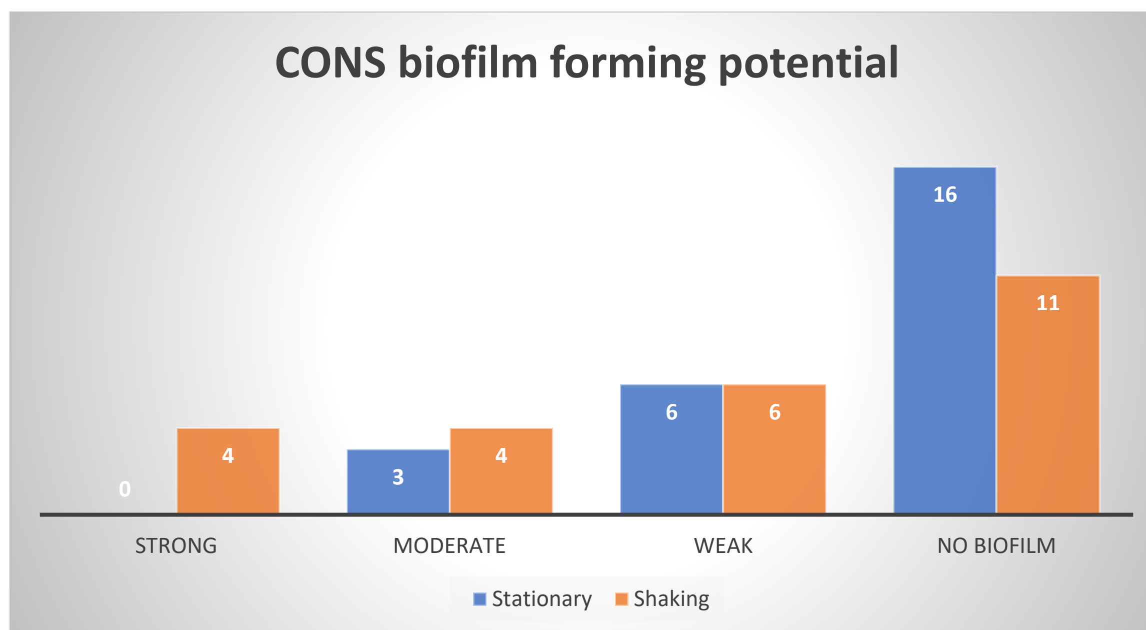


Figure 4. 13 CONS biofilm forming potential under static and shaking conditions

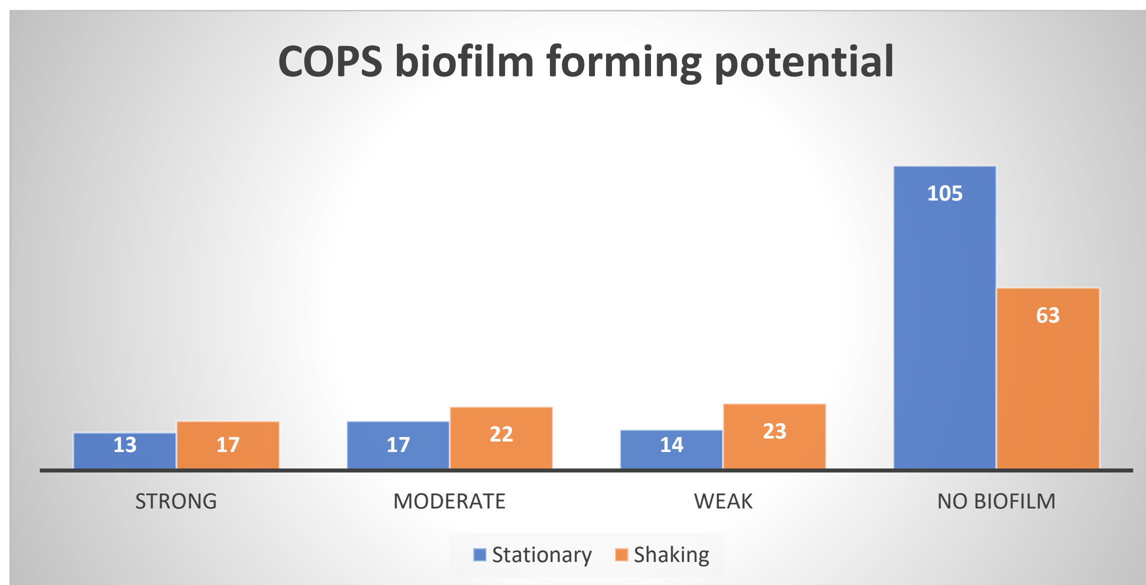


Figure 4. 14 COPS biofilm forming potential under static and shaking conditions

4.9 Statistical analysis results

Table 4. 2 Contingency table with frequencies

Biofilm Formation	Stationary Phase	Shaking Phase
Strong	12	21
Moderate	20	26
Weak	20	38
Non-biofilm producers	123	90

To calculate the p-value, chi-square test of independence was used. Assuming a significance level (α) of 0.05, if the resulting p-value is less than α, we can reject the null hypothesis and conclude that there is a significant difference between the proportions.

Using statistical software, the chi-square test yielded the following results:

Chi-square statistic: 10.9375

Degrees of freedom: 3

p-value: 0.0124

Therefore, the p-value is approximately 0.0124. Since this p-value is less than 0.05, we reject the null hypothesis and conclude that there is a significant difference between the proportions of biofilm producers under the stationary and shaking phases.

4.10 Detection of mecA Gene

Out of 72 MRSA isolates, *mecA* was detected in 38 isolates. In figure 4.15, the arrow points to a 285 bp amplicon generated by the *mecA* gene. The 285 bp (*mecA*) band can be observed in lanes 2, 4, 8, 9, and 10, whereas L represents a ladder of 100 bp molecular weight.

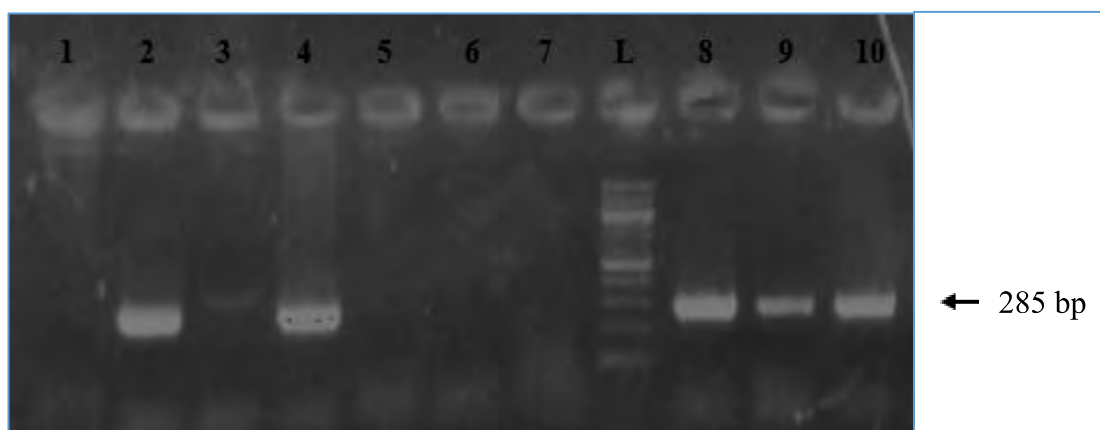


Figure 4. 15 A representative agarose gel showing *mecA* bands.

