MOLECULAR CHARACTERISATION OF *Staphylococcus aureus* SUBTYPES INFECTING POST-SURGICAL WOUNDS OF PATIENTS REPORTING TO KAKAMEGA COUNTY TEACHING AND REFERRAL HOSPITAL

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A Thesis Submitted in Partial Fulfillment for the Requirements for the Award of the Degree of Master of Science in Molecular Biology of Masinde Muliro University of Science and Technology

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DEDICATION

I dedicate this research work to my daughter, Gianna Nashipae Mogoi, for her budding curiosity in a world hungry for critical thinkers.

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ABSTRACT

Post-surgical wounds are progressively becoming hotspots for multi-drug resistant (MDR) *Staphylococcus aureus* infections globally. *S. aureus* has been attributed to more than 1.1 million hospital deaths as of 2019, marking hospitals as favorable environment for its survival and transmission. Reports indicate that the success of *S. aureus* stems from genetic diversity, attributed to the development of drug resistance and genetic modifications in its genome. However, the molecular structure and evolution of *S. aureus* to unravel its genetic diversity remains geographically limited. Information on dominant lineages of the pathogen in Western Kenya is needed. Therefore, the present cross-sectional study sought to molecularly characterise *S. aureus* sub-types infecting post-surgical sites of patients reporting to Kakamega County General Teaching and Referral Hospital.This study was based on patients in the surgical wards of Kakamega County General Teaching and Referral Hospital (KCGTRH). The study recruited seventy (70) consented patients from the surgical wards, presenting with post-surgical wound infections. Wounds were phenotypically examined and classified based on CDC scheme, and swabs taken aseptically. Standard microbiological cultures and biochemical tests were carried out on the swabs for microbial recovery. Antimicrobial sensitivity tests were carried out on the recovered isolates using the selected 15 antibiotics. Associations between antibiotic history, wound class, and organism isolated. was determined at $p < 0.05$ (Spearman Chi-square). Zymogen DNA extraction kit was used for DNA extraction from all the six strains which were resistant to Oxacillin and Ceftriaxone, a 3rd generation cephalosporin used as a prophylactic surgical treatment at the facility. The genomes were sequenced by HiSeq Illumina platform (NoVo gene Co., Ltd). Raw reads were trimmed (Unipro UGENE v.44.0), assembled (SPAdes and bowtie2). Sequences of each cluster of orthologous genes were aligned (MUSCLE), and locations of horizontally acquired genomic islands (GIs) in bacterial chromosomes mapped (Seward Gene Island Sniffer). Moreover, subtypes were determined (multi-locus sequence typing, MLST), and genetic diversity determined by sequence alignment and phylogenetic analysis (Mauve). Drug resistant genes (DRGs) and virulence genes were determined (Comprehensive Antibiotic Resistance Database- Resistant Gene Identifier, CARD-RGI), and the virulence factor database (VDFB) respectively. For modifications in DRGs, respective protein sequences were aligned (ClustalW-BioEdit v7.0.5.3) to identify mismatches in amino acid sequences. Among the 70 isolates, 24 (34.4%) was *S. aureus*. Class III wounds were predominated by *Pseudomonas aeruginosa*, while class IV wounds by *S. aureus* (p=0.001), and between wound class and previous history of exposure to antibiotics (p = 0.001). Sequence type (ST) 152 was the most predominant sub-type in circulation within the community and hospital environment. ST8 had an extended antimicrobial resistance, mostly due to mutated target proteins and regulators of efflux pumps. Five of the six isolates contained a 20.7-kb circular plasmid carrying *blaZ* (associated with resistance to β-lactam antibiotics). These five strains all belonged to the same sequence type, ST152. Despite the similarity of the plasmids of these isolates, whole genome sequencing revealed that the strains differed, depending on whether they were associated with hospital-acquired or community-acquired infections. Genomic islands contributed significantly to their virulence potential. Among the 24 *S. aureus* isolates, 25% were multi-drug resistant. Several antimicrobial resistant and virulence genes were discovered. The most striking evolutionary changes were observed in the UvrAB gene complex. This complex was conserved in the hospital-acquired isolates of ST152 but varied significantly in the community-acquired isolates of ST152 and in ST8.The presents a restricted cluster of drug resistance genes circulating within the study site, with a predominant subtype 152 among hospital and community settings and therefore, calls for urgent control measures.

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ABBREVIATIONS AND ACRONYMS

AMR	Anti-Microbial Resistant Genes
BA	Blood Agar
CA	Community Acquired
CAIs	Community Acquired Infections
CC	Clonal Complex
CDC	Centre For Disease Control
CLSI	Clinical Laboratory Standard Institute
CRO	Ceftriaxone
DRG	Drug Resistant Genes
DST	Drug Sensitivity Test
GIs	Genomic Islands
HA	Hospital-Acquired
HAI	Hospital-Acquired Infection
IRB	Institutional Review Board.
KCGTRH	Kakamega County General Teaching and Referral Hospital
MDR	Multi-Drug Resistant
MHA	Mueller Hinton Agar
MLST	Multi-locus Sequence Typing
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Sgar
OXA	Oxacillin
PBP	Penicillin Binding Protein
PCR	Polymease Chain Reaction
PSW	Post-Surgical Wounds
PASW	Predictive Analysis SoftWare
rRNA	Ribosomal Ribonucleic Acid
SCCmec	*Staphylococcal* Cassette Chromosome
SSI	Surgical Site Infection
STs	Sequence Types

OPERATIONALIZATION OF TERMS

AMR (Anti-Microbial Resistance) – The intrinsic ability of microorganisms to resist the effects of antimicrobial drugs used at the facility, therefore making surgical infections harder to treat.

Antibiotics – Medications that were used to treat bacterial infections by either killing bacteria or inhibiting their growth on the wounds.

BA (Blood Agar) – A nutrient-rich medium used for culturing bacteria- used in the research for detecting hemolysis.

CA (Community Acquired) – Infections acquired outside of healthcare facilities, by the time ptients were being admitted.

CAIs (Community Acquired Infections) – Infections contracted in the community rather than in a hospital or healthcare setting by time of admission.

CC (Clonal Complex) – A group of genetically related bacterial strains that share a common ancestor in the study.

Class I wounds- These are clean wounds; uninfected operative wounds where no inflammation was encountered, and the respiratory, alimentary or genital tracts were not entered.

Class II wounds- Contaminated wounds; these are operative wounds in which the respiratory, alimentary, genital or urinary tract was entered, under controlled conditions.

Class III wounds- These were contaminated wounds; These is where operations had major breaks in sterile technique, and incisions with acute, non-purulent inflammation.

Class IV wounds- These were dirty or infected wounds; old traumatic wounds with retained devitalised tissue and those which involved existing infection.

CRO (Ceftriaxone) – A third-generation cephalosporin antibiotic used to treat various bacterial infections, which was used as first line prophylaxis at the hospital.

DRG (Drug Resistant Genes) – Genes that provide bacteria with resistance to antimicrobial agents, and which were detected in the study..

DST (Drug Sensitivity Test) – A test which was used to determine the susceptibility of study bacteria to different antibiotics panel.

Gene – A unit of heredity composed of DNA that encodes functional molecules- proteins, and coding for antimicrobial resistance seen in the study.

Genetic Diversity – The variety of genetic material within a population of bacteria, which accounted for species-level variability of sequenced genomes in the study.

GIs (Genomic Islands) – Large segments of DNA in bacterial genomes which were acquired through horizontal gene transfer. They contained genes related to antibiotic resistance or virulence.

HA (Hospital Acquired) – Infections that are contracted within a hospital setting, within 72 hours of admission.

HAI (Hospital Acquired Infection) – An infection which was acquired in the hospital, typically occurring 72 hours after admission and often associated with *S. aureus*.

MDR (Multi-Drug Resistant) – *S. aureus* strains that were resistant to multiple classes of antibiotics.

MLST (Multi-Locus Sequence Typing) – A molecular typing method which was used to characterise study strains based on the sequences of multiple housekeeping genes.

MRSA (Methicillin-Resistant *Staphylococcus aureus*) – *S. aureus* that was resistant to methicillin and other beta-lactam antibiotics.

MSA (Mannitol Salt Agar) – A selective medium which was used for isolating and identifying *Staphylococcus* species.

OXA (Oxacillin) – A beta-lactam antibiotic which was used to test for methicillin resistance in *Staphylococcus aureus*.

PBP (Penicillin-Binding Protein) – A group of proteins identified and that are involved in bacterial cell wall synthesis and are targets of beta-lactam antibiotics.

Plasmid – A small, circular piece of DNA which was found in study bacteria, and which carried antibiotic resistance genes between cells.

PSW (Post-Surgical Wounds) – Wounds that occurred after surgical procedures on the sampled patients.

SCCmec (Staphylococcal Cassette Chromosome mec) – A mobile genetic element that carried the mecA gene, which conferred methicillin resistance in *Staphylococcus* reference species.

SSI (Surgical Site Infection) – An infection that occurred at or near a surgical incision site of the study participants.

STs (Sequence Types) – Unique genetic identifiers which were assigned to the study bacterial strains based on MLST analysis.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

For decades, global reports have identified surgery as one of the many risk factors for the infections by *Staphylococcus aureus*, recovered from surgical sites (Scalise *et al.,* 2016; Thimmappa *et al.,* 2021). Surgical site infections (SSIs) are those infections which occur following surgery, usually attributed to bacterial or fungal infection. Globally, SSIs have been identified as a major contributor to morbidity and long hospital stays (Aiken *et al.,* 2020). Africa bears a disproportionate burden of these infections, with estimates suggesting that the prevalence ranges between 7% and 20%. This has been reported to be significantly higher than in high-income countries (Allegranzi *et al.,* 2018). This problem is projected to compound, drawing from limited resources, inadequate strategies for infection control and antimicrobial resistance (Aiken *et al.,* 2020).

S. aureus strains have been implicated in leading these infection surges, thus a clinical concern (Okeke *et al.,* 2021). While continental data highlights this challenge in public health, there is still a scarcity of data by country. In Kenya, reports emerging indicate a worrying trend with referral hospitals documenting SSI incidences attributed to *S. aureus* (Dinda *et al.,* 2013; Nyasinga *et al.,* 2020; Mogoi *et al.,* 2024), However, systematic epidemiological studies in many Kenyan hospitals remain scarce. This creates a gap in understanding the local infection and transmission pattern of *S. aureus* wound infections.

Consequently, pathogens such as *S. aureus* remain a significant healthcare/infection problem (Falagas *et al.,* 2013). The occurrence and transmission of SSIs is therefore of concern, even for patients undergoing minor operations. In their study of 643 post-operative patients in a

referral facility in Ethiopia, Gebissa *et al.,* (2021) observed that this problem is further exacerbated by the rise in bacterial pathogens which are resistant to antimicrobial agents and by the high incidence of immuno-suppressive HIV in the region. Collectively, these factors contribute to an escalating burden of hospital infections in Kenya (Nyasinga *et al.,* 2020; Wangai *et al.,* 2019), yet we have only limited information about how these infections are transmitted in the hospital setting.

Genetic diversity continues to strengthen the ability of a species to manage stressors in environments including exposure to antibiotics. Additionally, it remains a critical component in the adaptation and survival of bacteria for decades (Francino, 2012; Hanage, 2016; Reyes Ruiz *et al.,* 2020). Various bacterial inherited characteristics have helped to identify and classify them globally (Munita & Arias, 2016), between and among species. Interestingly, high genetic diversity analyses have revealed many bacterial infections with a wider variety of different traits. This has been implicated in adapting and changing environments, from hospital settings to community settings (diCenzo & Finan, 2017). Previous data show that hospitals provide a favorable setting for bacteria to acquire genetic variations, attributed to frequent exposure to- and misuse of antibiotics (Mustapha *et al.,* 2022; Lieberman *et al.,* 2014). These antibiotics are usually used in the treatment of post-surgical wounds (Norman *et al.,* 2016; Orelio *et al.,* 2020).

Recently, there has been a surge in post-surgical wound infections in sub-Saharan Africa with *S. aureus* predominating these infections (Almuhayawi *et al.,* 2023). However, this comes with limited information on hospital transmission on which wound class mostly harbors *S. aureus.* Class III and IV wounds have been suspected to harbor a community of microorganisms in recent reports, suggesting microbial adaptation which may vary to strain

level. Moreover, different wound classes may provide opportunities for biofilm formation for certain microbial pathogens (Percival *et al.,* 2015: Firas Diban *et al.,* 2023). Class III wounds, being contaminated (Onyekwelu *et al.,* 2017) may be hotspots for *S. aureus*. Based on genome analyses, nevertheless, a few *Staphylococci* subtypes/sequence types (STs) have been reported.

Globally, clones of *S. aureus* have been reported including CC5, CC1, ST8 (CC8), CC22, CC30, CC45 and ST239 (CC239), to be a problem in infections management (Nyasinga *et al.,* 2020). In Kenya, ST152 has also been documented to be colonizing post-operative patients (Njenga *et al.,* 2022; Kyany'a *et al.,* 2019), where ST239 appears to predominate. However, data on any occurrences of the subtypes in Western Kenya is limited. Genomic analyses on *S. aureus* recovered from SSI to explain these transmissions in Western Kenya is therefore needed. Understanding the structure and function of the genome of multi-drug resistant *S. aureus* from Western Kenya will provide insights into strengthening infection prevention and control (IPC) programs in Kenya.

Staphylococcus aureus specifically remains a clinically significant pathogen attributed to skin and soft tissue infections (Tong *et al.,* 2015; Ansari *et al.,* 2019; Siddiqui, 2022; Makwela *et al.,* 2023), wound infections, pneumonia, and bacteremia in humans (Ladhani, 2004; Maina *et al.,* 2013). Their treatment and control proves a challenge due to emergence of antimicrobial resistance (AMR) globally (Taylor & Unakal, 2021). This state of AMR has been reported against trimethoprim (TMP), ampicillin (AMP), amoxicillin (AMX), cephazolin (CFZ), ceftazidime (CAZ), penicillin (PEN), oxacillin (OXA), cefuroxime (CXM), clindamycin (CLM), gentamicin (GEN), cephalothin (CEF), tetracycline (TET), trimethopan (TMP), erythromycin (ERY), ceftriaxone (CRO), and cefalexin (CEF) (Nwankwo, & Nasiru, 2011).

In 2019 alone, *S. aureus* was ranked as leading cause of deaths accounting for more than 1.1 million deaths globally among bacterial pathogens, making it an organism of interest and therefore a need for more research input on global burden of disease by *S. aureus* (Vos *et al.,* 2020).

Moreover, reports have indicated a rapid and an unprecedented resurgence of antibiotic resistance and virulence factors in the last decade (Deyno *et al.,* 2017; Alarjani & Skalicky, 2021) thus posing challenges to healthcare systems worldwide (Ventola, 2015; Dadgostar, 2019; Cheung *et al.,* 2021). Mutations at gene levels have been attributed to the development, acquisition and spread of drug resistant genes (DRGs) (Watford & Warrington, 2020; Munita & Arias, 2016). Common mutations have been reported at the DRGs attributed to *S. aureus*, including V291I and D327E in the gene *vanT* (Silver, 2017; Xu *et al.,* 2017),. More mutations have been implicated in resistance to fluoroquinolones in the gene *parC* (Pan *et al.,* 2002; de Oliveira *et al.,* 2019), and mutation S84L in *gyrA* (Korotetskiy *et al.,* 2023) have been reported globally. However, the information on the state of AMR and associated gene mutations in Western Kenya remains limited.

In the current work, a collection of *S. aureus* recovered from both hospital-acquired infections and community-acquired infections at a referral hospital in Kakamega, Western Kenya, is analysed. This institution has an established antimicrobial stewardship (AMS) program but like many comparable facilities in sub-Saharan Africa, is underfunded and underutilized. Recent developments in the fields of molecular biology and bioinformatics makes it possible to understand the molecular characteristics of *S. aureus* in resource limited settings of Western Kenya. Specifically, the research focused on post-surgical wound characteristics, genetic diversity of *S. aureus* subtypes, antimicrobial resistance, and drug

resistance gene mutations. This is significant because it allows for the identification of common *S. aureus* subtypes that may be responsible for the majority of infection burden. It also helps in discriminating among pathogens involved in infections, a better understanding of antimicrobial resistance determinants, and elucidation of possible transmission pathways. Only then can we understand bacterial antibiotic targets and how they change over time, (Beceiro *et al.,* 2013).

1.2 Statement of the problem

Surgical site infections (SSIs) remain the leading cause of post-operative morbidity and mortality globally, with disproportionately high prevalence in low-resourced healthcare settings like in sub-Saharan Africa. Narrowing down to East Africa, and specifically Kenya, these infections are a true representation of the challenge to infection prevention and control (IPC) initiatives. This comes about despite the region boasting over 1 million trained healthcare workers and a growing hospital network. *S. aureus* has consistently been mapped as one of the pathogens predominating these facilities, and implicated in post surgical wound infections. This has contributed to prolonged hospital stays, increased costs of treatment, and subsequent high mortality rates (Humphreys *et al.,* 2016; Pal *et al.,* 2019; Fernandez *et al.,* 2020). The clinical impact of this pathogen is amplified further by the capacity to develop resistance to multiple antibiotic classes, thus rendering empirical therapy obsolete (Dinda *et al.,* 2013; Aiken *et al.,* 2014; Fernandez *et al.,* 2020). Although routine culture and sensitivity methods exist, they are time-consuming and may not be effective in distinguishing between *S. aureus* subtypes circulating in many regional facilities, and also cannot detect critical virulence and resistance genes. This gap is further compounded by the limited application of molecular epidemiological tools in these hospitals, thus the nosocomial transmission of aggressive *S. aureus* clones go undetected. Moreover, recent reports underscore substantial genetic variability and divergence within *S. aureus* populations (Howden *et al.,* 2023), but most of this data is limited to high-income settings. This leaves the situation in Western Kenya largely unexplored. Indeed, the limited detailed molecular epidemiological data and genetic profiling of the pathogen cripples the infection control and surveillance, and the larger antimicrobial stewardship in this region. Therefore, there is an urgent need to characterise the antimicrobial resistance pattern, genetic diversity and evolutionary dynamics of *S. aureus* from post surgical wounds infections in Western Kenya. This study sought to

bridge this gap by availing data to help inform IPC strategies, in line with strengthening antimicrobial stewardship programs in resource-poor settings.

1.3 Justification of the study

Although *Pseudomonas aeruginosa* is also a predominant pathogen in wound infections (Qin *et al.,* 2022; Phan *et al.,* 2023), testing for *Staphylococcus aureus* remains important due to its prevalence (Phan *et al.,* 2023), antibiotic resistance patterns (Gurung *et al.,* 2020; Zheng *et al.,* 2021; Moges *et al.,* 2024), clinical significance, potential for mixed infections, and implications for infection control and tailored treatment (Tong *et al.,* 2015; Yu *et al.,* 2020). Despite *Staphylococcus aureus* coexisting with *P. aeruginosa* and other pathogens in a wound, methicillin-resistant *Staphylococcus aureus* (MRSA), has become a significant concern in healthcare settings (Gurung *et al.,* 2020; Zheng *et al.,* 2021). Testing for *S. aureus* in wound infections is important because MRSA infections can be challenging to treat and require specific antibiotics (Okwu *et al.,* 2019; Zheng *et al.,* 2021) and hence identifying MRSA early may allow for appropriate antibiotic therapy. *Staphylococcal* wound infections continue to spread to new areas and niches in Sub-Saharan Africa, and there exist indications of impending overlap to data-devoid areas like Kakamega (Dinda *et al.,* 2013). Furthermore, the treatment of wound infections has emerged as an important source for use of antibiotics. In low middle income countries (LMICs), culture and sensitivity tests are hard to come by and therefore patients with wound infection are put on antibiotics treatment with little information to guide the most appropriate drug of choice that would benefit the patient. This is further compounded by limited local molecular surveillance and treatment approaches. The availability of this information at the Kakamega County General Hospital is therefore urgent. Furthermore, the facility is the largest referral health facility in Kakamega county, Western Kenya, attending to approximately 15,000 patients monthly, including those undergoing

surgery. However, just like any other referral health facility in the region, insufficient funding has threatened to cripple health services and initiatives. Its importance in the region is vast since it has established the antimicrobial stewardship program. Therefore, the availability of data on microbial wound composition, genetic diversity and antimicrobial resistance is needed to inform the urgency in strengthening infrastructure.