Chapter 5

Discussion

Discussion

Staphylococci are commonly found from people's skin, noses and mucous membranes. They are able to withstand adverse conditions and are also present in the environment (Silva, Caniça, Capelo, Igrejas, & Poeta, 2020). Many studies reported that *staphylococci* are also present on the surfaces of public transport vhicles (J. Otter & G. L. French, 2009; J. A. Otter & G. L. French, 2009; Stepanovic, Cirković, Djukić, Vukovic, & Svabić-Vlahović, 2008; Yeh, Simon, Millar, Alexander, & Franklin, 2011). Among the most crucial virulence factors in *S. aureus* that are mutually reliant are biofilm and drug resistance (Manandhar, Singh, Varma, Pandey, & Shrivastava, 2018). In this work, antibiotic resistance and biofilm formation were investigated in bacteria that have previously been isolated from surfaces of several public transportation systems in Rawalpindi and Islamabad.

In the current research, a total of 175 isolates of *Staphylococci* were studied, out of which 142 were mannitol fermenter and 32 were non-mannitol fermenter.Typically mannitol fermenter and coagulase positive are considered *Staphylococcus aureus* (Abdulqader et al., 2012). It is reported that *S. aureus* is capable of fermenting mannitol and is coagulase positive, which differentiates it from other *Sthaphylococcus* species. In current study,121 isolates were both manitol positive and coagulase positive i.e. *Staphylococcus aureus* isolates. Our findings have been verified by a PubMed study that discovered 68% of buses had been contaminated with *S. aureus* and out of them, 63% were MRSA (Lutz et al., 2014). Another study found that *S. aureus* was the most often colonized *Staphylococcus* overall, with a 38.2% prevalence on environmental surfaces in fitness facilities (Dalman et al., 2019). While a study on MRCoNS in a university setting in Thailand reported that the prevalence of CoNS was higher than that of *Staph. aureus*, which contradicts our findings (Seng, Leungtongkam, Thummeepak, Chatdumrong, & Sitthisak, 2017). The spread of coagulase-positive *staphylococci* may be facilitated by inadequate infection control procedures or inadequate hand hygiene standards.

According to the previous studies, CONS are typically non-mannitol fermenters (Becker et al., 2014). *Staphylococcus epidermidis*, *Staphylococcus* haemolyticus, *Staphylococcus* warneri and *Staphylococcus simulans* are CONS that do not ferment mannitol (Carretto et al., 2005; Thakur, Nayyar, Tak, & Saigal, 2017). Only 3 of the 24 coagulase negative isolates included in the

current study were not mannitol fermenters. The remaining 21 Staphylococcal isolates were mannitol positive and coagulase negative. The literature reported that *Staphylococcus haemolyticus* and *Staphylococcus xylosus* are the *Staphylococcal* species that are mannitol fermenters yet coagulase negative (Becker et al., 2014; Thakur et al., 2017). A study revealed the discovery mannitol positive CONS from nasal swabs of medical professionals and students, which supported our findings (Shittu, Lin, Morrison, & Kolawole, 2006).

All staphylococcal isolates were tested for antibiotic sensitivity.One antibiotic from each class is used in accordance with CLSI 2023. Beta lactam antibiotics showed a high level of resistance.There was 84% resistance against pencillin. Cefoxitin or Oxalacin can be used to test for *mecA*-mediated resistance as per CLSI recommendations. Since methicillin isn't sold in stores. The CLSI recommends cefoxitin over oxacillin when utilizing the disk diffusion test to identify methicillin resistance in *S. aureus*. Cefoxitin results are much easier to understand than oxacillin results, making it more sensitive to detection of *mecA*-mediated resistance (Anand et al., 2009b; Broekema, Van, Monson, Marshall, & Warshauer, 2009).

In the current study, identification of methicillin/cefoxitin resistant isolates was done by applying cefoxitin breakpoints. When *mecA* is present, staphylococcal isolates may become resistant to cefoxitin.(Broekema et al., 2009). Methicillin/cefoxitin resistance was detected in 41%(72/175) isolates whereas 59% (103/175) isoaltes appeared to be sensitive to methicillin/cefoxitin. From 72 isolates which are methicillin resistant, 62 are MRSA and 10 are MRCoNS. A total of 422 (30.1%) of the 1400 samples collected from handrails in 55 motor vehicles in Serbia were positive for methicillin-resistant coagulase negative *Staphylococci* (Stepanović, Cirković, Djukić, Vuković, & Svabić-Vlahović, 2008). According to another research, 63% of the buses in well-known transportation service in the United States tested positive for MRSA (Lutz et al., 2014). Another French investigation revealed that MRSA was present in the environment of public transportation (Gaymard et al., 2016). This change in prevalence of MRSA may be due to changes in geographical location and hygiene practices.

The resistance to penicillin in our investigation is highest i.e., 82.86%. A study in Pakistan on clinical isolates of *S.aureus* reported 100% resistance against penicillin (Bukhari, 2011). Another study conducted on airborne staphylococci in 2013 isolated from Chinese metro stations reported 28% penicillin resistance (Zhou & Wang, 2013). A study on presence of environmental CoPS in

Veterinary Teaching Hospital in Mexico City shows 86% of *S. aureus* isolates were resistant to penicillin. CoPS isolates from wastewater in Iran were found to be the most resistant to penicillin, with 89% resistance (Ranjbar Omid, 2023). Another investigation on *Staphylococci* from clinical and nutrition samples in Algeria discovered that *S. aureus* resistance to penicillin was found to be 92.3% (Achek, 2018). High penicillin resistance can be due to the widespread use and over-prescription of penicillin antibiotics, leading to selective pressure that favors the survival of resistant strains.

The resistance rate against clindamycin, erythromycin and quinupristin/dalfopristin in our study was 57.14%, 52.57% and 47.02% respectively. A study on presence of environmental CoPS in Veterinary Teaching Hospital in Mexico City shows 47% of *S. aureus* isolates were resistant to erythromycin (Velázquez-Guadarrama, 2017). Another investigation on bacteria isolated from hand touch surfaces at bus stations in Uyo reported that 65% of S. aureus were erythromycin-resistant (John & Adegoke, 2018). Whereas a study in 2011 in Pakistan on clinical isolates reported 63% clindamycin resistance and 98% erythromycin resistance (Bukhari, 2011) A study conducted on airborne staphylococci in 2013 isolated from Chinese metro stations reported 23% clindamycin resistance and 30% erythromycin resistance(Zhou & Wang, 2013). Whereas a research done on cats in veterinary hospital in South Africa discovered that the proportion of samples resistant to clindamycin was 34.2% (Qekwana, 2017). This change in resistance may be due to prevalence of different stains in different geographical locations and in different environments. Additionally, variations in local healthcare practices, antibiotic usage patterns, and genetic exchange of resistance genes among bacterial populations can contribute to regional differences in resistance levels of these antibiotics.

The resistance to cefoxitin in our study on staphylococcal isolates from transportation was 41%. A study conducted in Ethiopia in 2022 found 27.1% cefoxitin resistance in *S. aureus* (Mekuriya, 2022). This variation in resistance may be caused by variations in the profile of antibiotic resistance in various geographic locations as well as variations in the predominant strain. The linezolid resistance in the current study was 47%. A study on healthcare acquired MRSA in India in 2015 showed 20% resistance against linezolid (Bhutia, 2015). The variation in resistance may be the consequence of several factors, including geographic differences, variations in resistance through time, circulating strains, and pharmaceutical practices in various countries.

In the current study, our transportation isolates show 40% resistance to rifampicin. A study in 2011 in Pakistan on clinical isolates reported 4.8% resistance against rifampicin (Bukhari, 2011). Whereas a study conducted on susceptibility profiles of *staphylococci* from children in rural eastern Uganda found almost all of the staphylococcal isolates are susceptible to rifampin (Kateete, 2020). The high levels of rifampicin resistance found in our study might be attributed to the misuse of antibiotics in our settings and differences in the prevalence of various strains in various environmental contexts and an overall rise in antibiotic resistance over time.

In our study, the resistance against trimethoprim/sulfamethoxazole was noted to be 32%. A study on clinical isolates in Pakistan in 2011 reported 96.1% SXT resistance (Bukhari, 2011). A study on presence of environmental CoPS in Veterinary Teaching Hospital in Mexico City shows 40% of *S. aureus* isolates were resistant to SXT. (Velázquez-Guadarrama, 2017) Studies on species identification and drug susceptibility profiles of *Staphylococci* found in healthy kids in Eastern Uganda demonstrate that *S. aureus* had 38% resistance to SXT (Kateete, 2020). This is because the antibiotic resistance profiles vary with time and also with region.

In our study, 20% resistance was noted against ciprofloxacin. A study on clinical isolates in Pakistan in 2011 reported 76% ciprofloxacin resistance (Bukhari, Ahmed, & Zia, 2011). Whereas A study conducted on airborne staphylococci in 2013 isolated from Chinese metro stations reported 2% ciprofloxacin resistance (Zhou & Wang, 2013). The variation in resistance may be the result of several factors, including geographic differences, variations in resistance through time, and difference in pharmaceutical practices.

The resistance to tetracycline in the current study, however, was 17%. A study on clinical isolates in Pakistan in 2012 found 56.9 % tetracycline resistance (Ullah, 2012). A study conducted on airborne staphylococci in 2013 isolated from Chinese metro stations reported 6% tetracycline resistance(Zhou & Wang, 2013). A study on presence of environmental CoPS in Veterinary Teaching Hospital in Mexico City shows 40% of *S. aureus* isolates were resistant to tetracycline (Velázquez-Guadarrama, 2017). Another investigation on bacteria isolated from hand touch surfaces at bus stations in Uyo reported that 60% of S. aureus were tetracycline resistant (John & Adegoke, 2018). This difference in resistance may be due to less use of tetracycline in our clinical practices.

19% of our staphylococcal isolates were resistant to nitrofurantoin. A study conducted on airborne staphylococci in 2013 isolated from Chinese metro stations reported 52% nitrofurantoin resistance(Zhou & Wang, 2013). A study investigated bacterial resistance to nitrofurantoin in diverse bacterial species isolated from dogs and cats, CoPS showed 100% susceptibility to nitrofurantoin for both animal species (Aurich, 2022). This rise in resistance against nitrofurantoin in our settings may be due to the excessive use of this antibiotic in clinical as well as veterinary practices.

Gentamycin resistance in the current investigation was less at 7%. A study on clinical isolates in 2011 from Pakistan reported 97.6% gentamicin resistance (Bukhari, 2011). In 2015, from India 16.6% resistance for gentamycin was recorded in various clinical specimens (Bhutia, 2015) Another research in Algeria also discovered that all *staphylococci* isolates obtained from food samples were sensitive to gentamicin, whereas *S. aureus* resistance to gentamicin in clinical isolates was 7.7% (Achek, 2018) All S. aureus were found to be gentamycin-susceptible in another study on bacteria isolated from hand-touch surfaces at passenger terminals in the Uyo metro area(John & Adegoke, 2018) This high variation is probably because of the circulating strains and pharmaceutical practices in different regions.

Resistance to chloramphenicol was shown to be least at 5.14% in our research. A study conducted in India in 2015 reported 13.3% chloramphenicol resistance in hospital acquired MRSA isolates (Bhutia, 2015). Another investigation on bacteria isolated from hand touch surfaces at bus stations in Uyo reported that 55% of S. aureus were chloramphenicol-resistant(John & Adegoke, 2018). Studies on species identification and drug susceptibility profiles of *Staphylococci* found in healthy kids in Eastern Uganda demonstrate that *S. aureus* had 3% resistance to chloramphenicol (Kateete, 2020). Different local antibiotic usage patterns, bacterial genetic changes, and HGT can be attributed to the varied frequency of chloramphenicol resistance.

aw. Similarly a study in 2011 on clinical isolates in Pakistan found that all 41.9% MRSA isolates were MDR. (Bukhari, 2011). A study of staphylococci isolates isolated from frequently handled surfaces in London discovered that 46.83% were multidrug resistant. (Cave, 2019)

The MTP assay is the widely used and generally recognized method for determining the production of biofilms. A screening method that is regarded as being highly sensitive, accurate,

and reproducible is the microtiter plate test.(Mott, Desveaux, & Guttman, 2018) It also has the advantage of being a quantitative method for assessing biofilm development.

Through the production of biofilms, multidrug resistant microbes can endure various conditions. Our study investigates the isolate's capacity to generate biofilm in static and shaking environments. The ability of isolates to produce biofilm varies under both circumstances. Out of 175 isolates, at static conditions,6.86% (12) were strong biofilm producers,11.43%(20) were moderate biofilm producers, 11.43%(20) were week biofilm producers and 70.29%(123) were no -biofilm producers. Whereas at shaking conditions, 12% (21) were strong biofilm producers,14.86%(26) were moderate biofilm producers, 21.71%(38) were week biofilm producers and 51.43%(90) were no biofilm producers. Whereas a research conducted in Ethiopia in 2022 on healthcare students found that 10.5% of *S. aureus* isolates were strong biofilm producers and 68.5% of the isolates were non-biofilm producers. (Mekuriya, 2022) *S. aureus* has been found to exhibit increased biofilm formation capability when exposed to shaking or turbulent circumstances as opposed to static or quiescent ones because of increased nutrient availability under shaking conditions.(Periasamy et al., 2012)

Under static and shaking conditions, only 5 of the isolates produced strong biofilm. While under the same conditions 74 of the isolates failed to produce any biofilm. Three of the isolates, however, developed strong biofilm under stationary conditions but none when shaken, and neither of them was *S. aureus*. However, it is impossible to say for sure whether all species of *Staphylococcus aureus* exhibit robust biofilm development under shaking circumstances based on the previous studies.

In order to determine whether there was a difference between the proportion of biofilm producers during the stationary and shaking phases, we conducted a statistical test and computed the p-value. By assuming a significance level α of 0.05, the p-value was roughly 0.0124. Thus, we came to the conclusion that there is a significant difference between the proportions of biofilm producers during the stationary and shaking phases.

By using the cefoxitin disk diffusion method, 72 of the 175 isolates were identified as MRSA. *MecA* is genotypically detected by PCR in 38(52.7%) of these 72 isolates. In 2011, a research on clinical isolates in Pakistan showed 462 MRSA by disk diffusion, of which 307 isolates had *mecA* detected by PCR.(Bukhari, 2011) In a 2014 an investigation in Makkah, reported 114

MRSA isolates, of which 100 had the *MecA* gene (Asghar, 2014) Similar results have also been reported in other studies.(Hoseini Alfatemi, 2014) This may be attributable to other diverse pathways of methicillin resistance, including the acquisition of the *mecB* and *mecC* genes, elevated PBP expression, and PBP mutation.

Conclusion

Conclusion

In this study, the alarmingly high rates of antibiotic resistance among Staphylococcus species isolated from public transportation vehicles are highlighted. In order to decrease the emergence of resistance, there is an urgent need for better antimicrobial stewardship and infection control strategies. The presence of MRSA in areas associated with public transportation acts as a possible reservoir for transmission. *Staphylococci* is a diverse group which contains both CoPS and CoNS. However, this study demonstrated that CoPS are significantly more common than CoNS in public transport. Both CoPS and CoNS exhibit resistance to almost all antibiotic classes, with penicillin showing highest resistance and chloramphenicol having the lowest resistance. Whereas79% of the isolates were shown to be MDR. Under shaking conditions, high biofilm-forming capacity is seen in isolates, and the number of both weak and strong biofilm formers nearly doubles. *MecA* is genotypically confirmed in 38 isolates by PCR out of the 72 methicillin-resistant isolates found by cefoxitin disk diffusion technique. The results highlight the significance of ongoing surveillance and study to better comprehend and address antibiotic resistance in environmental settings.

Future perspectives

Future perspectives

AMR, which affects the efficacy of many antibiotics and other antimicrobial therapies, is still a major problem on a global scale. Understanding the changing resistance patterns requires ongoing observation and monitoring of AMR developments. Better AMR surveillance in non-clinical settings is also necessary.