1.4. Objectives

1.4.1. General objective

To characterise molecularly *Staphylococcus aureus* subtypes infecting post-surgical wounds of patients reporting to Kakamega County Teaching and Referral Hospital in Western Kenya.

1.4.2. Specific objectives

i. To assess post-surgical wound infections for *Staphylococcus aureus* among patients reporting to Kakamega County Teaching and Referral Hospital.
ii. To determine the genetic diversity of *Staphylococcus aureus* subtypes recovered from post-surgical wounds of patients reporting to Kakamega County Teaching and Referral Hospital.
iii. To determine the antimicrobial resistance profile and genetic modifications of *Staphylococcus aureus* subtypes recovered from post-surgical wounds of patients reporting to Kakamega County Teaching and Referral Hospital.

1.4.3. Research questions

i. What is the prevalence of *Staphylococcus aureus* in post-surgical wound infections among patients at Kakamega County Teaching and Referral Hospital?

ii. What is the genetic diversity of *Staphylococcus aureus* infecting post-surgical sites at Kakamega County Teaching and Referral Hospital?

iii. What is the the antimcrobial resistance profile of *Staphylococcus aureus* infecting surgical wounds at Kakamega County Teaching and Referral Hospital?

1.5 Significance of the study

Detailed documentation of wound microbial composition is important for legal and regulatory compliance as required by the ministry of health of the Republic of Kenya and other countries. Moreover, understanding surgical wound microbial compositions and AMR resistance profiles of *S. aureus* will help in the targeted treatment of surgical wound infections at the facility and regionally. The general classes of the post-surgical wound infections will help understand and monitor the first-line post-surgical prophylaxes at the facility against *S. aureus* thus reducing treatment failures. The genetic structure of the organism- while unravelling gene mutations responsible for antimicrobial resistance to common antibiotics used at the facility- will help guide formulations of effective antibiotic strategies against post-surgical wound infections, research, and quality improvement. Although the importance of *Staphylococcus aureus* as a cause of nosocomial infections is well-documented worldwide, by employing whole genome sequencing and analysis, this study demonstrates a possibility of a well-established sub-type of *Staphylococcus aureus* in a health facility and community in Western Kenya. Moreover, this data potentially adds to the existing body of knowledge on the antimicrobial resistance structure, and the genetic makeup of *S. aureus* subtypes in Western Kenya and globally.

1.6 Limitations of the study

Following the review of the study, the following were the limitations encountered;

I. The first limitation of this hospital-based cross-sectional study stemmed from its design. For example, the scope only covered sampling after 4 weeks of post-operative procedure. This was attributable to the high rates of class III wounds infections that were reported. Other class I wound infections develop within a few days following surgery.

II. Another limitation stemmed from restrictions observed while picking the most populous microbe from the plates. Wounds have polymicrobial infections. However, this study has given a baseline assessment on which other studies assessing surgical wound infections can compare.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to surgical site infections

The Centers for Disease Control (CDC) defines surgical site infections (SSIs) as infections that occur at on near a surgical incision, usually within 30 days of a procedure, or within one year following an implant of a prosthetic material (CDC, 2017). Surgical sites and wounds are a major risk factor for the colonization success of such bacterial pathogens such as *S. aureus* (Mellinghoff *et al.,* 2018; Pal *et al.,* 2019; Paling *et al.,* 2018; Thimmappa *et al.,* 2021). Wounds from surgical sites are habitats for infecting pathogens that colonize and spread, delaying healing process. The CDC and the National Nosocomial Infections Surveillance (NNIS) ranked SSI as the third cause of these infections where in all the patients hospitalized, SSI accounted for 16% of all nosocomial infections (CDC, 2023). Moreover, global findings map the annual SSI incidence at 31000 to 35000 in all orthopedic procedures (Onyekwelu *et al.,* 2017; 1).

As per the need to identify the risk factors for nosocomial infections, the CDC categories SSIs according to the duration of occurrence. Any infection occurring within 30 days are regarded as superficial incision surgical sites, while those occurring up to 90 days of operative procedure are referred to as organ or space SSI. Among other, patients presenting with cardiothoracic surgery (Paling *et al.,* 2018), and diabetes are at a higher risk in Sub-Saharan Africa. However, those presenting with urological, those with lower extremity injuries and intra-abdominal do not have a higher risk of SSI (Onyekwelu *et al.,* 2017). Therefore, SSI predisposition stems from both major and minor operative procedures (Aiken *et al.,* 2013).

The infection duration of post-surgical infections can largely depend on the previous history of exposure to antibiotics (Zabaglo & Sharman, 2022). Previous exposure to an antibiotic can make an individual develop resistance to an antibiotic, which has been implicated to be caused by bacteria mutations drawing from misuse of these antibiotics in the facilities (Ventola, 2015; Llor & Bjerrum, 2014). This prolonged period of healing stemming from progression of bacterial infection, has been complimented with respective wound class to characterize wound infections among patients globally (Onyekwelu *et al.,* 2017). Class I wounds are considered clean, class II; clean and contaminated, class III; contaminated and class IV; dirty (Onyekwelu *et al.,* 2017; Herman & Bordoni, 2022). This information has been used to monitor and evaluate the effectiveness of most of first-line prophylaxes given to patients attending major and minor surgeries (Onyekwelu *et al.,* 2017; Mohareb *et al.,* 2021) in Africa. Nevertheless, the state of wound structures in Kenya and such area as Kakamega remains limited.

The time frame or cutoff value used to tell hospital acquired infections (HAIs) (nosocomial infections), from community-acquired infections (CAIs) has shown to vary depending on specific guidelines in each healthcare setting. Nevertheless, the general principle is that HAIs are those infections not present at the time of admission to hospital, but infection occurs up to 3 days (72 hours) after discharge, or up to 30 days after a surgical procedure. On the other hand, CAIs have been regarded as those infections detected within the first 48 hours of hospitalization (Chang *et al.,* 2011).

2.2 The role of *S. aureus* in surgical site infections

Staphylococcus aureus is a Gram-positive, facultative anaerobe which under a microscope, it appears as spherical cocci arranged in grape-like clusters (Wang *et al.,* 2012; Mancuso *et al.,* 2021). It is catalase- and coagulase positive, which is a distinguishing characteristic from other *Staphylococcus* species. Additionally, it possess a thick peptidoglycan cell wall responsible for its pathogenicity (Tong *et al.,* 2015), and has a genome size of approximately 2.81 Mb (Wang *et al.,* 2012).

The pathogen has been culprit in skin and soft tissue infection, especially those attributed to surgical site infections (Tong *et al.,* 2015). The pathogenicity if facilitated by a wide arsenal of virulence factors which include surface proteins which expedite adhesion, presence of toxins which cause damage to host tissues, and enzymes for the evasion of the immune system (Tong *et al.,* 2015). On these wounds, *S. aureus* delays healing and thus increase the risk of systemic infections and higher morbidity and mortality rates (Mancuso *et al.,* 2021). Additionally, strains like the methicillin-resistant *S. aureus* (MRSA) poses a serious threat, since resistance to multiple antibiotics complicates therapeutic interventions (Turner *et al.,* 2019). Since the pathogen is persistent in hospital environments, drawing from its rapid adaptation and horizontal gene transfer, it is the leading etiological agent of nosocomial wound infections globally and in Africa.

2.3 Antimicrobial resistance in *S. aureus*

2.3.1 Antimicrobial resistance and emergence of MRSA

Antimicrobial resistance, a global health and development threat (WHO, 2021), is defined as the ability of microbial pathogens to not only evolve, but also develop mechanisms enabling them resistant to the antimicrobials which were once effective in treating them. In so doing, research has shown that these medications become ineffective against them, hence a problem in controlling infections by these microbes (Dadgostar, 2019; Ventola, 2015). Reports have shed light into the occurrence of antimicrobial resistances which generally stems from the common one, misuses of antibiotics, natural selection, gene transfers, and microbial biofilm formation.

Since the inception and WHO recommendation of drugs like Penicillin against *S. aureus*, reports indicate the occurrence of *blaZ* gene in plasmids, with capabilities of neutralizing penicillin (Guo *et al.,* 2020). This comes among other genes conferring resistances to Erythromycin and Gentamycin (Guo *et al.,* 2020; Vestergaard *et al.,* 2019). In *S. aureus*, it has been thought that resistance to drugs develops when a bacterium acquires DRGs through horizontal gene transfer. Since most of these genes are located in plasmids, the transfer of mutations to such genes as gyrA and gryB is possible. These genes are implicated to resistance against fluoroquinolones and the over-expression of norA- a gene encoding efflux pump mechanism (Guo *et al.,* 2020; Mancuso *et al.,* 2021). Moreover, *S. aureus* is attributed to the acquisition of resistance to tetracyclines and trimethoprim-sulphamethoxazole via the different combination of mechanisms. Recently, Clindamycin has shown potential to prevent the spread of infections with excellent pharmacokinetics especially in cases where macrolide antibiotic resistance is noted. However, numerous observations indicate a propensity of *S. aureus* to launch antibiotic resistance against Clindamycin globally (Timsina *et al.,* 2021).

Information about the state of antibiotic resistance of *S. aureus* in Western Kenya is therefore needed.

Genetic factors have been attributed as the leading cause of natural selection and gene transfer mechanisms. In natural selection, for example, microbes have shown leverage their high reproduction rates to bring forth offspring bearing slight genetic variations, responsible for gene mutations or modifications which confer resistance and survive via selective pressure. For gene transfer, there is an exchange of a modified genetic material like drug resistant genes encoding drug targets, between strains (Tao *et al.,* 2022).

Amidist bacterial ability to adapt, the ministries of health across the globe, led by the Center for Disease Control (CDC) have been at the forefront of combating AMR incidence. The recommended antibiotics against microbial infections continue to roll out, in line with infection control programs (IPCs). For treatment of post-surgical wound infections, the World Health Organization (WHO) recommends parenteral administration of surgical antimicrobial prophylaxis (Ierano *et al.,* 2017), which includes 1-2g of Cefazolin (CEF), a first-generation cephalosporin. Moreover, a combination of beta-lactam antibiotics and an aminoglycoside like gentamicin are recommended. Others include nafcillin, oxacillin, vancomycin, daptomycin and linezolid.

However, the emergence of MRSA has excluded the use of these antibiotic for *S. aureus* infections (Esposito *et al.,* 2023). Despite, hospitals especially those in lower- and middle-income countries continue to use these antibiotics mostly as first line prophylaxes for patients undergoing minor and major surgeries. In Kenya for instance, the ministry of health recommends 1g cephazolin as the agent of choice due to its long duration of action. A recent

study in a leading referral facility Kenya focused on a point prevalence survey where data on antibiotic use was abstracted from patient medical records, In this study, they reported that the most prescribed and misused antibiotic classes were penicillins (46.9%) followed by cephalosporins like ceftriaxone a third-generation cephalosporin (44.7%) (Godman *et al.,* 2019). This report shows further predisposition of patients recovering from surgery to antimicrobial resistance. The World Association of Infectious Diseases and Immunological Disorders (WAidid 2020) data indicates that there are newer antibiotics developed to navigate the present problem of antibiotic resistance.

2.3.2 Antimicrobial resistance incidence globally and Kenya

The CDC (2021) recognizes antimicrobial resistance as a global threat to prevention of persistent diseases (Ferri *et al.,* 2017; Dadgoster, 2019), putting millions of lives at risk (Ventola, 2015). The CDC further links antimicrobial resistance to an additional $1,400 to the normal hospital bill for patients undergoing treatment for bacterial infections, and estimated to increase over the years, which could cripple healthcare reforms in lower- and middle-income countries (LMICs) (Prestinaci *et al.,* 2015;Worldbank, 2017; Dadgoster, 2019). *Staphylococcus aureus* at the forefront, it is therefore imperative that more data on the microbe is availed, to help in its control and management.

The success of AMR incident can be traced back to easy travel routes people opt for internationally and locally, increase in income levels, agricultural use of antibiotics among others (Dadgoster, 2019; Ventola, 2015; Dhingra *et al.,* 2020). However reported, there exist numerous knowledge gaps on AMR in line with human and animal use of antibiotics, which form avenues for the development and success of AMR globally. Drug resistance incidence has become a substantial clinical menace progressively, amidst the discovery and inception of

among others, beta-lactam antibiotics. However, MRSA proved to be a lead to resistance against these beta-lactams starting with vancomycin, a non-beta lactam but alternative to penicillin at the time (Ventola, 2015). This informed the onset of the seemingly never-ending antibiotic resistance problem globally, with pathogens showing resistance to basically all antibiotics to ever be developed.

Antimicrobial resistance has been reported in Ethiopia (Dilnessa & Bitew, 2016), where majority (50.5%) of the 1,360 *S. aureus* isolates were MDR strains. Furthermore, about 96.4% of them were resistant to Penicillin, 11.9% to Clindamycin and 5.1% to Vancomycin. Studies in Kenya have reported a spike in antimicrobial resistance in healthcare settings. A recent study conducted at the Kenyatta National hopsital looked at antibiotic susceptibility pattern of *S. aureus* from clinical isolates. Here, the researchers reported resistant to common antibiotics like Vancomycin, Trimethoprim and Tetracycline (Gitau *et al.,* 2018). Other studies have also attributed *S. aureus* resistance to trimethoprim at 97%, to cefoxitin (84.6%), clindamycin (75%) and Tetracycline (76%), recovered from surgical wounds (Salmanov *et al.,* 2019).

2.3.3 Treatment challenges posed by multi-drug resistant *S. aureus*

In her part 1 report of the antibiotic resistance crisis, Ventola, (2015) attributes infection incidences by bacterial pathogens like *S. aureus* due to unavailability of newer drugs designed against them. The shortage of newer drugs cripples the fight against AMR pathogens globally. There is a sharp decline in the development of newer antibiotics by pharmaceutical industries owing to regulatory and economic obstacles, with the decline in the number of antibiotic production and approval over the past decades.

Recently, studies have reported a correlation between AMR and high healthcare costs globally. This has been attributed to bacterial spontaneous evolution and mutational incidences owing to horizontal gene transfers among strains (Dadgoster, 2019). AMR-related deaths globally are escalating, with recent reports linking 1.1 million deaths to *S. aureus* infections alone (GBD, 2019). Moreover, it is estimated that this rate may triple by the year 2050, if no efforts are put to attempt understand the trends of this pathogen, among other reported ones. It is estimated that 4.15 million AMR-related mortality rates are projected to occur in Africa, by 2050, thus putting Sub-Saharan Africa on the limelight (Dadgoster, 2019; Gebissa *et al.,* 2021). Moreover, majority of reported infections in hospitals are thought to be community acquired, while catheter site infections from surgery contributing to most of these infections (Fernandez *et al.,* 2020).

2.4 Molecular typing and characterization of *S. aureus*

2.4.1 Importance of molecular techniques in understanding pathogen diversity

Molecular typing has enabled the unraveling of the genome of *S. aureus*, to enable an understanding of the diversity of the pathogen, in line with clinical importance. Traditional culture methods are valuable but are limited in the ability to discriminate between closely related bacterial strains and to detect virulence and resistance genes. On the contrary, molecular typing techniques like multi-locus sequence typing (MLST), Whole genome sequencing (WGS) and resistant gene identifying (RGI) provide high resolution data into the genetic diversity.

Moreover, these techniques enable the mapping of evolutionary relationships and pathogen transmission dynamics (Quainoo *et al.,* 2017). Indeed, these approaches go far to identify a specific clonal lineage associated with a disease outbreak and detecting mobile genetic

elements which confine resistance genes (Tagini & Greub, 2017). Importantly, the application of these tools in resource-poor settings hold immense potential for guiding policy on infection control, surveillance programs and antimicrobial stewardship. This is importance since in the long run, it will help reveal hidden reservoirs for resistance and transmission-two things which conventional diagnostics often overlook (Anyanwu *et al.,* 2021).

2.4.2 Detecting plasmids and genetic islands in *S. aureus*

Plasmids have been reported as key factors in the epidemiology and pathology of *S. aureus* globally. Moreover, several studies have attributed *S. aureus* plasmid to the carriage of *blaZ* gene (Guo *et al.,* 2020; Pennone *et al.,* 2022), perhaps indicating the ability of the plasmid and blaZ gene to co-exist and replicate autonomously. Recently a study observed, a carriage of a 20.7-kb plasmid in *S. aureus* in Egypt which appeared to confine *blaZ* gene (Mores *et al.,* 2021), indicating the presence of beta-lactam resistance in Africa. Moreover, the highly mosaic plasmid structures observed in these studies may suggest highly frequent recombination processes, since some plasmids are only associated with certain resistance and virulence genes (McCarthy & Lindsay, 2012). This further implies an association with their location on transposable elements like Tn552 and cadDx on insertion sequence (IS), enabling them jump from a strain to another (Rowland, 1989). First reported as a tool for bacterial transfer of genes horizontally (Juhas *et al.,* 2009), genomic islands (GIs) are part of the three avenues for bacterial evolution including mutations and rearrangements of genes. Moreover, *S. aureus* genomic islands facilitate horizontal transfer of genes, and have been reported globally (Novick & Ram, 2017). GIs are shown to be capable of integrating into chromosomes of the bacterial pathogens via conjugation and perhaps transduction. Thus based on the structure of a GI in line with genes it carries, a GI can be a factor in promotion of survival of a pathogenic bacteria like *S. aureus*.

A basic GI will have unique genomic features different from those found in the entire chromosome. Furthermore, studies have shown that they will have tRNA inserted, while harboring integrases, insertion sequences (ISs) and transposases. Depending on the gene content, a GI have been implicated as being of several types including symbiotic, metabolic, resistance and pathogenicity (Novick & Ram 2017). Apart from a bacterium transferring genes via GI, it can also transfer a part of its own chromosomes hence contributing to the general evolution process. Elsewhere, it has been reported that the chromosomal DNA transferred can reshape the recombination and subsequently, antigenic variation and antibiotic resistance (Moon *et al.,* 2016; Rao *et al.,* 2020). Therefore, understanding the GI, plasmid and chromosome structure of *S. aureus* can help map out the survival and adaptation success of the pathogen attributed to infections in the region.

2.4.3 Genome diversity of *S. aureus* subtypes

Multi-locus sequence typing and phylogenetics offer for a powerful and efficient tool to unravelling clonal sequence types (ST) and relatedness of organisms like *S. aureus* respectively. This is based on a collection of the seven house-keeping genes which include arcC – carbamate kinase, aroE – shikimate dehydrogenase, glpF – glycerol kinase, gmk – guanylate kinase, pta – phosphate acetyltransferase, tpi – triosephosphate isomerase, and yqiL – acetyl coenzyme A acetyltransferase. Clonal diversity and lineages have a role in determining infections outcome of wounds and surgical site infections (Shettigar & Murali, 2020). Globally, several sequence types of *S. aureus* have been reported where variants of CC97 and CC151 lead in both community and healthcare settings. Here, CC97 have shown to harbor up to seven STs including ST3274, ST3273, ST352, ST151, ST398, ST2187 and ST3028 (Thomas *et al.,* 2021), on wounds, all bearing enterotoxin genes and resistant to some common antibiotics of Clindamycin, Penicillin, Erythromycin and Tetracycline.

Moreover, a whole genome-wide analysis of ST72 from global sources of wound infections has revealed a spike in resistance against antimicrobials and carriage of virulence potency (Batool *et al.,* 2021). Data presented here offers insights into the global and local distribution of *S. aureus* STs. Many STs have been discovered both in animal and human reservoirs. For instance, ST5, ST398 and ST34 have been found in both animals and humans, but with ST22 and ST398 highly confined in animals, which when found in hospital infections, are evidence for community acquired infections (Salgueiro *et al.,* 2020).

Some STs are geographically confined. For instance, recent studies in Egypt revealed burden of ST152 (Mores *et al.,* 2021), ST239 and ST8 (CC8) (Montelongo *et al.,* 2022) ST22 and CC5, CC30 in Western Portugal (Salgueiro *et al.,* 2020). In Kenya however, ST152, ST39, ST241 and ST8 (CC8) colonizing healthcare settings (Kyany'a *et al.,* 2019; Njenga *et al.,* 2022) and ST239 (Aiken *et al.,* 2014), have been reported. Previously, STs 5, 8, 22, 30, 88, 152, 239, 241, 789, and 4705 were reported to colonize wounds in Kenya (Njenga *et al.,* 2022), including surgical, showing increased infection incidences therefore prolonging wound healing. This study was conducted in two facilities in Kenya, where DNA from isolates from wounds swabs were sequenced. Though reported in the country, the state of ST diversities to explain the occurrence of these clones in Western Kenya is not known.