Future research recommendations include the following:

- Strain level identification of isolates
- Screening of antimicrobil resistance genes other than *mecA*
- Molecular identification of biofilm forming genes
- Constant monitoring of genomic evolution of *Staphylococci* strains to spot novel virulence markers, mobile genetic elements, and resistance genes.
- Identification of Vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) by their MIC values.
- Determination of antibiotic resistance in other gram-positive bacteria, such as enterococcus, from transportation

References

REFERENCES

Abbas, A., Srivastava, P., & Nirwan, P. S. (2015). Prevalence of MLSB Resistance and Observation of erm A & erm C Genes At A Tertiary Care Hospital. *J Clin Diagn Res, 9*(6), Dc08-10. doi:10.7860/jcdr/2015/13584.6112

Abdulqader, H., Bakr, S., & Al-Hamdi, K. (2012). *Bacteriological and Immunological Aspects of Psoriasis. Evidence of superantigenic Staphylococcal Exotoxin in induction of Psoriasis*.

Achek, R., Hotzel, H., Cantekin, Z., Nabi, I., Hamdi, T. M., Neubauer, H., & El-Adawy, H. (2018). Emerging of antimicrobial resistance in staphylococci isolated from clinical and food samples in Algeria. *BMC Research Notes, 11*(1), 663. doi:10.1186/s13104-018-3762-2

Aggarwal, S., Stewart, P. S., & Hozalski, R. M. (2015). Biofilm Cohesive Strength as a Basis for Biofilm Recalcitrance: Are Bacterial Biofilms Overdesigned? *Microbiol Insights, 8*(Suppl 2), 29-32. doi:10.4137/mbi.s31444

Ahmad-Mansour, N., Loubet, P., Pouget, C., Dunyach-Remy, C., Sotto, A., Lavigne, J. P., & Molle, V. (2021). Staphylococcus aureus Toxins: An Update on Their Pathogenic Properties and Potential Treatments. *Toxins (Basel), 13*(10). doi:10.3390/toxins13100677

Ali Alghamdi, B., Al-Johani, I., Al-Shamrani, J. M., Musamed Alshamrani, H., Al-Otaibi, B. G., Almazmomi, K., & Yusnoraini Yusof, N. (2023). Antimicrobial resistance in methicillin-resistant staphylococcus aureus. *Saudi J Biol Sci, 30*(4), 103604. doi:10.1016/j.sjbs.2023.103604

Almeida, R. J., & Jorgensen, J. H. (1983). Identification of coagulase-negative staphylococci with the API STAPH-IDENT system. *J Clin Microbiol, 18*(2), 254-257. doi:10.1128/jcm.18.2.254-257.1983

Anand, K. B., Agrawal, P., Kumar, S., & Kapila, K. (2009a). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. *Indian J Med Microbiol, 27*(1), 27-29.

Anand, K. B., Agrawal, P., Kumar, S., & Kapila, K. (2009b). COMPARISON OF CEFOXITIN DISC DIFFUSION TEST, OXACILLIN SCREEN AGAR, AND PCR FOR mecA GENE FOR DETECTION OF MRSA. *Indian J Med Microbiol, 27*(1), 27-29. doi:https://doi.org/10.1016/S0255-0857(21)01748-5

Argemi, X., Hansmann, Y., Prola, K., & Prévost, G. (2019). Coagulase-Negative Staphylococci Pathogenomics. *Int J Mol Sci, 20*(5). doi:10.3390/ijms20051215

Bailey, C. J., Lockhart, B. P., Redpath, M. B., & Smith, T. P. (1995). The epidermolytic (exfoliative) toxins of Staphylococcus aureus. *Med Microbiol Immunol, 184*(2), 53-61. doi:10.1007/bf00221387

Baird-Parker, A. C. (1963). A classification of micrococci and staphylococci based on physiological and biochemical tests. *J Gen Microbiol, 30*, 409-427. doi:10.1099/00221287-30-3-409

Baird-Parker, A. C. (1965). Staphylococci and their classification. *Ann N Y Acad Sci, 128*(1), 4-25. doi:10.1111/j.1749-6632.1965.tb11626.x

Becker, K., Heilmann, C., & Peters, G. (2014). Coagulase-negative staphylococci. *Clin Microbiol Rev, 27*(4), 870-926. doi:10.1128/cmr.00109-13

Berlanga, M. (2010). Brock Biology of Microorganisms (11th edn). Michael T. Madigan, John M. Martinko (eds). *International Microbiology; Vol. 8, Núm. 2 (2005); 149-150*.

Bhakdi, S., & Tranum-Jensen, J. (1991). Alpha-toxin of Staphylococcus aureus. *Microbiol Rev, 55*(4), 733-751. doi:10.1128/mr.55.4.733-751.1991

Bonjean, M., Hodille, E., Dumitrescu, O., Dupieux, C., Nkoud Mongo, C., Allam, C., . . . Lina, G. (2016). Disk Diffusion Testing for Detection of Methicillin-Resistant Staphylococci: Does Moxalactam Improve upon Cefoxitin? *J Clin Microbiol, 54*(12), 2905-2909. doi:10.1128/jcm.01195-16

Branda, S. S., Chu, F., Kearns, D. B., Losick, R., & Kolter, R. (2006). A major protein component of the Bacillus subtilis biofilm matrix. *Mol Microbiol, 59*(4), 1229-1238. doi:10.1111/j.1365-2958.2005.05020.x

Briandet, R., Herry, J., & Bellon-Fontaine, M. (2001). Determination of the van der Waals, electron donor and electron acceptor surface tension components of static Gram-positive microbial biofilms. *Colloids Surf B Biointerfaces, 21*(4), 299-310. doi:10.1016/s0927-7765(00)00213-7

Broekema, N. M., Van, T. T., Monson, T. A., Marshall, S. A., & Warshauer, D. M. (2009). Comparison of cefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in Staphylococcus aureus in a large-scale study. *J Clin Microbiol, 47*(1), 217-219. doi:10.1128/jcm.01506-08

Bryers, J. D. (2008). Medical biofilms. *Biotechnol Bioeng, 100*(1), 1-18. doi:10.1002/bit.21838

Bukhari, S. Z., Ahmed, S., & Zia, N. (2011). Antimicrobial susceptibility pattern of Staphylococcus aureus on clinical isolates and efficacy of laboratory tests to diagnose MRSA: a multi-centre study. *J Ayub Med Coll Abbottabad, 23*(1), 139-142.

Cafini, F., Thi Le Thuy, N., Román, F., Prieto, J., Dubrac, S., Msadek, T., & Morikawa, K. (2017). Methodology for the Study of Horizontal Gene Transfer in Staphylococcus aureus. *J Vis Exp*(121). doi:10.3791/55087

Capoor, M. N., Ruzicka, F., Schmitz, J. E., James, G. A., Machackova, T., Jancalek, R., . . . Slaby, O. (2017). Propionibacterium acnes biofilm is present in intervertebral discs of patients undergoing microdiscectomy. *PLoS One, 12*(4), e0174518. doi:10.1371/journal.pone.0174518

Carolus, H., Van Dyck, K., & Van Dijck, P. (2019). Candida albicans and Staphylococcus Species: A Threatening Twosome. *Front Microbiol, 10*, 2162. doi:10.3389/fmicb.2019.02162

Carretto, E., Barbarini, D., Couto, I., De Vitis, D., Marone, P., Verhoef, J., . . . Brisse, S. (2005). Identification of coagulase-negative staphylococci other than Staphylococcus epidermidis by automated ribotyping. *Clin Microbiol Infect, 11*(3), 177-184. doi:10.1111/j.1469-0691.2004.01052.x

Carter, A. P., Clemons, W. M., Brodersen, D. E., Morgan-Warren, R. J., Wimberly, B. T., & Ramakrishnan, V. (2000). Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature, 407*(6802), 340-348. doi:10.1038/35030019

Chalmers, S. J., & Wylam, M. E. (2020). Methicillin-Resistant Staphylococcus aureus Infection and Treatment Options. *Methods Mol Biol, 2069*, 229-251. doi:10.1007/978-1-4939-9849-4_16

Chan, C. X., Beiko, R. G., & Ragan, M. A. (2011). Lateral transfer of genes and gene fragments in Staphylococcus extends beyond mobile elements. *J Bacteriol, 193*(15), 3964-3977. doi:10.1128/jb.01524-10

Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. *Virulence, 12*(1), 547-569. doi:10.1080/21505594.2021.1878688

Conan, P. L., Podglajen, I., Compain, F., Osman, M., Lebeaux, D., & Flamarion, E. (2021). Renal abscess caused by Panton-Valentine leukocidin-producing Staphylococcus aureus: report of an unusual case and review of the literature. *Infect Dis (Lond), 53*(2), 131-136. doi:10.1080/23744235.2020.1856920

Corrente, M., Ventrella, G., Greco, M. F., Martella, V., Parisi, A., & Buonavoglia, D. (2013). Characterisation of a catalase-negative methicillin-resistant Staphylococcus aureus isolate from a dog. *Vet Microbiol, 167*(3-4), 734-736. doi:10.1016/j.vetmic.2013.09.020

Craft, K. M., Nguyen, J. M., Berg, L. J., & Townsend, S. D. (2019). Methicillin-resistant Staphylococcus aureus (MRSA): antibiotic-resistance and the biofilm phenotype. *Medchemcomm, 10*(8), 1231-1241. doi:10.1039/c9md00044e

Dalman, M., Bhatta, S., Nagajothi, N., Thapaliya, D., Olson, H., Naimi, H. M., & Smith, T. C. (2019). Characterizing the molecular epidemiology of Staphylococcus aureus across and within fitness facility types. *BMC Infectious Diseases, 19*(1), 69. doi:10.1186/s12879-019-3699-7

Danese, P. N., Pratt, L. A., & Kolter, R. (2000). Exopolysaccharide production is required for development of Escherichia coli K-12 biofilm architecture. *J Bacteriol, 182*(12), 3593-3596. doi:10.1128/jb.182.12.3593-3596.2000

Del Pozo, J. L., Rouse, M. S., & Patel, R. (2008). Bioelectric effect and bacterial biofilms. A systematic review. *Int J Artif Organs, 31*(9), 786-795. doi:10.1177/039139880803100906

Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A., & Stobberingh, E. E. (2007). The molecular evolution of methicillin-resistant Staphylococcus aureus. *Clin Microbiol Infect, 13*(3), 222-235. doi:10.1111/j.1469-0691.2006.01573.x

Dickinson, G. M., & Bisno, A. L. (1989). Infections associated with indwelling devices: concepts of pathogenesis; infections associated with intravascular devices. *Antimicrob Agents Chemother, 33*(5), 597-601. doi:10.1128/aac.33.5.597

Diekema, D. J., Pfaller, M. A., Schmitz, F. J., Smayevsky, J., Bell, J., Jones, R. N., & Beach, M. (2001). Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis, 32 Suppl 2*, S114-132. doi:10.1086/320184

Duran, N., Ozer, B., Duran, G. G., Onlen, Y., & Demir, C. (2012). Antibiotic resistance genes & susceptibility patterns in staphylococci. *Indian J Med Res, 135*(3), 389-396.

Eliopoulos, G. M., & Huovinen, P. (2001). Resistance to Trimethoprim-Sulfamethoxazole. *Clinical Infectious Diseases, 32*(11), 1608-1614. doi:10.1086/320532

Emaneini, M., Bigverdi, R., Kalantar, D., Soroush, S., Jabalameli, F., Noorazar Khoshgnab, B., . . . Taherikalani, M. (2013). Distribution of genes encoding tetracycline resistance and aminoglycoside modifying enzymes in Staphylococcus aureus strains isolated from a burn center. *Ann Burns Fire Disasters, 26*(2), 76-80.

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol, 14*(9), 563-575. doi:10.1038/nrmicro.2016.94

Foster, T. J. (2017). Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. *FEMS Microbiology Reviews, 41*(3), 430-449. doi:10.1093/femsre/fux007

Foster, T. J. (2019). The MSCRAMM Family of Cell-Wall-Anchored Surface Proteins of Gram-Positive Cocci. *Trends Microbiol, 27*(11), 927-941. doi:10.1016/j.tim.2019.06.007

Freer, J. H., & Arbuthnott, J. P. (1982). Toxins of Staphylococcus aureus. *Pharmacol Ther, 19*(1), 55-106. doi:10.1016/0163-7258(82)90042-0

Furuhata, K., Ishizaki, N., Sogawa, K., Kawakami, Y., Lee, S., Sato, M., & Fukuyama, M. (2016). Isolation, Identification and Antibacterial Susceptibility of Staphylococcus spp. Associated with the Mobile Phones of University Students. *Biocontrol Sci, 21*(2), 91-98. doi:10.4265/bio.21.91

Gaymard, A., Pichon, M., Degaud, M., Tasse, J., Dupieux, C., & Laurent, F. (2016). Methicillin-resistant Staphylococcus aureus in the environment of public transport: data from the metropolitan network in Lyon, France. *International Journal of Antimicrobial Agents, 48*(4), 459-462. doi:https://doi.org/10.1016/j.ijantimicag.2016.07.012

Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. *Front Cell Infect Microbiol, 10*, 107. doi:10.3389/fcimb.2020.00107

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol, 2*(2), 95-108. doi:10.1038/nrmicro821

Hall, C. W., & Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol Rev, 41*(3), 276-301. doi:10.1093/femsre/fux010

Harris, L. G., Foster, S. J., & Richards, R. G. (2002). An introduction to Staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review. *Eur Cell Mater, 4*, 39-60. doi:10.22203/ecm.v004a04

Heilmann, C. (2011). Adhesion mechanisms of staphylococci. *Adv Exp Med Biol, 715*, 105-123. doi:10.1007/978-94-007-0940-9_7

Howden, B. P., Giulieri, S. G., Wong Fok Lung, T., Baines, S. L., Sharkey, L. K., Lee, J. Y. H., . . . Stinear, T. P. (2023). Staphylococcus aureus host interactions and adaptation. *Nature Reviews Microbiology, 21*(6), 380-395. doi:10.1038/s41579-023-00852-y

Huh, K., & Chung, D. R. (2016). Changing epidemiology of community-associated methicillin-resistant Staphylococcus aureus in the Asia-Pacific region. *Expert Rev Anti Infect Ther, 14*(11), 1007-1022. doi:10.1080/14787210.2016.1236684

Hussain, M., Wilcox, M. H., & White, P. J. (1993). The slime of coagulase-negative staphylococci: biochemistry and relation to adherence. *FEMS Microbiol Rev, 10*(3-4), 191-207. doi:10.1111/j.1574-6968.1993.tb05867.x

Jamrozy, D., Coll, F., Mather, A. E., Harris, S. R., Harrison, E. M., MacGowan, A., . . . Peacock, S. J. (2017). Evolution of mobile genetic element composition in an epidemic methicillin-resistant Staphylococcus aureus: temporal changes correlated with frequent loss and gain events. *BMC Genomics, 18*(1), 684. doi:10.1186/s12864-017-4065-z

Jin, M., Rosario, W., Watler, E., & Calhoun, D. H. (2004). Development of a large-scale HPLC-based purification for the urease from Staphylococcus leei and determination of subunit structure. *Protein Expression and Purification, 34*(1), 111-117. doi:https://doi.org/10.1016/j.pep.2003.10.012

John, O., & Adegoke, A. (2018). Bacteriological Evaluation of Hand Contact Surfaces at Bus Terminals in Uyo Metropolis. *Journal of Pure and Applied Microbiology, 12*, 1187-1193. doi:10.22207/JPAM.12.3.18