2.5 Detection of virulence and resistance determinants

Different bacterial pathogens have evolved different mechanism of averting evading antimicrobial infections. The most common mechanissm include acquiring drug resistant genes (DRGs) and virulence genes. By way of sequencing and annotating their genomes, it has become possible to map out these genes and their respective mechanisms of action (Quainoo *et al.,* 2017; Tagini & Greub, 2017). For instance, *P. aeruginosa,* which causes

wound infections is implicated to carry intrinsic resistance to various antibiotics including A, B, C and D classes of beta-lactamases, with risk of transferring this resistance capabilities to other strains like *S. aureus* (Hwang & Yoon, 2019; Pang *et al.,* 2019). This pathogen has been discovered to use efflux pump over-expression, altering the permeability of membrane to the drugs and perhaps mutating genes thus blocking the diffusion mechanism for antibiotics across its cell membrane (Langendonk *et al.,* 2021). *P. auruginosa* has also shown resistance against Ceftazidime and Cefepime (Sader *et al.,* 2017), by employing such endogenous lactamases as AmpC beta-lactamase among others (Pachori *et al.,* 2019). However, as previously reported, such resistances have been managed with the proper use of Fosfomycin and a combination of Cephalosporins and Penicillin (Ontong *et al.,* 2021).

Bacterial antibiotic resistance mechanism comes about upon prolonged exposure to the same antibiotics (Sun *et al.,* 2019). These resistances can be natural (intrinsic and induced) or acquired (Mancuso *et al.,* 2021). In intrinsic case, a bacterial pathogen will develop resistance naturally to such antibiotics as Vancomycin and Ampicillin, some 1st and 2nd generation cephalosporins (Reygaert, 2018; Sandner-Miranda *et al.,* 2018), without relying on previous exposure to any antibiotics. Furthermore, natural resistance can be induced by acquisition of genes from other strains (Ben *et al.,* 2019), especially polymicrobial infections on surgical wounds. On the other hand, acquired resistance is induced by mutations and DNA transfer (Mancuso *et al.,* 2021). Studies have reported that several mutations can be a consequence of nosocomial infections, where mutant strains develop resistance over time of exposure to the same panel of antibiotics, and then via vertical transfer, the progeny acquires the genes (Reygaert, 2018; Friedrich, 2019). Moreover, conjugations, transformation and transposition collectively known as horizontal gene transfer from other strains between nosocomial and community-acquired infections can enable the transfer of these genes among strains infecting

wounds, which develop from simple to complex wounds (Anyaphat Srithanasuwan *et al.,* 2023).

As reported recently, several mechanisms have been employed by these common bacterial pathogens to counter effects of the drugs they are exposed to (Mancuso *et al.,* 2021). This is a combination of several complex of mechanisms, which minimize effects of these antibiotics, aided by the acquisition of the drug resistant genes (DRGs). Here, the main mechanisms in *S. aureus* have been observed to be drug inactivation, drug efflux pump and drug target alteration. Moreover, the efflux pump flushes out any antibiotic molecule absorbed into the cell, as the target alteration is steered by mutational changes occurring in nucleotide sequences of an antibiotic target like the cell membrane (Reygaert, 2018; Benkő *et al.,* 2020). For common antibiotics prescribed, *S. aureus* was first repoted to use antibiotic inactivation and alteration of antibiotic target against penicillins and aminoglycosides, antibiotic trapping for vancomycin and efflux pumps for tetracycline and fluoroquinolones (Pantosti *et al.,* 2007). With the knowledge on how these mechanisms work against the antibiotics, and on which pathogens, it is the clear path on understanding the emergence of DRGs among hospital and even community acquired infections (Kapoor *et al.,* 2017; Schroede *et al.,* 2017).

2.5.1 Mutational hotspots in drug resistant genes of *S. aureus*

The ongoing diagnostic and treatment problems of post-surgical bacterial infections stem from the existence of mutational landmarks within the genomes of the infecting bacteria pathogens (Dean *et al.,* 2014). This incidence has been attributed to point mutations implicated at gene level within the pathogens (Gao *et al.,* 2010). Chromosomal-encoded genes have shown to be the natural hotspots for these mutations. Mutations in the DNA gyrase gene sequence, commonly known as *gyrA* and *gyrB* genes, have enabled resistance of

S. aureus to antibiotics targeting DNA replication mechanism of DNA gyrase enzyme like fluoroquinolones (Razieh Pourahmad Jaktaji & Ehsan Mohiti, 2010). Mutations in the QRDR sections of the two subunits for each protein, GyrA and GyrB, are more frequent in clinical isolates resistant to quinolones. Resistance mutations in *gyrA* and *parC* genes have been implicated in *Escherichia coli* resistant to quinolones (Johnning *et al.,* 2015). However, information on this region of mutations in *S. aureus* remains globally scattered.

Similarly, (Razieh Pourahmad Jaktaji & Ehsan Mohiti, 2010), codon deletions and other mutations on *gyrA* including S162A, E596D, E856D and T860N, have been reported in *S. aureus* ATCC BAA-39 strain. This incidence has also been observed in strain-specific mutations like mutation S84L in *E. coli* (Korotetskiy *et al.,* 2023). Among other mutations in the chromosomes, multiple mutations like substitution S80T, mutation V291I and D3227E in the *vanT* gene, and Glycerol-3-phosphate transporter, *glpT* have been reported (Silver, 2017). These and other genome modifications in *S. aureus* isolates in Western Kenya remain unknown. Therefore, this study sought to bridge this gap by identifying strain specific mutations in the drug resistance genes, which are the driving forces behind drug resistance in the region.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was conducted at the surgical wards of Kakamega County Teaching and Referral Hospital (KCTRH), the largest referral hospital in Kakamega County **(Appendix I).** Kakamega town is located on 0.28215°N, 34.75400°E and lying about 30 Kilometers North of the Equator. KCTRH is the largest of health facilities in Kakamega County but also serves the neighboring counties of Vihiga, Bungoma and Busia. It attends to 15,000 patients monthly and has a bed capacity of 400. The hospital is located on 1 Kilometer from Kakamega CBD (central business district) and is also 1 Kilometer from the Kakamega – Kisumu Road on the way to Shinyalu Centre.

3.2 Study design

This was a cross-sectional hospital-based study and was conducted between March 2021 and February 2022. In this study, inpatients were considered patients attending care, whose clinical case required that they are admitted for closer monitoring by the caregivers, while outpatient isolates were from patients treated at the facility and did not need admission. In this study, the cutoff value for HAIs was surgical wound infection occurring up to 3 days (72 hours) after discharge, or up to 30 days after a surgical procedure. These were infections not present at the time of admission to hospital. On the other hand, infections present or incubating at the time of hospital admission or that develop within the first 48 hours of admission were considered CAIs. In some cases, CAIs also referred to infections that were acquired outside of healthcare facilities in the community setting. In this study, the abbreviations ID was hospital acquired isolate from general surgical wards, OD ─

community acquired isolate from patients that attended a general surgical ward and IB ─ admitted patient with hospital acquired infection from post-natal wards.

At the time of the study, the facility's surgical wards were organized from ward one to ward ten based on specialization, like the postnatal wards that handles women who have had C-section, orthopedic and other surgical conditions. The facility also has three theatres; where two are in Kakamega Hospital, and one Kakamega Provincial Hospital maternity theatre.

3.2.1 Inclusion and exclusion criteria

The identification for recruitment of patients was based on clinical records with the help of clinicians. After consenting to the study, patients presenting with post-surgical infections occurring up to 4 weeks after minor or major operation, were included. Patients with community acquired infections like open fractures and furuncles were excluded. Sample collection was done by certified clinicians.

3.2.2 Study population

Study population mainly involved both inpatients and outpatients receiving care after minor or major operative procedures at the facility. This involved both male and female, of ages between 2-75 years who had undergone surgery.

3.2.3 Sample determination

In this study, purposive sampling was used which targeted patients who presented with infections following surgery at the facility. Since patient recruitment depended on the occurrence of SSIs during the period of study, a predetermined sample size could not be established. This study was therefore based on the Central Limit Theorem (CTL) to justify adequacy of the sample in line with statistical inferences. According to the CTL, when independent random variables are aggregated, their normalized sum approximates a normal distribution, as there is an increase in sample size. This is regardless of the original distribution of population (Ghasemi & Zahediasl, 2012). Other empirical evidence suggests that a sample size of more than 30 is generally sufficient for the distribution of sampling of the mean, to approximate normality (Norman, 2010). Therefore, the number of samples analysed in this study was considered adequate enough for statistical analyses, as far as CTL assumptions are considered.

3.2.4 Study variables

In this study, study variables were primarily for objective one. The dependent variable was wound classes observed, and independent variables were length of hospital stay, organism isolated and antibiotic history among the patients.

3.3 Data collection methods

3.3.1 Assessment of post-surgical wound infections for *S. aureus*

In this section, all recommended standard microbiological techniques and practices were adhered to for quality data. Sampling was 1 hour after daily cleaning and disinfecting routine. Clinical and demographic data were acquired from Consenting patients **(Appendix II),** using a pre-tested questionnaire **(Appendix III),** and previous patient information on files. Question four was the basis for making study conclusion for objective one. This study had an interpreter for Kiswahili. Age, gender, ward and type of operation, SSI and extent of infection, length of hospital stays, and antibiotic history were all included. Wounds were examined and classified as class I – IV (Herman & Bordoni, 2022), and samples were taken by cleaning the SSI with sterile gauze and normal saline. Pus was collected in Amies transport media with two sterile cotton swabs and processed at the facility's microbiology lab within 2 hours of collection (Koneman *et al.,* 1997).

The first and second swabs were used to examine Gram stains and recover any isolates. Four media were used: Nutrient agar (BD Difco, India) and Blood Agar (BD Difco, India) for recovery, and Mannitol Salt Agar (HiMedia, India), and MacConkey agar (HiMedia, India) for identification. Incubations were primarily for 24 hours at 35-37 °C. Gram stain was used discriminate between two groups: Gram positives and Gram negatives. For biochemical tests, Simmons citrate test (HiMedia, India), coagulase test (BD Difco, India), motility indole lysin (MIL) (HiMedia, India), and Triple sugar iron (TSI) (HiMedia, India), were used as phenotypic confirmatory tests. Control strains were *S. aureus* ATCC 25923, *Pseudomonas aeruginosa strain* ATCC 27853*, E. coli* ATCC 25922 and *Klebsiella spp* ATCC- BAA 1706.

3.3.2 Determination of the genetic diversity of *S. aureus* subtypes

Multi-drug resistant *S. aureus* were selected for whole genome sequencing (WGS). *Staphylococcus aureus* resistant to oxacillin and especially with minimum inhibitory concentrations (MIC) ≥ 4ugmL^{-1}, have been shown to be resistance to methicillin (MRSA).

3.3.2.1 DNA isolation and sequencing

DNA was isolated using a Zymogen genomic DNA extraction kit (17062 Murphy Ave., Irvine, CA 92614) according to the manufacturer's instructions. For optimal performance, beta-mercaptoethanol, was added to the genomic lysis buffer to a final dilution of 0.5 %(v/v) *i.e*., 500 µl per 100 ml.

The integrity, quality, size, and quantity of the isolated genomic DNA from the selected *S. aureus* strains were determined using the NanoDrop 2000c spectrophotometer (Thermo Scientific, USA) at the optical wavelengths of 260 nm and 280 nm and by electrophoresis on 1% agarose gel (Thermo Scientific, USA**).** Low quality reads were filtered by reverse and forward adapters **(Table 3.1).**

Table 3.1: Forward and reverse primers for PCR reactions.

Adapter	**Sequence**
Forward	5' Adapter: 5'AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
Reverse	3' Adapter: 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGATGACTATCTCGTATGCCGTCTTCTGCTTG-3'

Sequencing was done via the Illumina HiSeq 2500 platform (Novogene Co., Ltd, China). Genomic DNA was sheared into short fragments (end-repaired and A-tailed) then further using Illumina adapters as shown in **table 3.1**, they were ligated. Library quantification was performed using Qubit, and library size distribution was assessed using a Bioanalyzer. Paired-end sequencing was conducted on an Illumina HiSeq 2500 platform (Novogene Co., Ltd). Raw, paired-end reads were trimmed using a pipeline implemented in Unipro UGENE v.44 (Okonechnikov *et al.,* 2012), based on CASAVA 1.8 with default settings for adapter removal, read trimming, and quality filtering. The quality Q-score threshold for trimming and filtering was set at 20.

3.3.2.2 Sequence assembly and annotation

For genome assembly, a combinatory approach of *de novo* assembly using SPAdes v3.15.3 (Bankevich *et al.,* 2012), and DNA read mapping against a reference sequence by Bowtie2 (Langmead & Salzberg, 2012), was used. *S. aureus* ATCC BAA-39 [CP033505.1] was selected as the reference genome as a representative of multidrug resistant organism with multiple inserts of virulence and antibiotic resistance gene including the SCCmec cassette specific for the methicillin resistant MRSA clonal line (Joubert *et al.,* 2019). Samtools 1.13 was utilized for sorting the resulting alignments of the trimmed DNA reads against the reference genome. Then, the default consensus sequence algorithm with an automatic gap removal implemented in Unipro UGENE v.44.0 was used to produce assembled sequences from the sorted alignments. In parallel, *de novo* assembled contigs were ordered against the reference sequence using the function Move Contigs function of Mauve v.20150226 (Darling *et al.,* 2004). Contigs, which were not aligned against the reference sequence, were identified as plasmid contigs using the mlplasmids 1.0.0 tool (Arredondo-Alonso *et al.,* 2018) and the mob_recon function from the Mob-Suite v3.1.7 utility (https://github.com/phac-nml/mob-suite) (Robertson & Nash, 2018) with the default parameter setting. Ordered contigs were concatenated into a single sequence.

The function ProgressiveMauve of the Mauve v.20150226 with match seed weight parameter set to 15 was used to align the sequence of concatenated contigs (termed subject sequence) against the consensus sequence obtained by read mapping (termed reference sequence). Identified gaps in the subject sequence were stored in a separate file using function Export Gaps of the Mauve v.20150226. An in-house program GapClosure (Reva, 2024) (https://zenodo.org/records/10837027) was written on Python 3 to patch the gaps of the

subject sequence by respective sequences of the reference sequence. Program GapClosure ignored gaps shorter than 10 bp. Subsequently, the original reads were mapped against the assembled sequences to verify the patches followed by obtaining new consensus sequences. Possible duplications of contigs in the assembled genomes were identified by the nucmer, rclT, and show-coords functions of MUMmer 4.0.0rc1 (Kurtz *et al.,* 2004).

Quality of genome assembly was controlled by CheckM v.1.0.18 (Chklovski *et al.,* 2023) provided at the KBase Web-portal (https://www.kbase.us/). The consensus sequences were annotated using RAST Server (https://rast.nmpdr.org/; (Overbeek *et al.,* 2013)) with the automatic error-fixing option enabled. For genome annotation, *Staphylococcus aureus* (taxid: 1280) was set as the target bacterial genus with genetic code 11. Other parameters of the RAST annotation robot were set by default. All assemblies were rearranged using BioPython v1.81 to start at the position of 400 bp upstream of the *dnaA* chromosomal replication initiator in leading strands, corresponding to the chromosomal replication origins. The resulting chromosomal and plasmid sequences were submitted to the NCBI repository in FASTA format. They were automatically annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP-6.6) (Tatusova *et al.,* 2016).

3.3.2.3 Determination of sequence types and phylogenetics

To determine sequence types (STs), the FASTA chromosomal sequence files were uploaded to PubMLST at https://pubmlst.org/organisms/*Staphylococcus*-aureus (Jolley *et al.,* 2018). In the sequenced genomes, clusters of orthologous genes (COGs) were predicted using the program OrthoFinder with default parameters (Emms & Kelly, 2019). The alignment of each COG's sequences was performed using MUSCLE (Edgar, 2004). Gblocks (Talavera &

Castresana, 2007) was used to remove ambiguous parts of the alignments. Subsequently, BioPython v1.81 scripts were employed to concatenate the COG alignments into a superstring alignment. This superstring alignment was utilized for further phylogenetic inferences using a Neighbor-Joining algorithm, implemented in Neighbor (part of the PHYLIP package 3.69) (https://evolution.genetics.washington.edu/phylip.html), with *S. aureus* ATCC BAA-39 [CP033505], *S. epidermidis* ATCC 12228 [NC_004461], and *S. saprophyticus* ATCC 15305 [NC_007350] serving as references for out-grouping. To obtain a finer-grained analysis of the relationships between the isolates, the core gene set common to all six isolates and to three reference genomes was first identified. This yielded a collection of 1,839 clusters of orthologous genes (COGs). For each isolate, these genes were then translated into corresponding amino acid sequences, aligned separately, and the alignments were concatenated to yield an assembly comprising 475,577 amino acid residues

3.3.2.4 Determination of plasmids and barcording

Sequence identity of plasmids was estimated by aligning their nucleotide sequences using the ClustalW algorithm implemented in the program BioEdit v7.2.5 (Hall, 1999), which produced a sequence identity table for the created alignment. The SeqWord Gene Island Sniffer was employed to identify horizontally-acquired Genomic Islands (Bezuidt *et al.,* 2009; Reva, 2024). Gene annotation of predicted GIs was checked individually to exclude false predictions of genomic regions containing housekeeping genes with alternative k-mer patterns, such as clusters of rRNA, genes for ribosomal RNA and giant genes with multiple repeats. All the programs mentioned above were run with default parameter settings.

For DNA barcoding, multiple alignment was performed for *S. aureus* with UniproUGENE software. This enabled comparison of species-specific sequences from the study isolates to the reference sequence library, so that genes (barcodes) responsible for significant species-level genetic variability and divergence are determined. Identification of highly variable genes shared by the selected *S. aureus* isolates was performed using the program Barcoder (Rotimi *et al.,* 2018). Pipeline for these analyses is shown in **Appendix IV.**

3.3.3 Determination of the antimicrobial resistance and genetic modifications

Each of the recovered *S. aureus* isolates were treated with a panel of 15 antibiograms for AMR profiles determination, which were used at the facility according to CLSI 2022 guidelines (CLSI M100-S32). At the time of sampling, the facility was commonly using Ceftriaxone as first line prophylaxis for patients undergoing surgery. Antibiotics tested were cefalexin (30µg), erythromycin (15µg), gentamicin (10µg) from Oxoid (UK). Others were ceftriaxone (30 µ g), trimethopan (30 µ g), tetracycline (30 µ g), cephalothin (30 µ g), clindamycin (2µg), cefuroxime (30µg), oxacillin (30µg), penicillin (10µg), ceftazidime (30µg), cephazolin (30µg), amoxicillin (20µg), ampicillin (10µg), and trimethoprim (1.25µg) from BD Difco, (US). Inhibition zones were determined in millimeters (mm) **(Appendix V).** Quality control (QC) strain was *S. aureus* ATCC 29213 in line with CLSI guidelines (2020). For future accession, the isolates were stored in -80C in trypticase soy broth supplemented with 15% v/v glycerol.

Zones of inhibition for antibiotics were determined by measuring zone of clearing around the antibiotic disk in millimeters. *S. aureus* strains resistant to more than 12 antibiotics were then

sequenced. Antibiotic resistance genes were identified using a local installation of the RGI v.6.0.3 software (Alcock *et al.,* 2020). Virulence-associated genes were screened through the Virulence Factor Database (VFDB) (Chen *et al.,* 2011) using a local installation of Abricate v.1.0.1. Both software tools, Abricate and RGI, were run with default parameter settings. Specifically, Abricate was configured for a minimum coverage of 80% and minimum identity of 90%. Assembled genome sequences in FASTA format were used as inputs for the programs Abricate and RGI. To ensure that the strain-specific appearance of various virulence and drug resistance factors was not attributed to errors in genome assembly, the genomic sequences of all isolates were aligned using Mauve v.20150226 against the longest chromosomal sequence of strain ID003. Corrections were made to these genomes using the GapClosure tool. Further verification process involved mapping the original DNA reads back to the amended sequences. The resulting consensus sequences were then re-annotated using the RAST Server (Univesity of Pretoria).