Kaplan, J. B., Izano, E. A., Gopal, P., Karwacki, M. T., Kim, S., Bose, J. L., . . . Horswill, A. R. (2012). Low levels of β-lactam antibiotics induce extracellular DNA release and biofilm formation in Staphylococcus aureus. *mBio, 3*(4), e00198-00112. doi:10.1128/mBio.00198-12

Katayama, Y., Ito, T., & Hiramatsu, K. (2000). A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. *Antimicrob Agents Chemother, 44*(6), 1549-1555. doi:10.1128/aac.44.6.1549-1555.2000

Kuipers, A., Stapels, D. A. C., Weerwind, L. T., Ko, Y. P., Ruyken, M., Lee, J. C., . . . Rooijakkers, S. H. M. (2016). The Staphylococcus aureus polysaccharide capsule and Efb-dependent fibrinogen shield act in concert to protect against phagocytosis. *Microbiology (Reading), 162*(7), 1185-1194. doi:10.1099/mic.0.000293

Lakhundi, S., & Zhang, K. (2018). Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev, 31*(4). doi:10.1128/cmr.00020-18

Lewis, K. (2001). Riddle of biofilm resistance. *Antimicrob Agents Chemother, 45*(4), 999-1007. doi:10.1128/aac.45.4.999-1007.2001

López, D., Vlamakis, H., & Kolter, R. (2010). Biofilms. *Cold Spring Harb Perspect Biol, 2*(7), a000398. doi:10.1101/cshperspect.a000398

Lorian, V., Zak, O., Suter, J., & Bruecher, C. (1985). Staphylococci, in vitro and in vivo. *Diagn Microbiol Infect Dis, 3*(5), 433-444. doi:10.1016/0732-8893(85)90082-3

Lowy, F. D. (1998). Staphylococcus aureus infections. *N Engl J Med, 339*(8), 520-532. doi:10.1056/nejm199808203390806

Luo, A., Wang, F., Sun, D., Liu, X., & Xin, B. (2021). Formation, Development, and Cross-Species Interactions in Biofilms. *Front Microbiol, 12*, 757327. doi:10.3389/fmicb.2021.757327

Lutz, J. K., van Balen, J., Crawford, J. M., Wilkins, J. R., 3rd, Lee, J., Nava-Hoet, R. C., & Hoet, A. E. (2014). Methicillin-resistant Staphylococcus aureus in public transportation vehicles (buses): another piece to the epidemiologic puzzle. *Am J Infect Control, 42*(12), 1285-1290. doi:10.1016/j.ajic.2014.08.016

Lyon, B. R., & Skurray, R. (1987). Antimicrobial resistance of Staphylococcus aureus: genetic basis. *Microbiol Rev, 51*(1), 88-134. doi:10.1128/mr.51.1.88-134.1987

Majumdar, R. K., & Gupta, S. (2020). Isolation, identification and characterization of Staphylococcus sp. from Indian ethnic fermented fish product. *Lett Appl Microbiol, 71*(4), 359-368. doi:10.1111/lam.13362

Manandhar, S., Singh, A., Varma, A., Pandey, S., & Shrivastava, N. (2018). Biofilm Producing Clinical Staphylococcus aureus Isolates Augmented Prevalence of Antibiotic Resistant Cases in Tertiary Care Hospitals of Nepal. *Front Microbiol, 9*. doi:10.3389/fmicb.2018.02749

Matthews, K. R., Roberson, J., Gillespie, B. E., Luther, D. A., & Oliver, S. P. (1997). Identification and Differentiation of Coagulase-Negative Staphylococcus aureus by Polymerase Chain Reaction. *Journal of Food Protection, 60*(6), 686-688. doi:https://doi.org/10.4315/0362-028X-60.6.686

Menestrina, G., Dalla Serra, M., Comai, M., Coraiola, M., Viero, G., Werner, S., . . . Prévost, G. (2003). Ion channels and bacterial infection: the case of beta-barrel pore-forming protein toxins of Staphylococcus aureus. *FEBS Lett, 552*(1), 54-60. doi:10.1016/s0014-5793(03)00850-0

Michalik, M., Samet, A., Podbielska-Kubera, A., Savini, V., Międzobrodzki, J., & Kosecka-Strojek, M. (2020). Coagulase-negative staphylococci (CoNS) as a significant etiological factor of laryngological infections: a review. *Ann Clin Microbiol Antimicrob, 19*(1), 26. doi:10.1186/s12941-020-00367-x

Mlynarczyk-Bonikowska, B., Kowalewski, C., Krolak-Ulinska, A., & Marusza, W. (2022). Molecular Mechanisms of Drug Resistance in Staphylococcus aureus. *Int J Mol Sci, 23*(15). doi:10.3390/ijms23158088

Momeni, B. (2018). Division of Labor: How Microbes Split Their Responsibility. *Curr Biol, 28*(12), R697-r699. doi:10.1016/j.cub.2018.05.024

Monaco, M., Pimentel de Araujo, F., Cruciani, M., Coccia, E. M., & Pantosti, A. (2017). Worldwide Epidemiology and Antibiotic Resistance of Staphylococcus aureus. *Curr Top Microbiol Immunol, 409*, 21-56. doi:10.1007/82_2016_3

Monroe, D. (2007). Looking for chinks in the armor of bacterial biofilms. *PLoS Biol, 5*(11), e307. doi:10.1371/journal.pbio.0050307

Mott, G. A., Desveaux, D., & Guttman, D. S. (2018). A High-Sensitivity, Microtiter-Based Plate Assay for Plant Pattern-Triggered Immunity. *Mol Plant Microbe Interact, 31*(5), 499-504. doi:10.1094/mpmi-11-17-0279-ta

Mulder, J. G. (1995). A simple and inexpensive method for the identification of Staphylococcus epidermidis and Staphylococcus hominis. *Eur J Clin Microbiol Infect Dis, 14*(12), 1052-1056. doi:10.1007/bf01590938

Nicola, F. G., McDougal, L. K., Biddle, J. W., & Tenover, F. C. (1998). Characterization of erythromycin-resistant isolates of Staphylococcus aureus recovered in the United States from 1958 through 1969. *Antimicrob Agents Chemother, 42*(11), 3024-3027. doi:10.1128/aac.42.11.3024

Noble, W. C. (1997). Antibiotic resistance in the staphylococci. *Sci Prog, 80 (Pt 1)*, 5-20.

Ohta, T., Hirakawa, H., Morikawa, K., Maruyama, A., Inose, Y., Yamashita, A., . . . Hayashi, H. (2004). Nucleotide substitutions in Staphylococcus aureus strains, Mu50, Mu3, and N315. *DNA Res, 11*(1), 51-56. doi:10.1093/dnares/11.1.51

Oliveira, D., Borges, A., & Simões, M. (2018). Staphylococcus aureus Toxins and Their Molecular Activity in Infectious Diseases. *Toxins (Basel), 10*(6). doi:10.3390/toxins10060252

Otter, J., & French, G. L. (2009). Bacterial contamination on touch surfaces in the public transport system and in public areas of a hospital in London. *Lett Appl Microbiol, 49*, 803-805. doi:10.1111/j.1472-765X.2009.02728.x

Otter, J. A., & French, G. L. (2009). Bacterial contamination on touch surfaces in the public transport system and in public areas of a hospital in London. *Lett Appl Microbiol, 49*(6), 803-805. doi:https://doi.org/10.1111/j.1472-765X.2009.02728.x

Otto, M. (2013a). Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection: Staphylococcal commensal species such as Staphylococcus epidermidis are being recognized as important sources of genes promoting MRSA colonization and virulence. *Bioessays, 35*(1), 4-11. doi:10.1002/bies.201200112

Otto, M. (2013b). Community-associated MRSA: what makes them special? *Int J Med Microbiol, 303*(6-7), 324-330. doi:10.1016/j.ijmm.2013.02.007

Pandey, R., Mishra, S. K., & Shrestha, A. (2021). Characterisation of ESKAPE Pathogens with Special Reference to Multidrug Resistance and Biofilm Production in a Nepalese Hospital. *Infect Drug Resist, 14*, 2201-2212. doi:10.2147/idr.s306688

Parsek, M. R., & Singh, P. K. (2003). Bacterial biofilms: an emerging link to disease pathogenesis. *Annu Rev Microbiol, 57*, 677-701. doi:10.1146/annurev.micro.57.030502.090720

Paul, S. I., Rahman, M. M., Salam, M. A., Khan, M. A. R., & Islam, M. T. (2021). Identification of marine sponge-associated bacteria of the Saint Martin's island of the Bay of Bengal emphasizing on the prevention of motile Aeromonas septicemia in Labeo rohita. *Aquaculture, 545*, 737156. doi:https://doi.org/10.1016/j.aquaculture.2021.737156

Paulitsch-Fuchs, A. H., Bödendorfer, B., Wolrab, L., Eck, N., Dyer, N. P., & Lohberger, B. (2022). Effect of Cobalt-Chromium-Molybdenum Implant Surface Modifications on Biofilm Development of S. aureus and S. epidermidis. *Front Cell Infect Microbiol, 12*, 837124. doi:10.3389/fcimb.2022.837124

Peetermans, M., Verhamme, P., & Vanassche, T. (2015). Coagulase Activity by Staphylococcus aureus: A Potential Target for Therapy? *Semin Thromb Hemost, 41*(4), 433-444. doi:10.1055/s-0035-1549849

Periasamy, S., Joo, H.-S., Duong, A. C., Bach, T.-H. L., Tan, V. Y., Chatterjee, S. S., . . . Otto, M. (2012). How <i>Staphylococcus aureus</i> biofilms develop their characteristic structure. *Proceedings of the National Academy of Sciences, 109*(4), 1281-1286. doi:doi:10.1073/pnas.1115006109

Prechtl, S., Roellinghoff, M., Scheper, R., Cole, S. P., Deeley, R. G., & Lohoff, M. (2000). The multidrug resistance protein 1: a functionally important activation marker for murine Th1 cells. *J Immunol, 164*(2), 754-761. doi:10.4049/jimmunol.164.2.754

Rapacka-Zdonczyk, A., Wozniak, A., Nakonieczna, J., & Grinholc, M. (2021). Development of Antimicrobial Phototreatment Tolerance: Why the Methodology Matters. *Int J Mol Sci, 22*(4). doi:10.3390/ijms22042224

Reyes-Robles, T., & Torres, V. J. (2017). Staphylococcus aureus Pore-Forming Toxins. *Curr Top Microbiol Immunol, 409*, 121-144. doi:10.1007/82_2016_16

Rivas, J. M., Speziale, P., Patti, J. M., & Höök, M. (2004). MSCRAMM--targeted vaccines and immunotherapy for staphylococcal infection. *Curr Opin Drug Discov Devel, 7*(2), 223-227.

Rossi, C. C., Pereira, M. F., & Giambiagi-deMarval, M. (2020). Underrated Staphylococcus species and their role in antimicrobial resistance spreading. *Genet Mol Biol, 43*(1 suppl 2), e20190065. doi:10.1590/1678-4685-gmb-2019-0065

Sabaté Brescó, M., Harris, L. G., Thompson, K., Stanic, B., Morgenstern, M., O'Mahony, L., . . . Moriarty, T. F. (2017). Pathogenic Mechanisms and Host Interactions in Staphylococcus epidermidis Device-Related Infection. *Front Microbiol, 8*, 1401. doi:10.3389/fmicb.2017.01401

Santos-Lopez, A., Marshall, C. W., Scribner, M. R., Snyder, D. J., & Cooper, V. S. (2019). Evolutionary pathways to antibiotic resistance are dependent upon environmental structure and bacterial lifestyle. *Elife, 8*. doi:10.7554/eLife.47612

Sato, T., Ito, R., Kawamura, M., & Fujimura, S. (2022). The Risk of Emerging Resistance to Trimethoprim/Sulfamethoxazole in Staphylococcus aureus. *Infect Drug Resist, 15*, 4779-4784. doi:10.2147/idr.s375588

Schleifer, K. H., & Kroppenstedt, R. M. (1990). Chemical and molecular classification of staphylococci. *Soc Appl Bacteriol Symp Ser, 19*, 9s-24s. doi:10.1111/j.1365-2672.1990.tb01794.x

Schlievert, P. M., & Davis, C. C. (2020). Device-Associated Menstrual Toxic Shock Syndrome. *Clin Microbiol Rev, 33*(3). doi:10.1128/cmr.00032-19

Schmitz, F.-J., Krey, A., Sadurski, R., Verhoef, J., Milatovic, D., Fluit, A. C., & participants, f. t. E. S. (2001). Resistance to tetracycline and distribution of tetracycline resistance genes in European Staphylococcus aureus isolates. *Journal of Antimicrobial Chemotherapy, 47*(2), 239-240. doi:10.1093/jac/47.2.239

Seng, R., Leungtongkam, U., Thummeepak, R., Chatdumrong, W., & Sitthisak, S. (2017). High prevalence of methicillin-resistant coagulase-negative staphylococci isolated from a university environment in Thailand. *Int Microbiol, 20*(2), 65-73. doi:10.2436/20.1501.01.286

Sharaf, E., El-Sayed, W., & Abosaif, R. (2014). Lecithinase-Producing Bacteria from Commercial and Homemade Foods: Evaluation of Toxigenic Properties and Identification of Potent Producers. *Journal of Taibah University for Science, 8*, 207-215. doi:10.1016/j.jtusci.2014.03.006

Shi, M., Chen, X., Sun, Y., Kim, H. K., Schneewind, O., & Missiakas, D. (2021). A protein A based Staphylococcus aureus vaccine with improved safety. *Vaccine, 39*(29), 3907-3915. doi:10.1016/j.vaccine.2021.05.072

Shittu, A., Lin, J., Morrison, D., & Kolawole, D. (2006). Identification and molecular characterization of mannitol salt positive, coagulase-negative staphylococci from nasal samples of medical personnel and students. *Journal of Medical Microbiology, 55*(3), 317-324. doi:https://doi.org/10.1099/jmm.0.46072-0

Silva, V., Caniça, M., Capelo, J. L., Igrejas, G., & Poeta, P. (2020). Diversity and genetic lineages of environmental staphylococci: a surface water overview. *FEMS Microbiology Ecology, 96*(12). doi:10.1093/femsec/fiaa191

Snitser, O., Russ, D., Stone, L. K., Wang, K. K., Sharir, H., Kozer, N., . . . Kishony, R. (2020). Ubiquitous selection for mecA in community-associated MRSA across diverse chemical environments. *Nature Communications, 11*(1), 6038. doi:10.1038/s41467-020-19825-3

Somerville, G. A., & Proctor, R. A. (2009). The Biology of Staphylococci *Staphylococci in Human Disease* (pp. 1-18).

Sorlozano-Puerto, A., Lopez-Machado, I., Albertuz-Crespo, M., Martinez-Gonzalez, L. J., & Gutierrez-Fernandez, J. (2020). Characterization of Fosfomycin and Nitrofurantoin Resistance Mechanisms in Escherichia coli Isolated in Clinical Urine Samples. *Antibiotics, 9*(9), 534.