3.4 Data analysis

Data from clinical and laboratory data were fed into excel spreadsheet, cleaned, and then exported to the Predictive Analysis SoftWare (PASW) version 28 (Armonk, NY: IBM Corp) for analysis.The data was tested for normality using shapiro wilk test. Data from gram stain and biochemical tests was expressed as percentages for respective organisms.Continuous variables including the ratios of females and males was expressed as percentages, were expressed as means using descriptive statistics. Chi-square test was used to test if there was relationship between the zone of drug inhibitions. Correlation between wound class and duration of hospital stay, wound class and history of antibiotics and between wound class and age of the patients was performed at ($p < 0.05$). To test whether GIs contributed to the antimicrobial resistance profile among the CA and HA isolates of ST152, Kruskal-Wallis was

done to determine if there were any significant differences between the inhibition zones of OXA and CRO among the ST152 ($p \leq 0.05$).

3.5 Ethical considerations

Ethical approval was granted by the Masinde Muliro University of Science and Technology (MMUST) Institutional Ethical Review Committee (IERC) (MMUST/IERC/118/20), the Kakamega County General Teaching and Referral Hospital (KCGTRH) Ethics and Research Committee (ERC) (SBB/G/01-53199/2018), and the National Commission for Science, Technology and Innovation (NACOSTI) **(See appendix VI).**

CHAPTER FOUR

RESULTS

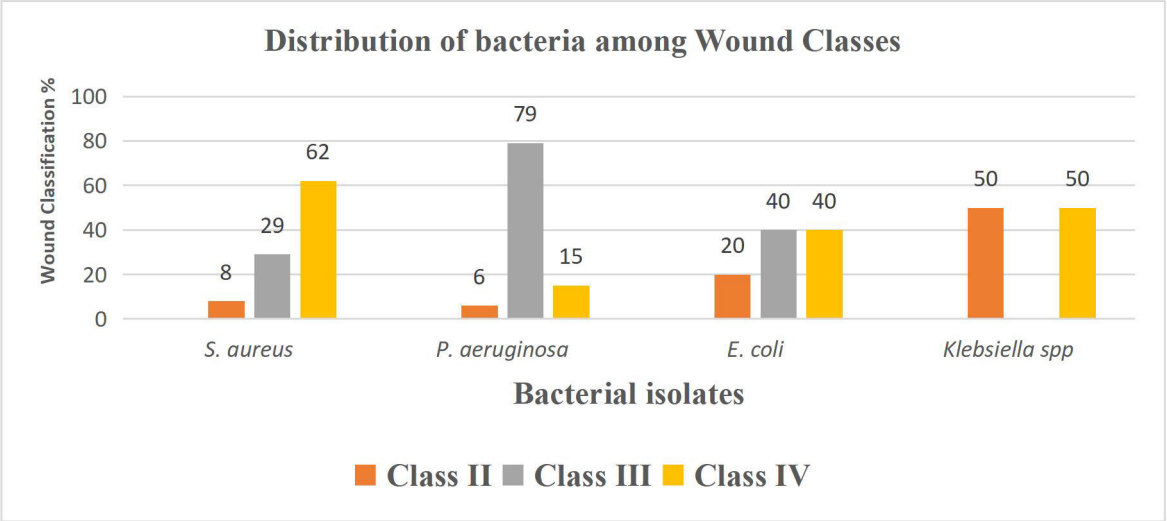
4.1 Assessment of post-surgical wound infections for *S. aureus*

4.1.1 Surgical patient characteristics and surgical wound classes

In this study, seventy (70) patients presenting with clinical post-surgical infections were enrolled where, 45.7% were female with median age 30 years. *S. aureus* appeared to progress infections amidst antibiotic history (Ceftriaxone, Penicillin, Floxapen, and Clindamycin) to beyond 30 days (4.2%). *E. coli* was the most infecting microbe for infections that took 6 to 10 days **(Figure 4.1 b).** Among the 70 samples, four post-surgical wound classes were observed; 51.4% were class III wounds, 35.7% class IV and 12.9% class II. Males aged between 11-30 years represented the highest (69.4%) with class III wounds; whereas females aged ≥ 31 years represented the highest 56% class IV wounds. The study observed no relationship between wound class and age of the patients *($p > 0.05$)* as shown in the chi-square test **(Table 4.1).**

Culture characteristics are shown in **appendix VII** and microbial biochemical tests in **appendix VIII**. This study observed that 24 (34%) of isolates *S. aureus* (Gram-positive), 33 (47.1%) *P. aeruginosa*, 5(7.1%) *E. coli* and 8(11.4%) *Klebsiella spp* (All Gram-negatives)**.** **P.** *aeruginosa* was most recovered from Class III wounds at 79%, followed by *E. coli* at 40%. *S. aureus* was recovered from class IV wounds the most at 62%, followed by *Klebsiella spp* and *E. coli* at 40% and 50% respectively, *P aeruginosa* was the least recovered in this class at 15% **(Figure 4.1a).** The study observed no statistical relationship between wound classes and gender of the patients ($p = 0.28$). Nevertheless, the study observed an association between wound class and organism isolated ($p = 0.001$), and history of antibiotics ($p = 0.001$). **(Table 4.1),** with *S. aureus* and *P. aeruginosa* being associated with class IV and III respectively**.**

a



b

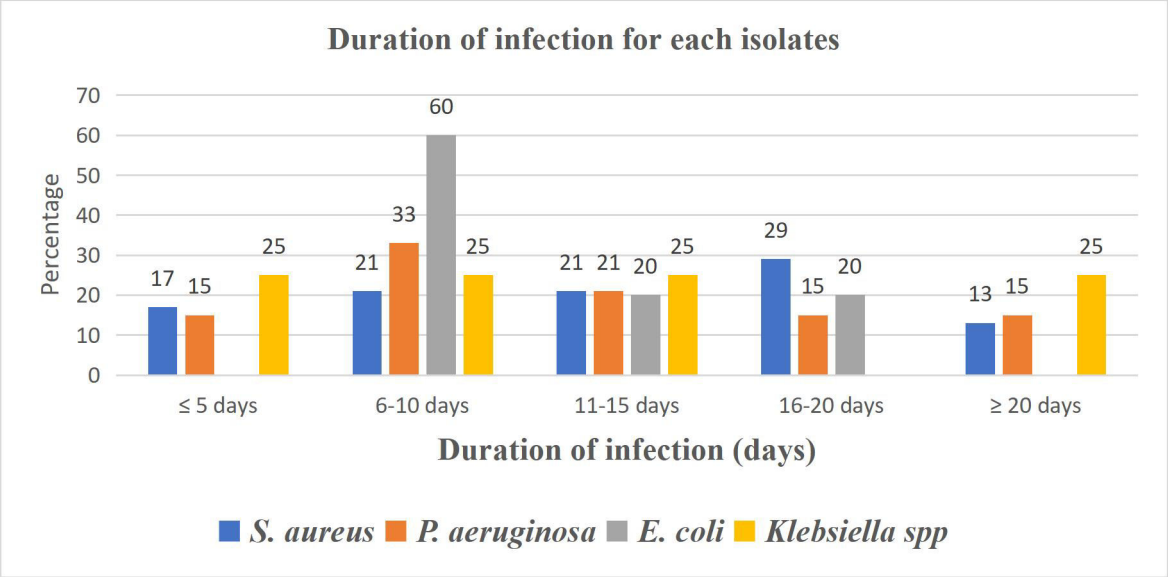


Figure 4.1. Categorization of the four bacterial pathogens recovered from surgical site infections**. (a)** Shows the frequency of bacterial isolates among the 70 patients presenting with surgical site infections; and **(b)** shows surgical wound and infection metadata for the 70 isolates categorized as 4 isolates. *P. aeruginosa* was the highest in class III infections and infections lasting for 1 week while *S. aureus* led in Class IV infections and infections lasting up to 1 month.

As shown in table 4.1 below, class III surgical wounds in males aged above 31 years were the majority observed. However, for antibiotic history, selected antibiotics ceftriaxone and floxapen were tested against wound classes for associations, as well as *S. aureus* and *P. aeruginosa.*

Table 4.1. General characteristics of study participants, showing comparison of various wound classes observed.

Patient Metadata		**Wound classes (n=70)**			
		Class II (12.9%)	**Class III (51.4%)**	**Class IV (35.7%)**	**Chi-square test (α = *0.05***
Gender	Male	4 (5.72%)	25 (35.75%)	14 (20.02%)	$\chi 2$ = 1.156566952 *p-value* = *0.282178664* df = 1
	Female	5 (7.15%)	11(15.73%)	11 (15.73%)	
Age years	≤ 10	2 (2.86%)	5 (7.15%)	5 (7.15%)	
	11-30	4 (5.72%)	12 (17.16%)	9 (12.87%)	
	≥ 31	3 (4.29%)	19 (27.17%)	11 (15.73%)	
Duration of infection (Days)	≤ 10	8(11.44 %)	14 (20.02%)	11 (15.73%)	
	11-30	1 (1.43%)	22 (31.46%)	14 (20.02%)	
	≥ 31	0	0	0	
Patient	Inpatient	8(11.44 %)	24 (34.32%)	19 (27.17%)	

admission	Outpatient	1 (1.43%)	12 (17.16%)	6 (8.58%)	
Clinical infection History	Lesions	0	0	1 (1.43%)	
	Catheter site	0	10 (14.3%)	6 (8.58%)	
	Diabetic patients	1 (1.43%)	1 (1.43%)	0	
	Septic wound	0	3 (4.29%)	10 (14.3%)	
	Multiple abscess	2 (2.86%)	0	2 (2.86%)	
	Chronic wounds	6 (8.58%)	20 (28.6%)	6 (8.58%)	
Antibiotic History	Ceftriaxone	5 (7.15%)	9 (12.87%)	13 (18.59%)	$\chi 2$ = 3644.540541 *p-value* = 0.001 df = 1
	Floxapen	2 (2.86%)	9 (12.87%)	6 (8.58%)	
	Penicillin	0	1 (1.43%)	0	
	Clindamycin	0	1 (1.43%)	1 (1.43%)	
	None	2 (2.86%)	16 (22.88%)	5 (7.15%)	
Organism isolated	*Staphylococcus aureus*	2 (2.86%)	6 (8.58%)	16 (22.88%)	$\chi 2$ = 20.27887701 *p-value* = 6.69355E-06 df = 1
	Pseudomonas aeruginosa	2 (2.86%)	28 (40.04%)	4 (5.72%)	
	Escherichia coli	1 (1.43%)	2 (2.86%)	2 (2.86%)	
	Klebsiella spp	4 (5.72%)	0	4 (5.72%)	

4.3 Determination of the genetic diversity of *Staphylococcus aureus* subtypes

Whole genome sequences of six selected isolates of *S. aureus* were obtained using Illumina MiSeq sequencing and assembly, as described in the methods section. Gel electrophoresis analysis was used to confirm genomic DNA in study samples **(Appendix IX).** Evaluation of quality of assembly revealed genome completeness ranging from 95% (OD028) to 98% (IB010 and ID029), and a contamination level of approximately 0.08% for all assemblies **(See Appendix X).**

The alignment of de novo contigs identified regions of similarities among the isolates**.** ID029 isolate showed a high variability among the sequences **(Appendix XI).** Colum range selected was 1-500. Reference sequence was *S. aureus* ATCC BAA-39. The whole genome annotated sequences of the isolates were deposited at the National Centre for Biotechnology Information (NCBI). Accession numbers of the genomes, CheckM assembly quality parameters and other supporting information are in **Appendix X.**

4.3.1 Multi-locus sequence typing of the isolates

Multi-locus sequence typing (PubMLST) analysis characterized study isolates into two groups. The isolates were assigned to sequence types following a PubMLST analysis. ID029 belongs to ST8, whereas all other selected isolates belong to ST152, including the hospital-acquired (ID003, IB011, IB010) and community-acquired (OD001, OD028) strains.

4.3.2 Virulence determinants

The analysis identified multiple virulence-associated genes, as summarized in **Figure 4.2 and appendix XII**. The highest number of virulence genes was found in the reference MRSA strain *S. aureus* BAA-39, followed by the hospital-acquired isolates ID029 and ID003, which belonged to different MLST types. Unlike other ST152 isolates, strain ID003 contained the *cifAB* fibrinogen-binding proteins, the *hly/hla* alpha-hemolysin precursors, and the *lukF* Panton-Valentine leukocidin precursor, which were also found in ID029. Additionally, ID029 possessed the *hlgC* gamma-hemolysin component, but was absent in the other isolates. The community-acquired ST152 isolates, OD001 and OD028, have a smaller number of virulence-associated genes. OD001 lacks the *icaR* transcriptional regulator of intercellular adhesion, the IgG-binding protein *sbi*, and the staphylococcal enterotoxins K and G, encoded by *selK* and *selG*, respectively. The latter two genes were also absent in the genome of ID029, but the other isolates possessed all these genes.

Due to the stringent gene search parameters set by default in the program Abricate, several putative virulence associated genes were overlooked by this search. Specifically, while the program failed to identify virulence genes on the plasmids, an inspection of annotated genes revealed the presence of a cluster of staphylococcal enterotoxins R, J, T, and S in ID029's larger plasmid. Additionally, ID029's shorter plasmid harbors *qacA* multidrug efflux pump and its transcriptional regulator *qacR*.

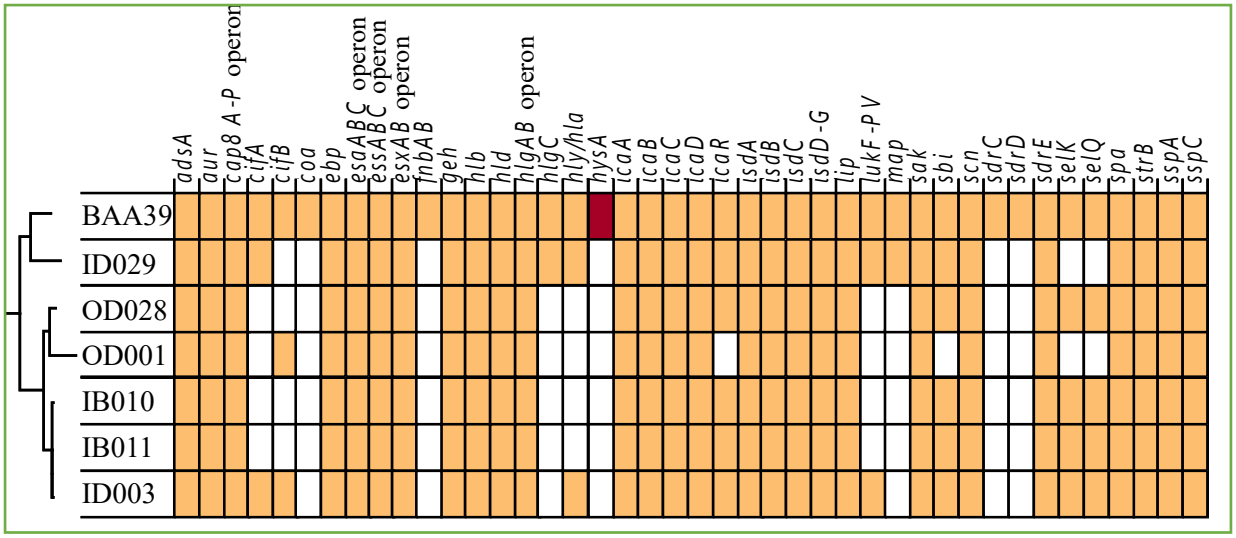


Figure 4.2. Distribution of virulence genes in the genomes of the selected *S. aureus* isolates and the reference strain *S. aureus* ATCC BAA-39. (A) Virulence genes identified by searching through the VFDB database using the program Abricate.

4.3.3 Determination of horizontally acquired genomic islands

Horizontally-acquired genetic islands (GIs) were identified using the SeqWord Sniffer program, which was designed to identify relatively large integrons and prophages. It measures differences in k-mer patterns of local sliding windows compared to the overall k-mer pattern of the chromosome. The predicted locations of GIs in the chromosomes of the selected *S. aureus* isolates are shown in **Figure 4.3**.

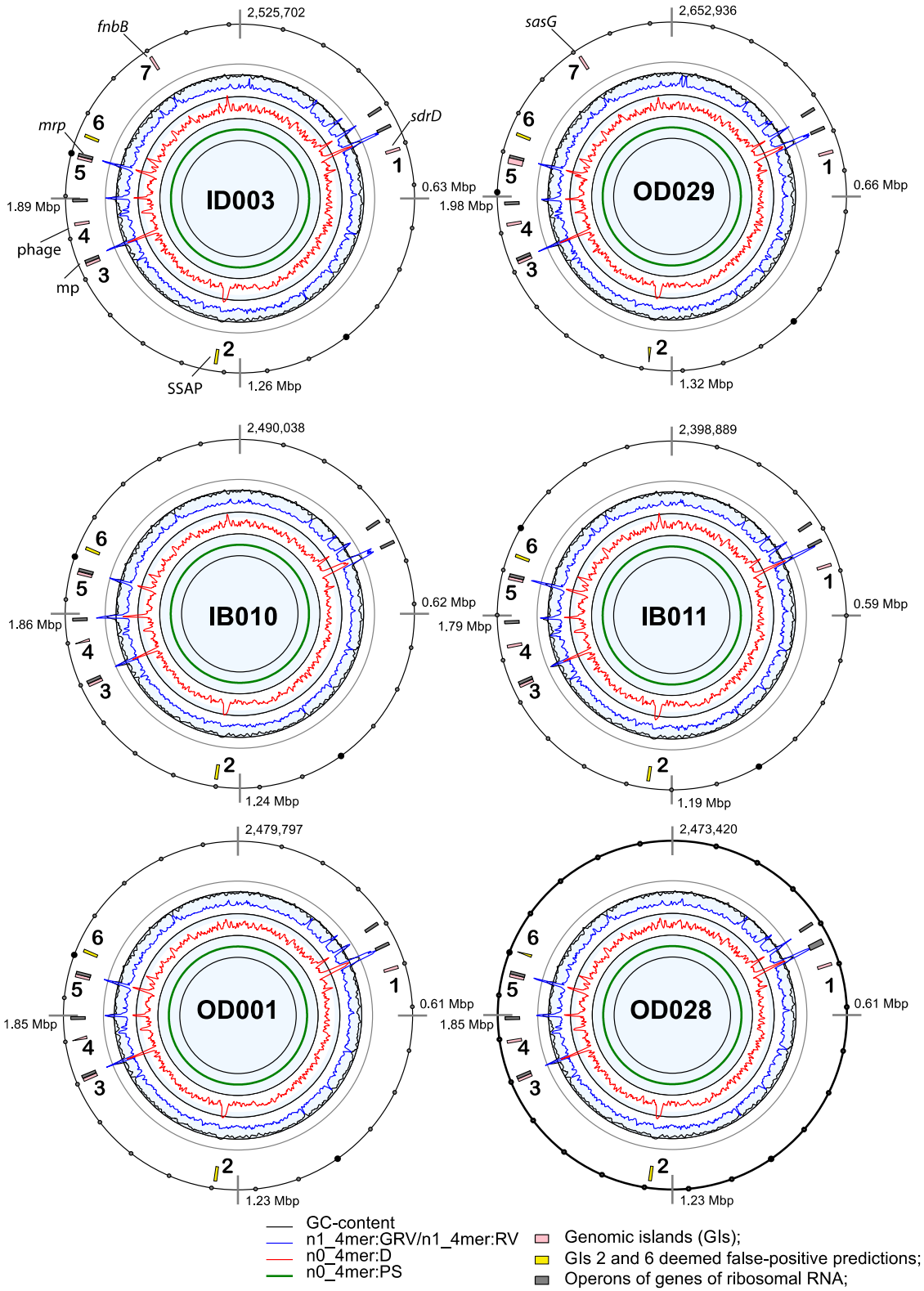


Figure 4.3. Atlas maps of distribution of genetic islands (GIs) on the chromosomes of the selected *S. aureus* isolates. Insert numbers of the GIs are labelled 1-6.

GIs locations and lists of genes found within GIs are shown in **Appendix XII**. The distribution of GIs in the selected isolates was then compared with that of the reference strain *S. aureus* ATCC BAA-39 (**Figure 4.4**). After comparing, it was found that the locations of horizontally acquired inserts in chromosomes of *S. aureus* are quite conserved. The regions in **Figure 4.4** are numbered from 1 to 10, following the order of the inserts in the reference genome, where the number of inserts is the largest.

Insert #1, located near the replication origin in the reference strain, corresponds to the SCCmec methicillin resistance cassette. This insert was specific to MRSA strains, as was Insert #2, which contains a gene encoding an exotoxin. GI #3 appears to be a historical insert containing the *sdrD* and *sdrE* adhesins. It is present in all *S. aureus* strains; however, the program did not identify it as a foreign insert in strain IB010, due to the similarity of its 4-mer pattern to the surrounding chromosomal loci. Region #4 encompasses genes for giant staphylococcal surface-anchored proteins, characterized by an alternative frequency of 4-mers.