Speer, B. S., Shoemaker, N. B., & Salyers, A. A. (1992). Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clin Microbiol Rev, 5*(4), 387-399. doi:doi:10.1128/cmr.5.4.387

Spoering, A. L., & Lewis, K. (2001). Biofilms and planktonic cells of Pseudomonas aeruginosa have similar resistance to killing by antimicrobials. *J Bacteriol, 183*(23), 6746-6751. doi:10.1128/jb.183.23.6746-6751.2001

Stepanovic, S., Cirković, I., Djukić, S., Vukovic, D., & Svabić-Vlahović, M. (2008). Public transport as a reservoir of methicillin-resistant staphylococci. *Lett Appl Microbiol, 47*, 339-341. doi:10.1111/j.1472-765X.2008.02436.x

Stepanović, S., Cirković, I., Djukić, S., Vuković, D., & Svabić-Vlahović, M. (2008). Public transport as a reservoir of methicillin-resistant staphylococci. *Lett Appl Microbiol, 47*(4), 339-341. doi:10.1111/j.1472-765x.2008.02436.x

Stevens, M., & Geary, C. (1989). Comparative evaluation of a latex test for the identification of Staphylococcus aureus. *Eur J Clin Microbiol Infect Dis, 8*(2), 153-156. doi:10.1007/bf01963902

Stewart, P. S., & Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *Lancet, 358*(9276), 135-138. doi:10.1016/s0140-6736(01)05321-1

Styers, D., Sheehan, D. J., Hogan, P., & Sahm, D. F. (2006). Laboratory-based surveillance of current antimicrobial resistance patterns and trends among Staphylococcus aureus: 2005 status in the United States. *Annals of Clinical Microbiology and Antimicrobials, 5*(1), 2. doi:10.1186/1476-0711-5-2

Su, M., Lyles, J. T., Petit Iii, R. A., Peterson, J., Hargita, M., Tang, H., . . . Read, T. D. (2020). Genomic analysis of variability in Delta-toxin levels between Staphylococcus aureus strains. *PeerJ, 8*, e8717. doi:10.7717/peerj.8717

Supersac, G., Piémont, Y., Kubina, M., Prévost, G., & Foster, T. J. (1998). Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of Staphylococcus aureus. *Microb Pathog, 24*(4), 241-251. doi:10.1006/mpat.1997.0192

Switalski, L. M., Rydén, C., Rubin, K., Ljungh, A., Höök, M., & Wadström, T. (1983). Binding of fibronectin to Staphylococcus strains. *Infect Immun, 42*(2), 628-633. doi:10.1128/iai.42.2.628-633.1983

Takahashi, H., Suda, T., Tanaka, Y., & Kimura, B. (2010). Cellular hydrophobicity of Listeria monocytogenes involves initial attachment and biofilm formation on the surface of polyvinyl chloride. *Lett Appl Microbiol, 50*(6), 618-625. doi:10.1111/j.1472-765X.2010.02842.x

Takahashi, T., Satoh, I., & Kikuchi, N. (1999). NOTE. *International Journal of Systematic and Evolutionary Microbiology, 49*(2), 725-728. doi:https://doi.org/10.1099/00207713-49-2-725

Tam, K., & Torres, V. J. (2019). Staphylococcus aureus Secreted Toxins and Extracellular Enzymes. *Microbiol Spectr, 7*(2). doi:10.1128/microbiolspec.GPP3-0039-2018

Thakur, P., Nayyar, C., Tak, V., & Saigal, K. (2017). Mannitol-fermenting and Tube Coagulase-negative Staphylococcal Isolates: Unraveling the Diagnostic Dilemma. *J Lab Physicians, 9*(1), 65-66. doi:10.4103/0974-2727.187926

Thomas, C. M., & Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat Rev Microbiol, 3*(9), 711-721. doi:10.1038/nrmicro1234

Thomas, V. C., & Hancock, L. E. (2009). Suicide and fratricide in bacterial biofilms. *Int J Artif Organs, 32*(9), 537-544. doi:10.1177/039139880903200902

Thurlow, L. R., Hanke, M. L., Fritz, T., Angle, A., Aldrich, A., Williams, S. H., . . . Kielian, T. (2011). Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J Immunol, 186*(11), 6585-6596. doi:10.4049/jimmunol.1002794

Trzcinski, K., Cooper, B. S., Hryniewicz, W., & Dowson, C. G. (2000). Expression of resistance to tetracyclines in strains of methicillin-resistant Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy, 45*(6), 763-770. doi:10.1093/jac/45.6.763

Tuchscherr, L., Löffler, B., Buzzola, F. R., & Sordelli, D. O. (2010). Staphylococcus aureus adaptation to the host and persistence: role of loss of capsular polysaccharide expression. *Future Microbiol, 5*(12), 1823-1832. doi:10.2217/fmb.10.147

Udo, E. E., Boswihi, S. S., Mathew, B., Noronha, B., & Verghese, T. (2021). Resurgence of Chloramphenicol Resistance in Methicillin-Resistant Staphylococcus aureus Due to the Acquisition of a Variant Florfenicol Exporter (fexAv)-Mediated Chloramphenicol Resistance in Kuwait Hospitals. *Antibiotics, 10*(10), 1250.

Uzun, B., Güngör, S., Pektaş, B., Aksoy Gökmen, A., Yula, E., Koçal, F., & Kaya, S. (2014). [Macrolide-lincosamide-streptogramin B (MLSB) resistance phenotypes in clinical Staphylococcus isolates and investigation of telithromycin activity]. *Mikrobiyol Bul, 48*(3), 469-476. doi:10.5578/mb.7748

Vasileiou, N. G. C., Chatzopoulos, D. C., Gougoulis, D. A., Sarrou, S., Katsafadou, A. I., Spyrou, V., . . . Fthenakis, G. C. (2018). Slime-producing staphylococci as causal agents of subclinical mastitis in sheep. *Vet Microbiol, 224*, 93-99. doi:10.1016/j.vetmic.2018.08.022

von Eiff, C., Arciola, C. R., Montanaro, L., Becker, K., & Campoccia, D. (2006). Emerging Staphylococcus species as new pathogens in implant infections. *Int J Artif Organs, 29*(4), 360-367. doi:10.1177/039139880602900405

von Eiff, C., Peters, G., & Heilmann, C. (2002). Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis, 2*(11), 677-685. doi:10.1016/s1473-3099(02)00438-3

Vuong, C., & Otto, M. (2002). Staphylococcus epidermidis infections. *Microbes Infect, 4*(4), 481-489. doi:10.1016/s1286-4579(02)01563-0

Vuotto, C., Longo, F., Balice, M. P., Donelli, G., & Varaldo, P. E. (2014). Antibiotic Resistance Related to Biofilm Formation in Klebsiella pneumoniae. *Pathogens, 3*(3), 743-758. doi:10.3390/pathogens3030743

Wang, C., Fang, R., Zhou, B., Tian, X., Zhang, X., Zheng, X., . . . Zhou, T. (2019). Evolution of resistance mechanisms and biological characteristics of rifampicin-resistant Staphylococcus aureus strains selected in vitro. *BMC Microbiol, 19*(1), 220. doi:10.1186/s12866-019-1573-9

Yamamoto, T., Nishiyama, A., Takano, T., Yabe, S., Higuchi, W., Razvina, O., & Shi, D. (2010). Community-acquired methicillin-resistant Staphylococcus aureus: community transmission, pathogenesis, and drug resistance. *J Infect Chemother, 16*(4), 225-254. doi:10.1007/s10156-010-0045-9

Yeh, P. J., Simon, D. M., Millar, J. A., Alexander, H. F., & Franklin, D. (2011). A diversity of Antibiotic-resistant Staphylococcus spp. in a Public Transportation System. *Osong Public Health Res Perspect, 2*(3), 202-209. doi:10.1016/j.phrp.2011.11.047

Yılmaz, E. Ş., & Aslantaş, Ö. (2017). Antimicrobial resistance and underlying mechanisms in Staphylococcus aureus isolates. *Asian Pacific Journal of Tropical Medicine, 10*(11), 1059-1064. doi:https://doi.org/10.1016/j.apjtm.2017.10.003

Zhou, F., & Wang, Y. (2013). Characteristics of antibiotic resistance of airborne Staphylococcus isolated from metro stations. *Int J Environ Res Public Health, 10*(6), 2412-2426. doi:10.3390/ijerph10062412

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