GIs #5 and #8, identified as transposable elements Tn1 and Tn2 in **Figure 4.4**, are associated with clusters of tRNA genes upstream of rRNA clusters. The terminal genes of these inserts were identical, and consisted of a membrane-associated PTS transporter subunit and the transposase IS1181 annotated as the ‘mobile element protein’. Trails of integrated genes were found on the chromosome upstream of the transposase. In Tn1, these genes were conserved, including two *glnQ*-like ABS transporters and the *queG* epoxyqueuosine reductase. In contrast, Tn2 remained active, capable of integrating additional horizontally transferred gene cassettes, as evidenced by the larger size of these regions in the reference genome and in ID029. The genomes of all strains examined in this study contained the *fmtB*/*mrp* cell wall biosynthesis and *femD* phosphoglucosamine mutase genes involved in methicillin resistance. These genes are located at the 5'-terminal part of the inserts, opposite the transposases.

Upstream of the transposase, an arginase, a bacterial checkpoint controller *disA*, and the diadenylate cyclase *spyDAC* were found. The presence of intermediate genes varied across different strains. ID029, for example, harbored several additional copies of *fmtB*/*mrp*-like genes and an uncharacterized secreted protein was found in the reference strain.

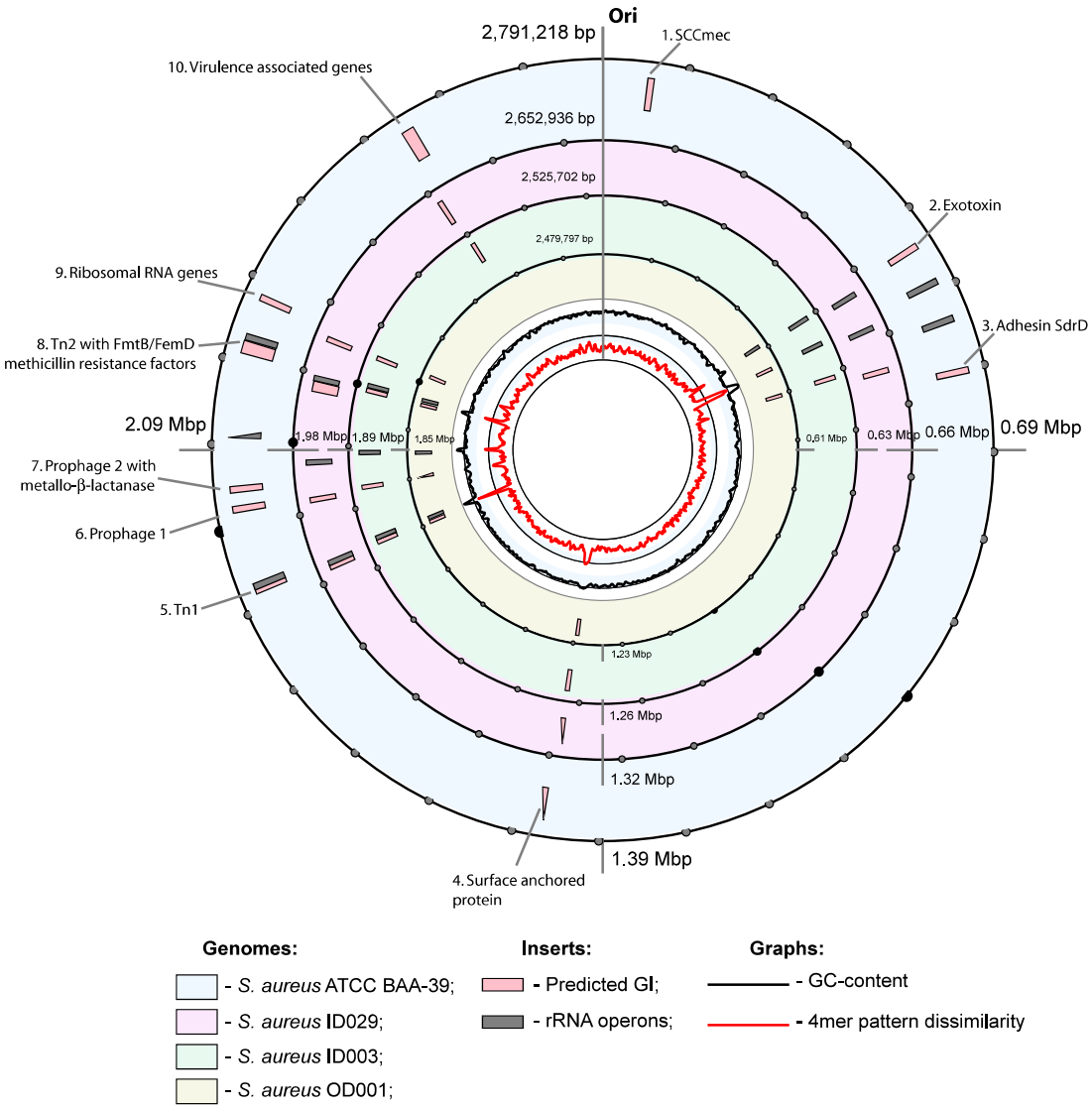


Figure 4.4. Distribution of genetic islands (GIs) on the chromosomes of *S. aureus*. The outermost ring represents the chromosome of the reference strain *S. aureus* ATCC BAA-39, which is followed by graphical representations of the chromosomes of the isolates ID029 (represents ST8 genotype); ID003 (represents hospital isolates of ST152); and OD001(represents community isolates of ST152). All chromosomes were aligned to start from putative replication origin (Ori) locations, which were selected 400 bp upstream of the genes for chromosomal replication initiator protein DnaA. Locations of inserts of GIs and rRNA clusters are depicted respectively by pink and dark grey boxes. Fluctuations of GC-content and 4-mer pattern dissimilarities in 5 kbp sliding windows are shown by black and red-colored histograms, respectively.

GI #6 is identified as a historical prophage with remnants of phage-associated genes. Nearby, the reference strain also harbors an additional prophage, GI #7, which includes a metallo-beta-lactamase superfamily domain protein alongside phage genes. This insert was not found in other *S. aureus* strains sequenced in this study. Region #9 represents another false positive prediction common to all *S. aureus* strains, featuring a cluster of ribosomal protein genes. This DNA locus is distinguished by an alternative 4-mer nucleotide usage pattern.

GI #10 likely serves as an active chromosomal hotspot that facilitates new genetic acquisitions through horizontal gene transfer. Both the reference strain BAA-39 and ID029 contain the *sasG* virulence-associated cell-wall-anchored protein here, which plays a role in binding to the squamous nasal epithelium, followed by several transcriptional regulators, enzymes involved in the UTP-glucose-1-phosphate transformation, and various fibronectin-binding proteins. ID003 is the sole isolate within the ST152 group featuring an insertion at this locus, which includes multiple genes for fibronectin-binding proteins and one UTP-glucose-1-phosphate uridylyltransferase gene. Significantly, the strain ID003 from the ST152 group shares this characteristic with ID029. All the virulence genes identified within the GIs were not detected by the Abricate search against the VFDB database, conducted with the default stringent identity and coverage parameters.

4.3.4 Plasmid sequence alignment and comparison

Plasmids were found in all the *S. aureus* isolates selected for this study **(Appendix X).** Alignments of the plasmid sequences with gene locations are shown in **Figure 4.5**. In congruent with MLST typing, the isolates of ST152 had almost identical plasmids of ~20,760 bp **(Figure 4.6).** The most immediately obvious feature is that the plasmids of all ST152 isolates showed 99% identity of their nucleotide sequences. These homologous plasmids encode 23 genes, including pairs of recombinases and transposases. The transposases flank a

cassette of six genes comprising class A β -lactamase (*blaZ*), β -lactam sensor/signal transducer (*blaR1*), possible penicillinase repressor (*blaI*), and three genes of unknown function.

All of the *S. aureus* strains from NCBI containing these plasmid sequences belonged to ST152. Two smaller plasmids were found in the ST8 isolate, ID029. The longer of the two shared several homologous segments with the ST152-associated plasmid. However, instead of a *blaZ*-containing cassette, it contained four *staphylococcal* enterotoxin-encoding genes. The smaller ID029 plasmid carried an efflux transporter *qacA* involved in antiseptic resistance (**Figure 4.5).**

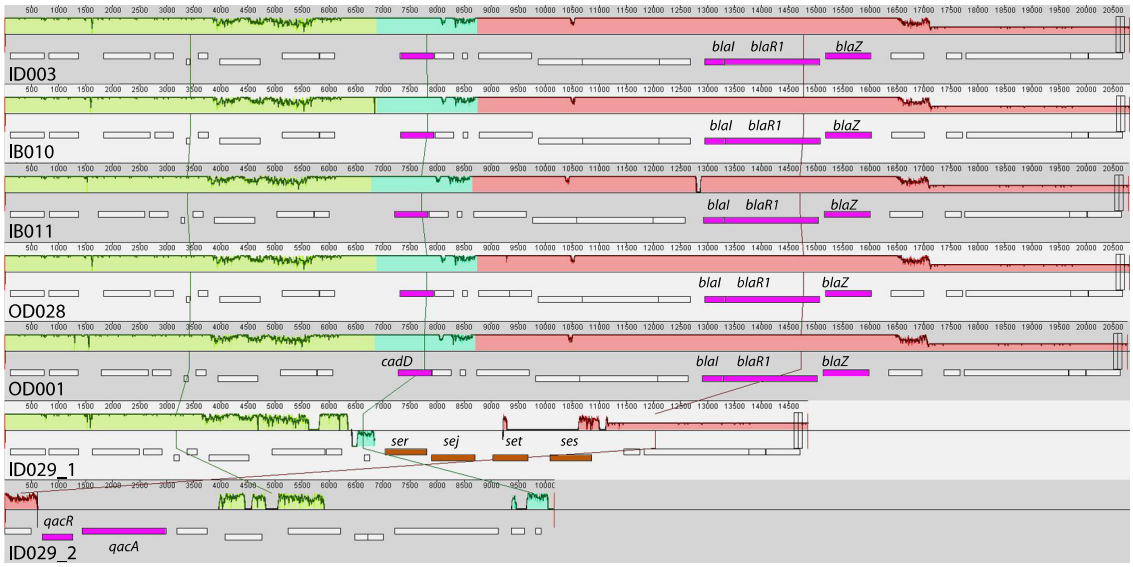


Figure 4.5. Plasmid alignment showing various genes and virulence factors among the isolates. Alignment of the plasmid sequences found in the *S. aureus* isolates (generated by Mauve). Note the clear similarity in terms of gene content and synteny of the ST152 group plasmids (ID003, IB010, IB011, OD028 and OD001). Pink color depicts antibiotic resistance genes; brown - virulence genes. Colored histograms show the level of DNA identity between plasmid sequences. Different colors of the histograms depict different Maximum Unique Match (MUM) regions identified in the plasmid sequence alignment. The transposon cassette is designed to maximize on mobility of genes. Moreover, plasmid recombination genes not only bear the mobility capabilities, but also enable insertion of genes into chromosomal DNA of other strains. Isolate belonging to ST8 carried virulence genes *S. aureus* enterotoxin A and TN552 in its plasmid.

Another significant finding is that two smaller plasmids were found in the isolate *S. aureus* ID029 of ST8 **(Figure 4.6).** The longer plasmid of 14,849 bp was genealogically linked to the plasmids of the ST152 isolates as they shared several homologous segments carrying two plasmid recombination genes and several hypothetical genes, which could be parts of the replication/dissemination machinery of these plasmids (**Figure 4.5**). However, instead of *blaZ* containing cassette, the plasmid of the strain *S. aureus* ID029 carried four genes encoding *Staphylococcal* enterotoxins **(Figure 4.5).**



Figure 4.6 Circular plasmid maps of the 6 *S. aureus* isolates in the study. The maps show various restriction enzyme sites for insertion of acquired genes, and open reading frames (ORFs). Isolate ID029 had two plasmids of 14,849 bp and 10,162 bp. The average plasmid size of the other isolates is 20.7 kb. All ST152 isolates contained the same set of restriction enzymes, which varied with the ST8 isolate. Moreover, *blaZ* gene in all ST152 isolates was located in 888-bp from the origin of replication.

4.3.5 Determination of phylogenetic relationship among the isolates

In total, 1,839 clusters of orthologous genes (COGs) were predicted by homology search through the genomes of the isolates and the three selected reference genomes: *S. aureus* ATCC BAA-39 [CP033505], *S. epidermidis* ATCC 12228 [NC_004461] and *S. saprophyticus* ATCC 15305 [NC_007350]. The phylogenetic tree **(Figure 4.7)** was constructed by Neighbor-Joining algorithm using the concatenated alignments of translated protein sequences of all homologous genes of 475,577 amino acid residues in total. The inpatient, and outpatient isolates of *S. aureus* were grouped into two distinguishable sub-clusters with the cluster of ST152 isolates.

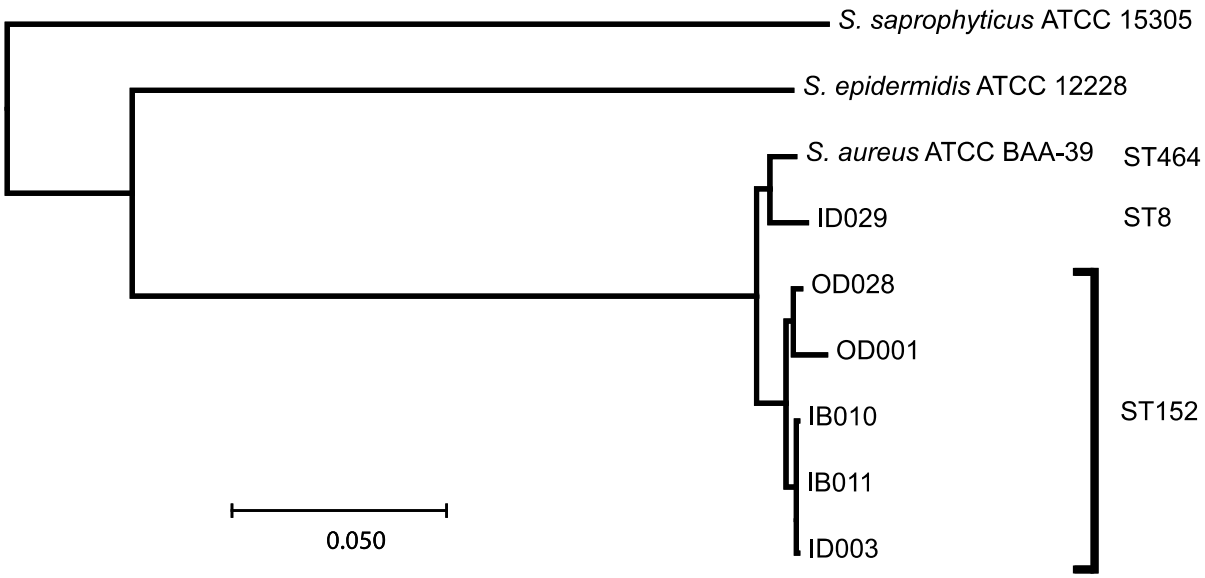


Figure 4.7. Phylogenetic tree of *S. aureus* isolates (cf. three ATCC reference strains) with MLST of each isolate indicated. Note the sub-clustering of the two community-acquired isolates (OD028 and OD001) and the three hospital-acquired isolates (ID003, IB011 and IB010) within ST152. This tree were based on amino acid sequences (concatenated alignments of all homologous proteins). Scale bar represents evolutionary distances between aligned protein sequences calculated using the JTT substitution model.

4.3.6 DNA barcoding

DNA barcoding **(Appendix XIII)** revealed two genes, uvrA and uvrB which further diversified the compared strains to two sub-groups **(Figure 4.8).** However, uvrB appeared in congruent with the phylogenetic tree **(Figure 4.7)**

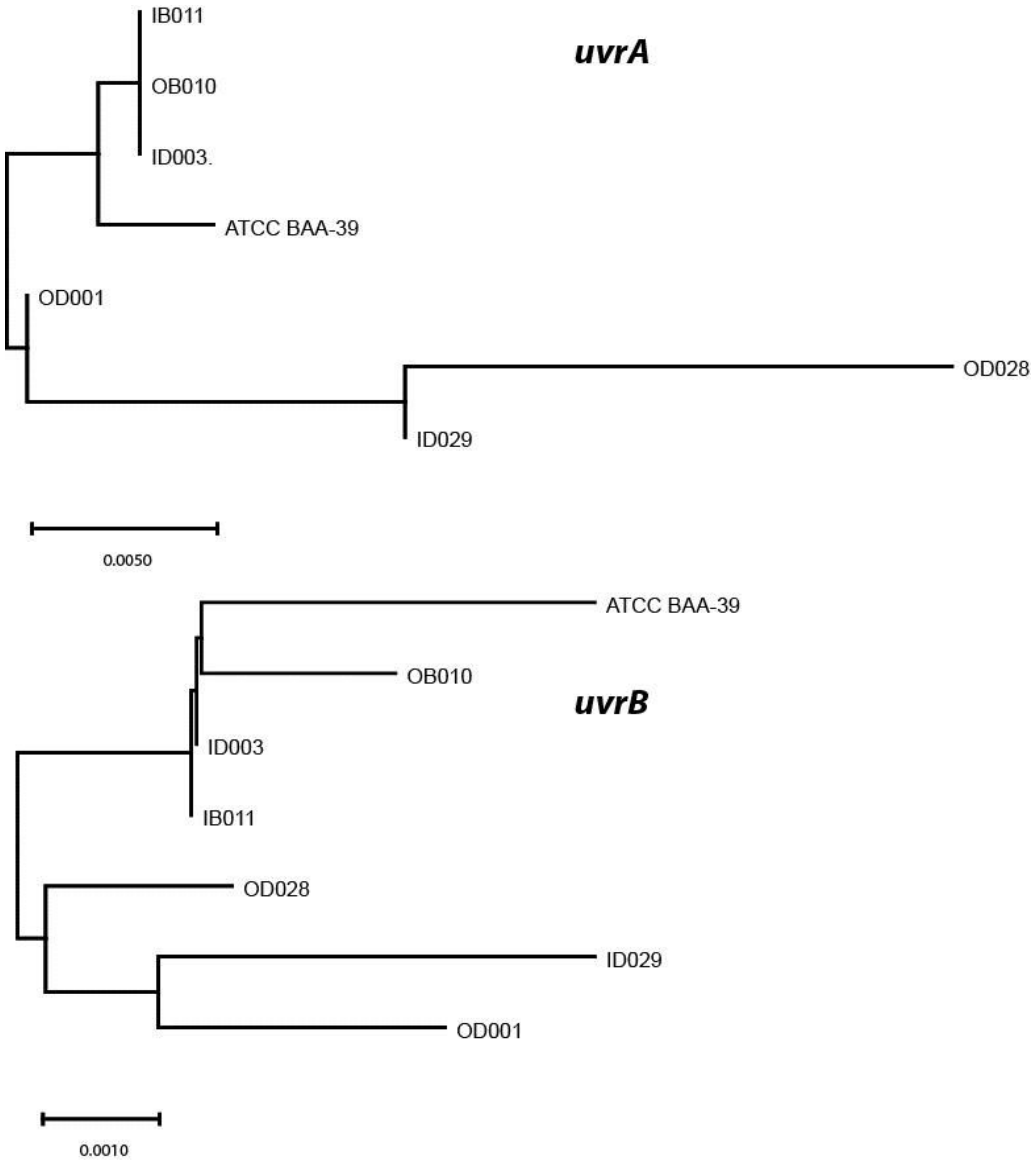


Figure 4.8. Dendrograms of sequence similarity of UvrAB proteins of the sequenced *S. aureus* isolates. The clustering of the isolates in line with the two genes suggests species-level genetic variability and divergence.

4.4 Determination of the antimicrobial resistance and genetic modification

The diversity of the 15 antibiotics in this study stemmed from the ability to elicit different destructive effects on the pathogens, via different modes of actions.

The antibiotic sensitivity test summary is shown (**Figure 4.9 and 4.10)**. *S. aureus* was highly resistant to Cephazolin, Cefuroxime and Ampicillin (95%). 37.5% isolates were resistant to more than 11 antibiotics. Only 25% were resistant to more than 12 antibiotics **(Figure 4.11 and Appendix XIV).** Six isolates were resistant to the commonly preferred Ceftriaxone and Oxacillin as first line prophylaxis for patients under surgery.

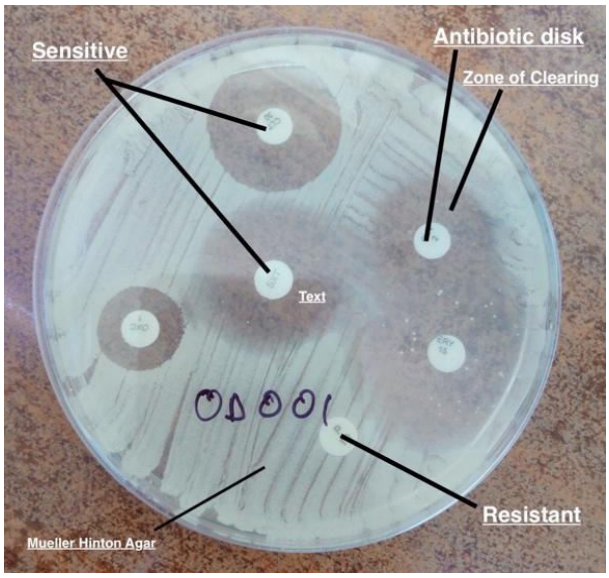


Figure 4.9. Sample culture plate showing the AST on Muller Hinton agar, on one super-resistant strain, OD001.

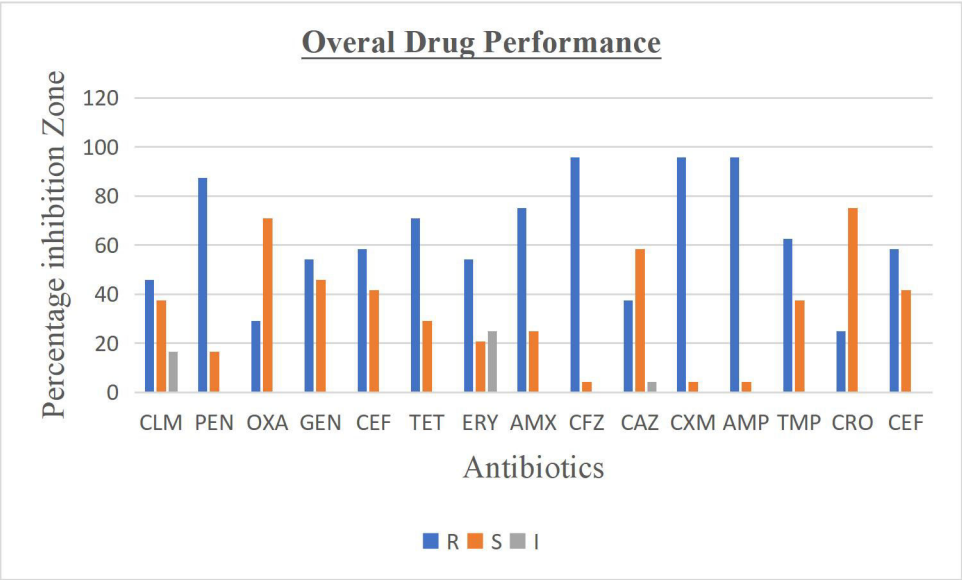


Figure 4.10. Antimicrobial sensitivity test summary showing performance of various antibiogram tested. The highest resistance was observed against CFZ, CXM, and AMP. The isolates were highly sensitive to OXA and CRO.

KEY: R- Resistant, S- Sensitive, I-Intermediate; Clindamycin (CLM), Penicillin (PEN), Oxacillin (OXA), Gentamicin (GEN), Cephalothin (CEF), Tetracycline (TET), Erythromycin (ERY), Amoxicillin (AMX), Cephazolin, (CFZ), Ceftazidime (CAZ), Cefuroxime (CXM), Ampicillin (AMP), Trimethopan (TMP), Ceftriaxone (CRO), Cephalothin (CEF).

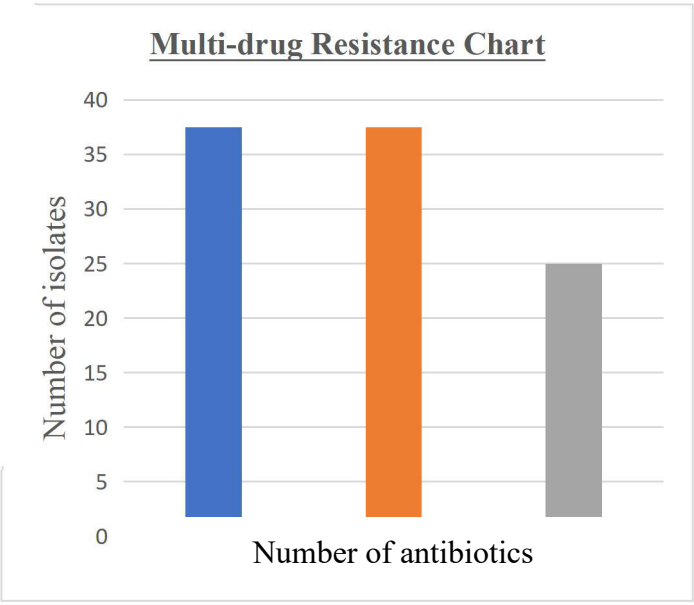


Figure 4.11 Chart showing multi-drug resistance among the study isolates of *S. aureus*.

4.4.2 Determination of antimicrobial resistance genes

From CARD-RGI analysis, several drug resistant genes were found on the chromosomes of the isolates **(Appendix XV).** All these genes were broad spectrum efflux pumps. Several genes associated with antimicrobial resistance were encoded on the chromosomes of each isolate (**Figure 4.12**). Most of these genes, which encode broad-spectrum efflux pumps, were found in all isolates, whereas *arlSR* and *mgrA* were absent or incomplete in the community isolates OD001 and OD028. In contrast, the reference strain *S. aureus* ATCC BAA-39 contained additional efflux pumps, *tet(45)* and *tet(K)*, as well as the SCCmec methicillin resistance cassette with genes *mecA* and *mrcR1*. These genes were absent in the genomes of the selected strains **(Appendix XVI).**

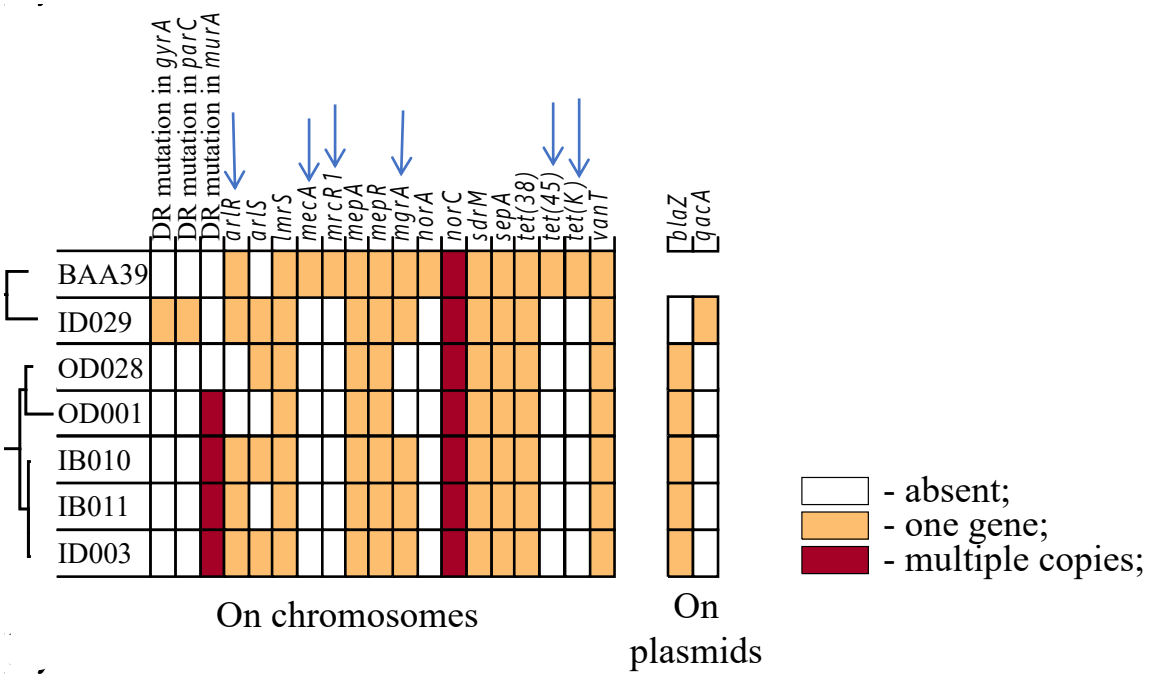


Figure 4.13. Distribution of drug resistance genes in the genomes of the selected *S. aureus* isolates and the reference strain *S. aureus* ATCC BAA-39. Antibiotic resistance genes and mutations identified by searching through the CARD database using the program RGI.

There were no significant differences among the zones of antimicrobial inhibition between CRO and OXA of CA and HA isolates of ST152 ($p = 0.150$) nor between their means ($p = 0.900$) (**Table 4.2**).

Table 4.2. Associations between the means of drug inhibition zone of OXA and CRO among the ST152 isolates.

		Oxacillin (OXA)	**Ceftriaxone (CRO)**
CA ST152	OD001	20	18
	OD028	20	18
	MEAN	**20**	**18**
HA ST152	ID003	20	18
	IB010	25	24
	IB011	18	18
	MEAN	**21**	**20**
Chi-Square Test of association between means **P < 0.05**		**df= 1, p-Value = 0.900**	
Kruskal-Wallis Test of significant differences between inhibition zones **(p<0.05, DF = 1)**	**N**	6	6
	Median	20	18
	Mean Rank	7.8	5.2
	Z-Value	1.28	-1.28
	H-value	2.08	
	P-Value	0.15	

There was no significant differences between the means of drug inhibition zones between OXA and CRO ((p-value = 0.900), and also among the inhibition zones (p-value = 0.15).

The study did not find plasmid encoded- nor chromosomally encoded *blaZ* gene DRG in the ST8 (ID029) isolate. However, all the ST152 carried the 888-bp *blaZ* gene on the 20.7-kb circular plasmid **(Figure 4.13).**

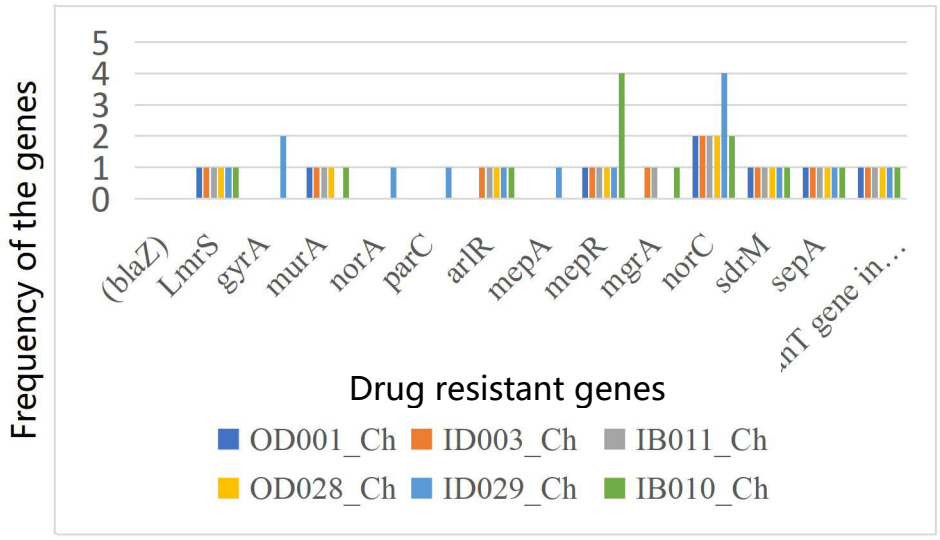


Figure 4.13. Chromosome encoded DRGs among each isolate. Although *blaZ* was only plasmid encoded in ST152, gyrA, parC, mepA and norA mutational factors were only in ID029 isolate. Moreover, sdrM, sepA and VanT appeared in all the isolates.

Antibiotic efflux appeared to be the most utilized mechanism (60%) of antibiotic resistance, followed by antibiotic target alteration on the pathogen (30%). Antibiotic inactivation appeared to be the least (10%) among the mechanisms (Figure 4.14).

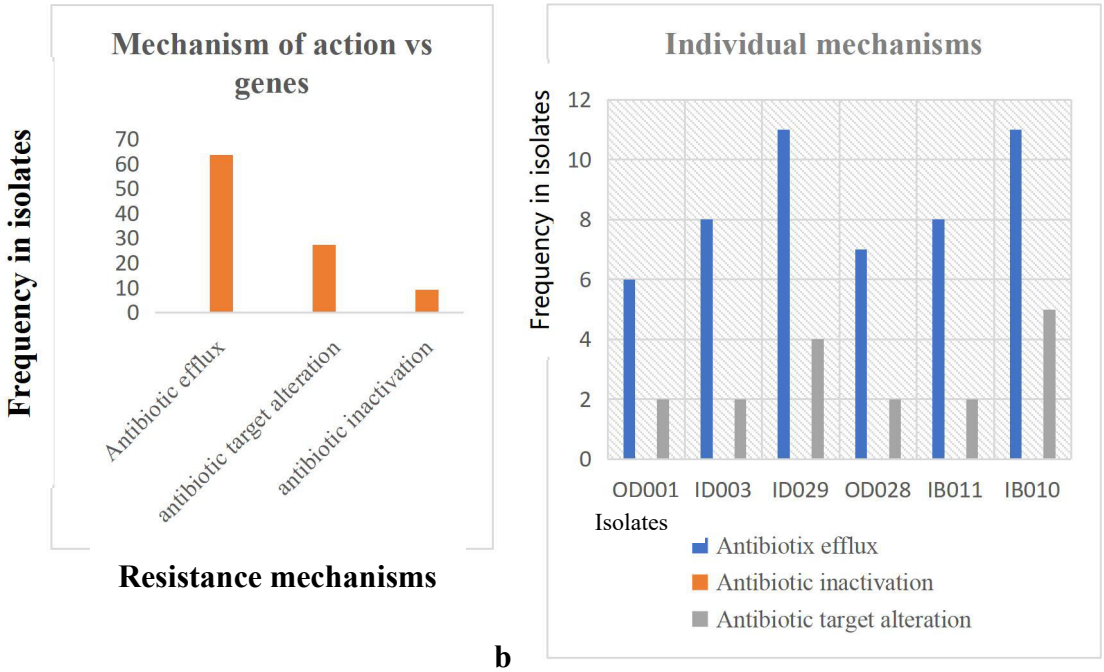


Figure 4.14. Different resistance mechanisms in the identified DRGs. Antibiotic efflux was utilized by 60% of genes while inactivation was used by 10% of all genes annotated **(a).** Plasmids encoded gene, *blaZ*, utilized antibiotic inactivation mechanism only. IB010 & ID029 isolates utilize antibiotic efflux mostly **(b).**

4.4.3 Determination of genetic modifications in the drug resistant genes

Several mutations associated with acquired antibiotic resistance were discovered in the selected strains using the program RGI. An important mutation was observed in *glpT* gene of the ID029 isolate (V89T). *S. aureus* ID029 (ST8) also had two other mutations in GyrA DNA gyrase subunit and ParC DNA topoisomerase IV subunit A (S80Y) **(Figure 4.15).**

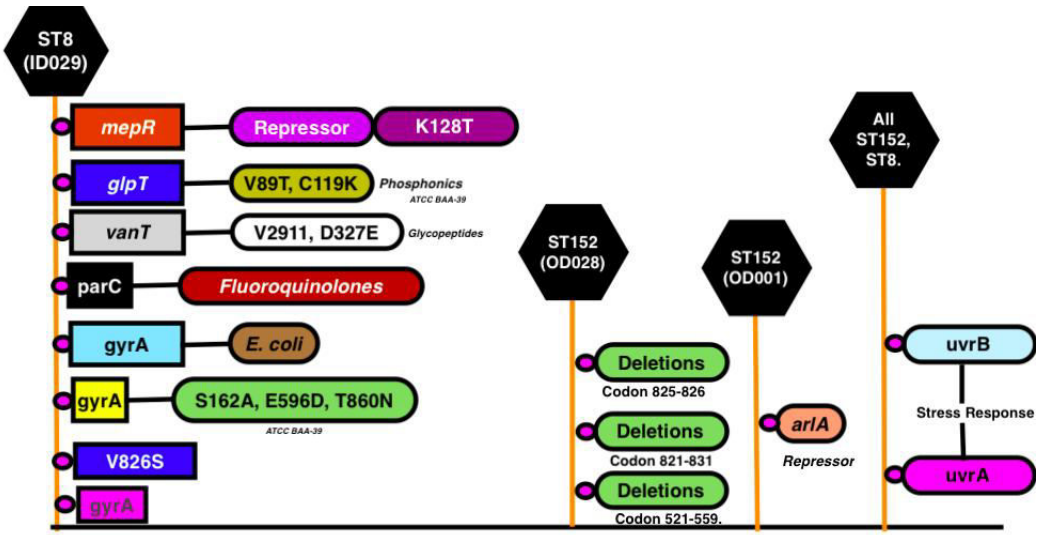


Figure 4.15. Mutations and Genetic modifications in the drug resistant genes of study isolates.

CHAPTER FIVE

DISCUSSION

5.1 Assessment of post-surgical wound infections for *S. aureus* among patients

In this study, majority of patients who presented with post-surgical wound infections were males. However, other studies on surgical wound infections in the region have shown that females report a high number of patients in this category of infection. For example, Dinda *et al.,* (2013) in a study conducted in the Aga Khan University Hospital, Kenya, on patients admitted in the general surgery wards, females recorded a higher numer of infections. The gender distribution of post-surgical wound infections varies across studies due to a complex interplay of demographic factors, types of surgeries performed, risk factors, hygiene practices, immune responses, healthcare access, and research methodologies (Mohan *et al.,* 2023). Gender distribution of patients undergoing surgeries can also vary depending on the population being studied and can geographically determine the number of patients, where a high number of males can be higher in a region due to a higher proportion of male patients seeking surgical care (Alkhaldi *et al.,* 2022). Moreover, certain surgeries may be more common in one gender owing to anatomical differences or health conditions. For example, studies have shown that gynecological surgeries are predominantly performed on females, while prostate surgeries are specific to and are on the rise in males (Cheng *et al.,* 2021; Polan & Barber, 2021). However, in this study, the higher number of males who presented with chronic wounds and catheter site infections can be explained by sociocultural or demographic factors affecting when patients seek surgical care. Therefore, the prevalence of these procedures can impact the gender distribution of infections.

In this study, class III wounds represented hotspots for bacterial infections. Previous studies have shown increased treatment costs due to high infection incidences (Ousey *et al.,* 2020),

attributed to extended hospital stays, addition medications to complement antibiotics, more diagnostic tests, and other complications, whose severity varies in line with the class of wound (Herman, 2022). Patient age is also an independent predictor of wound class infections, owing to such factors as immune system functions. These functions deteriorate with age, and skin integrity which makes skin vulnerable to attack by pathogens (Bernatchez & Bichel, 2023; Russell-Goldman & Murphy, 2020). This therefore predisposes patients advanced in age or infants for wound being infected and colonizes by microbes.

Moreover, the association between the organism recovered and wound class observed in the study may suggest microbial adaptation which varies to strain level. In this study, the organiss recovered were *S. aureus, P. aeruginosa, E.coli and Klebsiella sp.* A choice of antibiotic used in the treatment of specific wound class has shown to exert selective pressure on the microbial population on the wound, and thus some organisms inhabit only certain class of wound (Baceiro *et al.,* 2013). Similarly in this study, Class II wounds were predomianntly infected by *Klebsiella* sp, Class III wounds by *P. aeruginisa*, and Class IV by *S. aureus*. Moreover, different wound classes may provide opportunities for biofilm formation for certain microbial pathogens (Percival *et al.,* 2015: Firas Diban *et al.,* 2023). Findings on *S. aureus* wound infections in this study agrees with previous studies (Mwita *et al.,* 2021; Endashaw Hareru *et al.,* 2022), that the infections continue to rise due to high prevalence of *S. aureus* in sub-Saharan region, and the presence of the isolated bacterial pathogens on the skin of patients, before or after undergoing major or minor operation, as reported elsewhere (Szultka-Młyńska *et al.,* 2021; Shakir *et al.,* 2021). Previous study in Kenya have reported on the predominant *S. aureus* infecting surgical sites (Dinda *et al.,* 2013; Fernandez *et al.,* 2020). The recent surge in MRSA infections in sub-Saharan Africa therefore necessitates the need for continuous analysis of the *S. aureus* isolates in an attempt to understand the structure of

the pathogen in the region. Nevertheless, this study agrees with previous studies that *S. aureus* and *P. aeruginosa* are the most commonly bacteria recovered from wound infections as previously reported (DeLeon *et al.,* 2014; Serra *et al.,* 2015).

However, in this study, *S. aureus* was the most predominant in class IV wounds. Previous data shows that *P. aeruginosa* and *S. aureus* may benefit each other by infecting wounds. Additionally, the host-derived matrix may be as significant as the bacterium-derived matrix in shielding the pathogens from some antibiograms (DeLeon *et al.,* 2014). In this study, it was observed that the recovery of *S. aureus* and *P. aeruginosa* from post-surgical wounds may have been influenced by their differential localization within the wound environment. Previous work has shown that *S. aureus* typically colonizes the superficial wound layers, while *P. aeruginosa* is confined to the deeper wound level (Serra *et al.,* 2015). This distribution is biologically plausible, since *S. aureus* growth depends on oxygen availability. *S. aureus* is found abundant at the wound surface (Balasubramanian *et al.,* 2017), whereas *P. aeruginosa* is capable of surviving in oxygen-deprived environments by utilizing alternative electron acceptors (Moradali *et al.,* 2017). Consequently, the predominance of one organism over the other in culture could partly reflect the depth and method of specimen collection. These observations underscore the importance of standardized sampling techniques, as superficial swabs may preferentially capture *S. aureus*, while deeper aspirates or biopsies are more likely to recover *P. aeruginosa*. These findings therefore highlight how wound microbiology is structured, not only by pathogen biology but also by methodological approaches in specimen collection.

5.2 Determination of the genetic diversity of *Staphylococcus aureus* subtypes

This study sought to determine the genetic diversity of the *S. aureus* that were recovered from the patients. From the *S. aureus* recovered, six were selected and sequenced based on their resistance patterns to oxacillin and ceftriaxone. This study's *S. aureus* displayed varied patterns of antimicrobial susceptibility, demonstrating their diversity. Generally, studies investigating *S. aureus* wound infections have shown that diverse *S. aureus* are involved in various infections. A study by Muhammad Ali Syed *et al.,* (2021) on clinical samples in Pakistan observed that approximately 25.3% of the clinical samples were positive for *S. aureus*; displaying 23 different *spa* types, where most isolates belonged to spa type t632 and t657 (9/66; 13.6% each spa type) and ST772-t657. Additionally, Rajiha *et al.,* (2023) on clinical samples in Ethiopia observed eight pulso-types groups (from A to I) among the methicillin susceptible *S. aureus* (MSSA) recovered. These findings thus show that *S. aureus* infections both at community or hospital associated settings are driven by diverse isolates (Tong *et al.,* 2015), therefore, calling for increased monitoring and surveillance of *S. aureus* at both the community and health facilities. This would facilitate tracking of antibiotic resistance, boosting infection control initiatives, including early warning systems and collaborative efforts to address AMR on a global scale.

In this work, 5 out of the 6 *S aureus* samples subjected to whole genome sequencing belonged to the same ST152 subtype. Subtype or sequence type ST152 represents a distinct lineage within the *S. aureus* population and therefore, grouping of the isolates to ST152 can be implicated to high genome similarity among the study strains, sharing a common ancestor (Ndahetuye *et al.,* 2021). Other studies have also reported a similar incidence of a dominant strain of ST152, attributed to clonal expansion when a single lineage of *S. aureus* proliferates, dominating the entire area or region (Ndahetuye *et al.,* 2021; Nyasinga *et al.,* 2020; Kyany'a

et al., 2019; Benear Apollo Obanda *et al.,* 2022). ST152 has also been implicated in increased virulence and antibiotic resistance in Sub-Saharan Africa (Osahon Obasuyi *et al.,* 2020). Therefore, the high representation of ST152 in this study is evidence for a localized outbreak and dissemination of a particular strain within a clinical setting, and thus should be of great concern.

Among the isolates sequenced in this study, it was observed that the number of the horizontally acquired GIs displayed by the isolates associated with hospital and community infections**.** Those associated community infections displayed 5 GIs, whereas those associated hospital infections displayed more GIs, ranging from 7 to 10 GIs, similar observation in Holden *et al.,* (2004) study which compared hospital acquired MRSA and community acquired MSSA. The differential distribution of large mobile elements harboring virulence and drug resistance could be responsible for the phenotypic differences observed among strains. This observation can be attributed to many factors involving evolutionary and selective pressures among the study isolates in different environments. Unlike isolates associated with community infections, isolates from hospital environments are constantly exposed to antibiotics and invasive procedures like surgeries (Prestinaci *et al.,* 2015). These factors have shown to contribute to the acquisition and therefore maintenance of specific genomic islands, drug resistance and virulence genes in the genomic islands (Juhas *et al.,* 2009). Moreover, the difference in environments can also vary transmission dynamics of the strains where, hospital associated strains are in closer contact within the hospital setting. This has been shown to greatly influence the genetic diversity and the exchange of GIs among the strains (Li & Wang, 2021). The Genomic islands characteristics shared among ST152 isolates which were closely related, may suggest their diversification and adaptation which impacts genome plasticity, evolution, and dissemination of DRGs among strains. Moreover, the GIs

contributed significantly to the virulence potential of the study strains, as was evidenced by the number and functions of the drug resistant genes and the mutation sites within the genes. A study by Ventola, (2005) showed that an increased rate of horizontal gene acquisition by the hospital acquired pathogens could enforce over appearance of highly virulent pathogens in hospitals.

Genomic analysis of the ST152 isolates, OD001 and OD028, revealed that the two missed three genes known as the Holliday junction resolvase/endonuclease and 30S ribosomal proteins S14 and S14-2 in the core part of their chromosomes. The Holliday junction resolvases play an important role in homologous recombination in *S. aureus* (Pereira *et al.,* 2013). The shorter plasmid of ID029 isolate carried *icaR* transcriptional regulator in addition to a homologous gene on the chromosome. This gene is potentially involved in the regulation of biofilm formation (Conlon *et al.,* 2002; Cerca *et al.,* 2008). However, this study did not confirm biofilm formation among these isolates. These recent studies have shown that, as part of icaADBC operon, this gene is involved in the active synthesis of polysaccharide intracellular adhesin (PIA), implicated in biofilm matrix formation (Arciola *et al.,* 2015; Nguyen *et al.,* 2020). Hospital strains bearing this gene are advantaged by the regulation of quorum sensing, allowing them to sense their population density and adjusting expression of *icaR* accordingly, facilitating the formation of PIA and biofilm (Rutherford & Bassler, 2012). Moreover, biofilm formation on medical devices like those used in surgery and implants have been attributed to chronic infection and therefore offers treatment challenges (Khatoon *et al.,* 2018) by protecting the pathogen against antimicrobials.

Further genomic analysis among the hospital and community related isolates in this study, showed increased frequency of bacteriophages and other mobile elements among isolates recovered from hospital environment rather than in the community environments. Isolates in the hospital setting are in closer contact within the hospital setting and thus better suited for the acquisition and maintenance of these genomic elements due to selective pressures and adaptation. Bacteriophages have been implicated in the horizontal gene transfers as well as phage defense systems like CRISPR-Cas in *S. aureus* (Varble *et al.,* 2019). Therefore, the presence of these genomic features means better evolution and adaptability of the *S. aureus* isolates in hospital setting, amidst frequent use of antibiotics as compared to community associated isolates, explaining the differences observed. This adaptation has involved many drug-resistant genes identified in this study, that is reflected by grouping the hospital acquired and the community acquired genomes into two separate sub-clusters in the phylogenetic tree**,** suggesting an ongoing micro-evolutionary adaptive events in this group of microorganisms.

Analysis of the uvrA and uvrB genes, which are involved in stress responses and adaptive mutations in bacteria (McKenzie *et al.,* 2000; Podlesek & Žgur Bertok, 2020) revealed variations in both amino acid substitutions and large deletions, which could make these proteins non-functional. UvrAB proteins are part of the SOS-response of *S. aureus* which identify lesions in chromosomal DNA caused by UV-irradiation and by other factors. These activate the DNA reparation machinery to fix the errors. An inactivation of genes encoding these proteins could lead to an increase in the rate of mutations (Ha & Edwards, 2021). While the mutator phenotype decreases viability of the microorganisms, it feeds the selection of beneficiary mutations allowing an adaptation to harsh environmental conditions (McKenzie *et al.,* 2000). Generally, these are known as evolutionary hotspots of *S. aureus* (Ha & Edwards, 2021). Evolutionary hotspots are specific regions in the genome of *S. aureus*

susceptible to frequent genetic alterations or modifications during evolution, making the strains adapt to new environments. These genes were conserved in the hospital acquired isolates of ST152, but a big number of variations was observed in the community acquired isolates of ST152 and in the strain *S. aureus* ID029 of ST8**.** According to these variations in uvrB, the hospital acquired isolates clustered together suggesting a distinct evolutionary pattern from the community acquired isolates.

5.3 Determination of the antimicrobial resistance and genetic modification

This study determined antimicrobial resistance profile of *S. aureus* to fifteen antibiotics commonly used in health facilities. The increased resistance to cephazolin, cefuroxime and ampicillin observed in this study has been previously reported in Africa (Eman Farouk Ahmed *et al.,* 2023). A similar trend of resistance to antimicrobials can be attributed to the continued adaptation of the pathogen to hospital setting, where antibiotics are heavily used compared to community settings (MacFadden *et al.,* 2018). In this study, this trend has also been observed where isolates associated with hospital setting showed a similar profile of resistance among themselves. Thus this calls for urgent control measures. Moreover, six of the 24 *S. aureus* isolates were resistant to ceftriaxone and oxacillin. Other studies have reported similar observations among *S. aureus.* Zehra *et al.,* (2017) observed that about 10-15% of their *S. aureus* strains were resistant to oxacillin (MIC 6-8 µg/ml) and about 60% of isolates in Pickering *et al.,* (2014) showed resistance to ceftriaxone. Resistance to a commony preferred antibiotics can have important implications to the infection control program and public health in a particular facility, these can range from limited treatment options for the pathogen's infections, increased risk of infection, prolonged hospital stays, and increased risk of complication as seen in this study's participants.

In this study, resistance of *S.aureus* to oxacillin was identified, implicating a serious health concern since resistant to oxacillin and especially with minimum inhibitory concentrations (MIC) ≥ 4ugmL^{-1}, have been shown to be resistance to methicillin (Siddiqui & Koirala, 2023). *blaZ* gene is an 846-bp gene which appears to be controlled by two other regulatory genes-repressor *blaI* and antirepressor *blaR1* (Takayama *et al.,* 2018). *blaR1* is a beta-lactam sensor, which induces resistance to beta-lactam antibiotics (Llarull *et al.,* 2011). A 95% resistance to cephazolin, cefuroxime and ampicillin in this study therefore can be explained by the presence of *blaZ* gene in the 5 out of the 6 *S. aureus* isolates (83.3%), belonging to ST152. This frequency of occurrence of the gene can be projected to all the recovered isolates since *blaZ* is often associated with resistance to beta-lactam antibiotics, which includes the cephalosporins. This study therefore suggests that the resistance levels observed in the isolates were from a highly resistant and dominant lineage of ST152, where antimicrobials are often used. However, the global pattern of AMR has been shown to vary on basis of healthcare conditions such as infection monitoring and prevention, and rationale antimicrobial usage by region or country (Falagas *et al.,* 2013). This may be attributed to antibiotic stewardship and awareness, surveillance and data collections on AMR and new antibiotic and therapies (Majumder *et al.,* 2020).

Moreover, in this study, whole genome sequences of the six hospital- and the community associated isolates of *S. aureus* characterized with multi-drug resistant phenotype were compared, as a representative of the 24 recovered isolates. The broad spectrum of resistance of the ST152 isolates can be explained to some extend by the presence of the *blaZ*-carrying plasmid. In contrast to these isolates, the strain *S. aureus* ID029 has developed an extended antibiotic resistance mostly due to mutations in *mepR, glpT, vanT, parC, gyrA*, and the efflux pump inhibitors. The size of this plasmid potentially outlines its similarity to other studies

and the importance in the horizontal dissemination of *blaZ* gene among all the ST152 isolates in the study (McCarthy & Lindsay, 2012).

The size of plasmids relative to DNA size is an important factor in bacterial transformation. For example, small plasmids are easily transferred horizontally. Smaller plasmids have fewer genes and thus can replicate and segregate more rapidly in host cell, while requiring low amounts of energy. Furthermore, the presence of *blaZ* gene in ST152 plasmids may indicate penicillin resistance among the isolates and an origin-specific genomic composition as first reported (Vesterholm-Nielsen *et al.,* 1999). This phenomenon has been implicated in diversity, evolution, conservation, and endemism of the microbe (Olsen *et al.,* 2006). The smaller plasmid of 10,162 bp in isolate of ID029 (ST8) could be a transposable element carrying an efflux transporter *qacA* involved in antiseptic resistance.

Although this study observed variation in the number of GIs carriage, there was no significant difference in the levels of resistance obtained by comparing the diffusion diameters of antimicrobial susceptibility tests. This could therefore imply that the identified genetic factors of antibiotic resistance may only partly explain the broad range of drug resistance, owing to co-evolution with antibiotics (Tognon *et al.,* 2017), diversity of resistance mechanisms among the strains, emergence of other novel resistance mechanisms and stress-induced response (Ranganathan *et al.,* 2020). Moreover, the study identified increased antibiotic efflux as the mostly utilised mechanism for drug resistance. While other studies have similarly observed this phenomenon among *S. aureus* isolates (Dashtbani-Roozbehani & Brown, 2021), antibiotic efflux can be implicated in reduced treatment efficacies, increased antibiotic resistances, and reduced antibiotic options against *S. aureus* (Amit Gaurav *et al.,* 2023).

The study identified mutations to play an important part in the resistance to antibiotics and disinfectants. In *parC* gene identified in ID029 (ST8) isolate, there was a mutation associated with resistance to fluoroquinolones, has been previously reported (Pan *et al.,* 2002; de Oliveira *et al.,* 2019; Nouri *et al.,* 2016). This mutation changes amino acid sequence of topoisomerase IV protein- involved in DNA replication, thus reducing the binding affinity of fluoroquinolones to the active site of the enzyme (Hooper & Jacoby, 2016). Another important mutation was observed in *glpT* gene of the ID029 isolate. In this isolate, the point mutations conferred resistance to Fosfomycin and phosphonic based antimicrobials including detergents (Castañeda-García *et al.,* 2009).

In summary, the study identified one special isolate, ID029, among the six isolates of *S. aureus* sequenced. Not only does it have the biggest genome, but also the biggest number of genomic islands and plasmids, making it versatile and perhaps more pathogenic by favorably evolving and adapting fast. Further, the study demonstrates that *S. aureus* ST152 is locally abundant among clinical isolates with a multi-drug-resistant phenotype in Kakamega, Western Kenya. All ST152 isolates carried similar plasmids encoding several antibiotic resistance genes, including the *blaZ* β-lactamase. Phylogenetic analysis and genome comparison suggest ongoing evolution among the ST152 isolates, leading to the segregation of hospital-acquired and community-acquired lineages. An inspection of PubMLST database records (https://pubmlst.org) revealed that out of 121 recorded ST152 isolates, 98 strains (81%) were isolated in African countries. Another multi-drug-resistant isolate, ID029, possessing two virulence plasmids, belongs to ST8. Pathogens of this genotype are common among hospital *S. aureus* isolates, as reported by Nyasinga *et al.,* (2020), in their review on the Eeidemiology of *Staphylococcus au reus* infections in Kenya. However, according to 3,726 records in the PubMLST database (https://pubmlst.org), the ST8 genotype is dominant

in the USA and Europe but rare in Africa. This observed geographic separation of *S. aureus* sequence types necessitates further study to determine whether it is associated with adaptation to specific human populations, or antibiotic treatment protocols in different regions. Another plausible explanation is that these rapidly evolving lineages have emerged recently and are dominant in the regions where they appear.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

S. aureus is an important pathogen associated with post-surgical wound (PSW) infections at KCTRH. Nevertheless, this study has demonstrated microbial diversity, involving different genera of bacterial pathogens infecting post-surgical wounds at the facility. Moreover, post-surgical wound class had an association with increased dominance with as specific microbe, with *S. aureus* being associated with class III wound types.

This study further demonstrated that *S. aureus* infecting PSWs at KCTRH are diverse, although further genomic analysis of oxacillin and ceftriaxone showed a dominant sequence type - ST152. Additionally, all ST152 carried similar 20,760 bp plasmids that possessed several antibiotic resistance and virulence genes including *blaZ* lactamase. This study also showed that genomic dissimilarity between the isolates of the same sequence type ST152 originated from different sources of isolation suggested an ongoing evolution of these pathogens, hence two different lineages. Thus, the selected microorganisms present a useful collection of isolates to study mechanisms of evolutionary divergence between closely related lineages of pathogens towards adaptation to specific environments and survival strategies.

Finally, this study demonstrated an occurrence of multidrug resistant *S. aureus* among microbes infecting PSW at KCTRH, and may be a consequence of genetic modifications in the drug resistant genes identified. GIs were observed as important drivers of antibiotic resistance and virulence development among *S. aureus* recovered in this study.

6.2 Recommendations

From the overall review of this study, the following recommendations can be deduced; there is need to sensitize patients and health care workers on good wound care and management. This is because of the increased numbers of class III, IV wounds and MDR infections observed in this study, to avoid infections by multi-drug resistant microbes. There is also a possibility of a strain of ST152 predominating hospital infections at the Kakamega County General Hospital, thus further multi-site studies need to verify this, to explore strategies for its control. Finally, the recovery of multi-drug resistant *S. aureus* strains haboring drug resistant genes from the post-surgical wounds at KCGTRH, underscores an urgent for continuous surveillance at genomic level, and antimcrobial stewarship.

6.2.1 Recommendations for future studies

Future mutli-site studies across Western Kenya and beyond are recommended to validate these findings, to map resistant genes circulating, and also inform infection control programs.

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APPENDIX

Appendix I: Map of study area



Map of Kenya showing the circled study area in Kakamega County (0°16'29N, 34°45'31E). (Adapted from Akech *et al.,* 2019)

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Appendix II: Study Consent Form

CONSENT FORM.

MOLECULAR CHARACTERISATION OF *Staphylococcus aureus* SUBTYPES INFECTING POST-SURGICAL WOUNDS OF PATIENTS REPORTING TO KAKAMEGA COUNTY TEACHING AND REFERRAL HOSPITAL

Investigator: Nyabera Mogoi Nicholas

INTRODUCTION

My name is Nicholas Mogoi, a Masters student at the Masinde Muliro University of Science and Technology. I am here to conduct a research on the above mentioned title. I kindly request you to cooperate with me regarding this exercise. Please the consent form below will be read out for you. I would be pleased if you accept my request to participate in this study.

THE PURPOSE OF THE STUDY

The aim of this study is to characterize the selected and recovered isolates from surgical wards, so that the common clonal lineages (populations / complexes) of *S. aureus* can be established and also the pattern and mechanism of antibiotic resistance that are attributed to nosocomial infections in hospital settings established.

PROCEDURE

Theis form is to obtain your permission to participate. If you agree to participate, a questionnaire will be administered to you and the interview will take between 10 and 20 minutes to complete. Sterile moistened swabs will be used to collect samples from your surgical site. The procedure is harmless and will be done aseptically in 3 minutes.

Two swabs will be obtained from you from each surgical wound.

RISKS AND BENEFITS

Apart from the inconveniences, this is a safe process with no risks whatsoever involved, and hardly any direct benefits for participation. However, data collected will be communicated back to the health facility for necessary action by the health authority. The information you provide here will help with strengthening surveillance and keeping track of emerging clones, strengthen infection control programs and ease economic burden that are incurred by prolonged hospital stay by patients and high cost antimicrobials in KCTRH. The data will also be used in thesis completion as part requirement by the university.

CONFIDENTIALITY

Participants have codes for identification. No data will be available to the public without explicit consent this research team.

CONTACT INFORMATION

For any inquiries;
Investigator: Mogoi Nicholas Nyabera
Telephone:+254717056379
Email: mogoinn67@gmail.com**;** nmogoi@mmust.ac.ke

The participant's submission:

Participant's identification code: ……..
Admission Ward ……
Signature of participant ……..
Name of interviewer……
Signature…… ……
Date……..

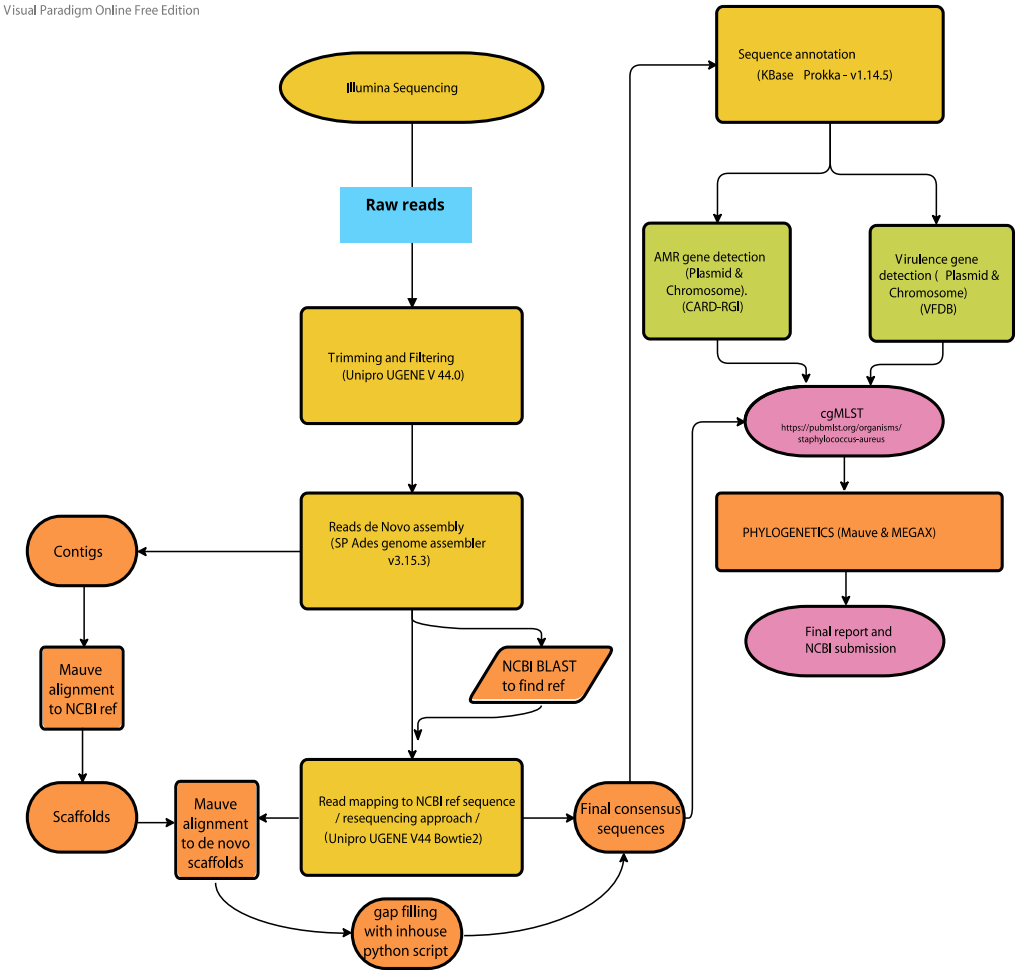
Appendix III: Study Questionnaire

SURGICAL WARD QUESTIONNAIR
SECTION I: <u>DEMOGRAPHIC DATA</u>
1. Patient Code …….....
2. Age ……
3. Gender (a) Male [ ] (b) Female [ ]

SECTION II: <u>CLINICAL DATA</u>
1. Admission (a) Inpatient [ ] (b) Outpatient [ ]
2. Admission ward (a) Postnatal [ ] (b) Orthopedic [ ] (c) Obstetrics [ ] (d) Gynecology [ ]
(e) Others [ ] (General).
3. Type of operation ……..
4. Surgical Site Infection (a) Yes [ ] (b) No [ ]
5. If yes, what is the extent of infection? (a) 1 week [ ] (b) 3 weeks [ ] (c) 4 weeks [ ] (d) More than 5 weeks [ ]
6. Presence of cancer (a) Yes [ ] (b) No [ ]
7. Length of Hospital Stay …….
8. Are you currently on antibiotics? (a) Yes [ ] (b) No [ ]

Thank you for participating in this study.

Appendix IV. Whole genome analysis pipeline

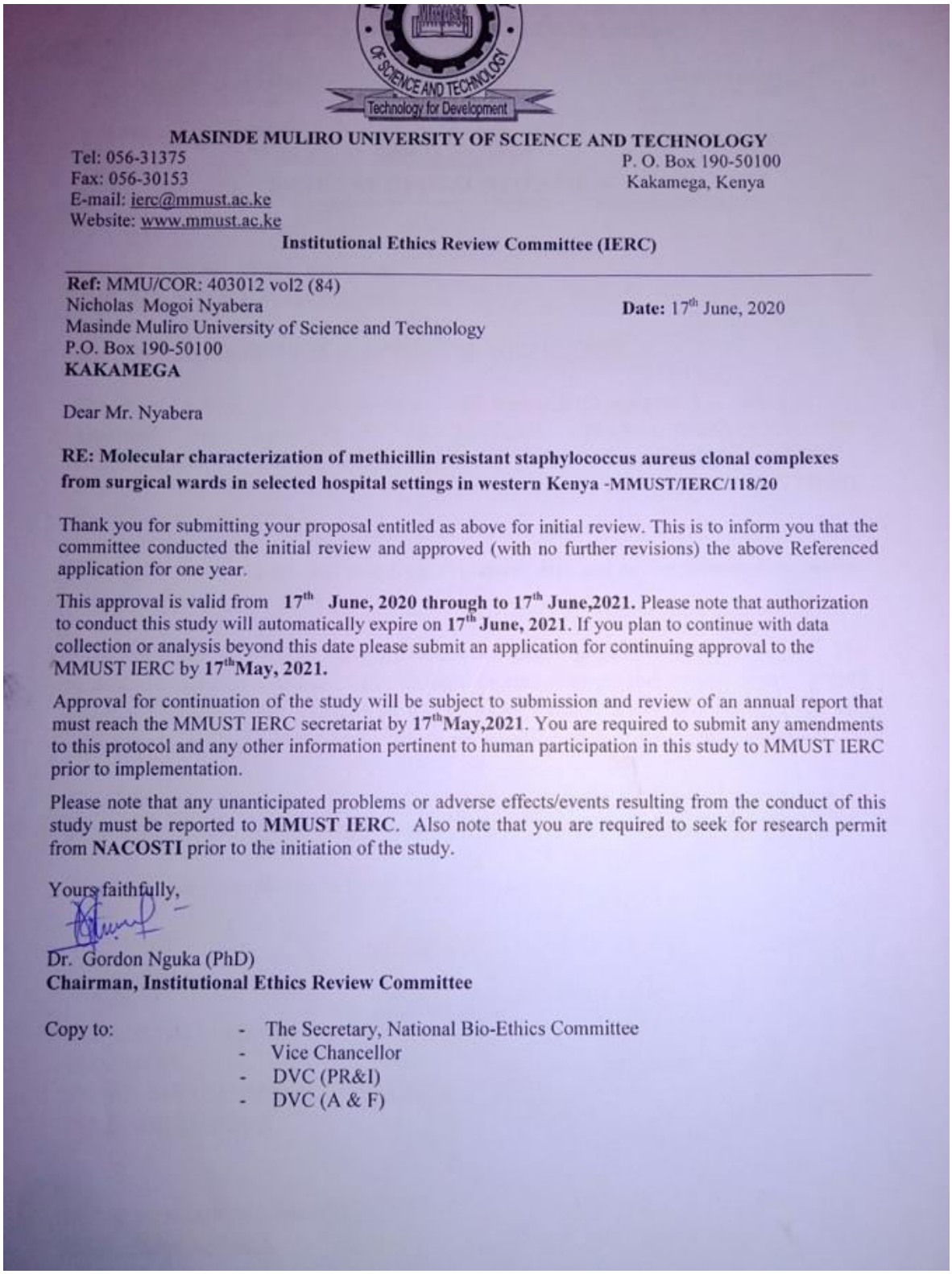


Appendix V: Antibiotic inhibition zone determination chart

Antimicrobial Agent	**Disc Content (μg)**	**Sensitive, S (mm)**	**Intermediate, I (mm)**	**Resistant, R (mm)**
1. Ampicillin (AMP)	10/10 μg	≥ 15	12-14	≤ 11
2. Amoxicillin (AMX)	20/10 μg	≥ 20	--	≤ 19
3. Ceftazidime (CAZ)	30 μg	≥ 18	15-17	≤ 14
4. Cephalothin (CEF)	30 μg	≥ 18	15-17	≤ 14
5. Cefalexin (CFX)	20/10 μg	≥ 20	--	≤ 19
6. Cephazolin), (CFZ)	30 μg	≥ 18	15-17	≤ 14
7. Clindamycin (CLM)	2 μg	≥ 21	15-20	≤ 14
8. Ceftriaxone (CRO)	30 μg	≥ 21	14-20	≤ 13
9. Cefuroxime (CXM)	30 μg	≥ 18	15-17	≤ 14
10. Erythromycin (ERY)	15 μg	≥ 23	14-22	≤ 13
11. Gentamicin (GEN)	10 μg	≥ 15	13-14	≤ 12
12. Oxacillin (OXA)	1 μg	≥ 13	11-12	≤ 10
13. Penicillin (PEN)	10 μg	≥ 29	--	≤ 28
14. Tetracycline (TET)	30 μg	≥ 19	15-18	≤ 14
15. Trimethoprim (TMP)	5 μg	≥ 16	11-15	≤ 10

This is the chart that was used to determine the antimicrobial sensitivity pattern among the study isolates, according to CLSI 2022 guidelines (CLSI M100-S32).

Appendix VI: Ethical and study approval letters



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190-50100
Kakamega, Kenya

Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 vol2 (84)

Date: 17th June, 2020

Nicholas Mogoi Nyabera
Masinde Muliro University of Science and Technology
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Nyabera

RE: Molecular characterization of methicillin resistant staphylococcus aureus clonal complexes from surgical wards in selected hospital settings in western Kenya -MMUST/IERC/118/20

Thank you for submitting your proposal entitled as above for initial review. This is to inform you that the committee conducted the initial review and approved (with no further revisions) the above Referenced application for one year.

This approval is valid from **17th June, 2020 through to 17th June,2021.** Please note that authorization to conduct this study will automatically expire on **17th June, 2021**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **17thMay, 2021.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC secretariat by **17thMay,2021**. You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/events resulting from the conduct of this study must be reported to **MMUST IERC**. Also note that you are required to seek for research permit from **NACOSTI** prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)
- DVC (A & F)

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
(MMUST)

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P.O Box 190
50100 Kakamega
KENYA

Directorate of Postgraduate Studies

Ref: MMU/COR: 509079

5th February, 2020

Nicholas Mogoi Nyabera
SBB/G/01-53199/2018
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Nyabera,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters proposal entitled: ***"Molecular Characterization of Methicillin Resistant staphylococcus Clonal Complexes from Surgical Wards in Selected Hospital Settings in western Kenya"*** and appointed the following as supervisors:

1. Dr. Anthony Sifuna - Department of Biological Sciences - MMUST
2. Dr. Patrick Okoth - Department of Biological Sciences - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences and Technology Graduate Studies Committee and Chairman, Department of Biological Sciences. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

DEAN
SCHOOL OF GRADUATE STUDIES
MASINDE MULIRO UNIVERSITY
OF SCIENCE & TECHNOLOGY
Date:..... Sign:.....

Prof. John Obiri
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **233986**

Date of Issue: **30/September/2025**

RESEARCH LICENSE



This is to Certify that Mr.. Nicholas Mogoi Nyabera of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kakamega on the topic: MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS CLONAL COMPLEXES FROM SURGICAL WARDS IN SELECTED HOSPITAL SETTINGS IN WESTERN KENYA for the period ending : 30/September/2026.

License No: **NACOSTI/P/25/4180389**

233986

Applicant Identification Number

Ag. Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

COUNTY GOVERNMENT OF KAKAMEGA

E-mail: wpgh15@yahoo.com
Telephone: Kakamega 0720295739
When replying, please quote:

REF: CGH/KAK/ERC/VOL.I/85



COUNTY GENERAL HOSPITAL
P.O. BOX 15-G.P.O-50100
KAKAMEGA

DATE: 19TH OCTOBER, 2020

MINISTRY OF HEALTH SERVICES

NICHOLAS MOGOI NYABERA
ERC NO. SSB/G/01-53199/2018

RE: RESEARCH PROPOSAL APPROVAL -112/10/2020

This is to inform you that the Ethics and Research Committee has reviewed and approved your work titled **"MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS CLONAL COMPLEXES FROM SURGICAL WARDS IN SELECTED HOSPITAL SETTINGS IN WESTERN KENYA "**

The approval is valid for one year from the above date and any continuation thereafter will necessitate a request for renewal.

Note that this approval is only for the work that you have submitted to us. The committee must be notified of any changes or amendments and serious or unexpected outcomes related to the study. You will be expected to submit a final report at the end of the study and may be requested to do a presentation of the same to the hospital.

This information will form part of the database that will be consulted in future when processing related research studies so as to minimize chances of study duplication.

Thank you for your interest in research in our institution.



For DR. AUSTIN S. AJEVI
CHAIRMAN
ETHICS AND RESEARCH COMMITTEE
CGH - KAKAMEGA

CC
The Medical Superintendent
CGH - KAKAMEGA

Appendix VII: Culture plate characteristics of various isolates recovered from surgical wound infections.

		
Plate 1: Greenish pigmentation of *Pseudomonas aeruginosa* on nutrient agar.	**Plate 2:** Pink to red colonies of *Klebsiellae* on MacConkey agar.	**Plate 3:** Yellow colonies of *Staphylococcus aureus* on mannitol salt agar.

The table shows some phenotypic characteristics of the identified isolates recovered from the surgical site infections of patients. Biochemical confirmatory tests were used to confirm these observed characteristics for each isolate.

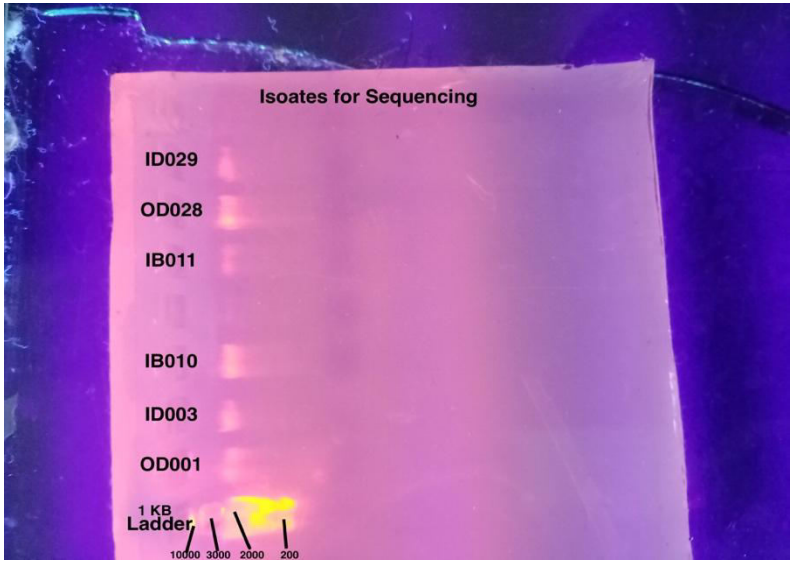
Appendix VIII: Biochemical and Gram stain tests

Isolate ID	**Biochemical Tests**					**Gram Stain**	**Inference**
	Simmons Citrate Test	Triple Sugar Iron	Coagulase Test	Motility Indole Lysin	Catalase		
S. aureus ATCC 25923	+	-	+	-	+	+	*S. aureus*
Pseudomonas aeruginosa strain ATCC 27853	+	-	-	-	+	-	*P. aeruginosa*
E. coli ATCC 25922	-	-	N/A	+	+	-	*E. coli*
Klebsiella spp ATCC-BAA 1706.	+	A/A	-	-	+	-	*Klebsiella spp*
OD 001	+	-	+	-	+	+	*S. aureus* confirmed
OD 002	+	-	+	-	+	+	*S. aureus* confirmed
ID 003	+	-	+	-	+	+	*S. aureus* confirmed
ID 004	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 005	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 006	+	-	-	-	+	-	*P. aeruginosa* confirmed
IB 007	-	-	N/A	+	+	-	*E. coli* confirmed
ID 008	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 009	+	-	+	-	+	+	*S. aureus* confirmed
IB 010	+	-	+	-	+	+	*S. aureus* confirmed
IB 011	+	-	+	-	+	+	*S. aureus* confirmed
ID 012	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 013	+	-	-	-	+	-	*P. aeruginosa* confirmed

ID 014	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 015	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 016	+	-	+	-	+	+	*S. aureus* confirmed
ID 017	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 018	+	-	+	-	+	+	*S. aureus* confirmed
ID 019	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 020	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 021	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 022	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 023	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
OD 024	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 025	+	-	+	-	+	+	*S. aureus* confirmed
ID 026	+	-	+	-	+	+	*S. aureus* confirmed
ID 027	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 028	+	-	+	-	+	+	*S. aureus* confirmed
ID 029	+	-	+	-	+	+	*S. aureus* confirmed
OD 030	-	-	N/A	+	+	-	*E. coli* confirmed
ID 031	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 032	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 033	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 034	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 035	+	-	+	-	+	+	*S. aureus* confirmed
OD 036	+	-	+	-	+	+	*S. aureus* confirmed
ID 037	+	-	+	-	+	+	*S. aureus* confirmed
OD 038	-	-	N/A	+	+	-	*E. coli* confirmed
ID 039	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 040	+	-	+	-	+	+	*S. aureus* confirmed
ID 041	+	-	+	-	+	+	*S. aureus* confirmed
ID 042	+	-	+	-	+	+	*S. aureus* confirmed
ID 043	+	-	+	-	+	+	*S. aureus* confirmed
ID 044	+	-	+	-	+	+	*S. aureus* confirmed

ID 045	+	-	+	-	+	+	*S. aureus* confirmed
ID 046	-	-	N/A	+	+	-	*E. coli* confirmed
ID 047	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 048	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 049	+	A/A	-	-	+	-	*Klebsiella spp* confirmed
ID 050	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 051	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 052	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 053	+	-	+	-	+	+	*S. aureus* confirmed
ID 054	-	-	N/A	+	+	-	*E. coli* confirmed
ID 055	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 056	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 057	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 058	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 059	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 060	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 061	+	-	+	-	+	+	*S. aureus* confirmed
ID 062	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 063	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 064	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 065	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 066	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 067	+	-	-	-	+	-	*P. aeruginosa* confirmed
ID 068	+	-	-	-	+	-	*P. aeruginosa* confirmed
OD 069	+	-	+	-	+	+	*S. aureus* confirmed
ID 070	+	-	-	-	+	-	*P. aeruginosa* confirmed

Appendix IX. Gel Electrophoresis



Agarose gel electrophoresis (1% agarose, Thermo Scientific, USA) showing genomic DNA isolated from *Staphylococcus aureus* clinical samples. The distinct high-molecular-weight bands indicate successful extraction of intact DNA with adequate quantity and quality for downstream molecular analysis. The ladder used was 1kb.

Appendix X: Sequence annotation summary of the 6 selected isolates.

Isolate ID	Replicon	Length (bp)	CDS	NCBI Accession	CheckM parameters	
					Completeness	Contamination
OD001	Chromosome	2,479,797	2,809	CP121234	95%	0.04%
	Plasmid	20,767	23	CP121235	NA	NA
ID003	Chromosome	2,525,702	2,608	CP118688	98%	0.08%
	Plasmid	20807	23	CP118689	NA	NA
IB011	Chromosome	2,398,889	2,439	CP121238	97%	0%
	Plasmid	20785	23	CP121239	NA	NA
OD028	Chromosome	2,473,420	2,632	CP121240	95%	0.08%
	Plasmid	20808	24	CP121241	NA	NA
ID029	Chromosome	2,652,936	2,860	CP121242	96%	0.12%
	Plasmid_1	14849	19	CP121243	NA	NA
	Plasmid_2	10162	13	CP121244	NA	NA
IB010	Chromosome	2,490,038	2,540	CP121236	98%	0.99%
	Plasmid	20814	23	CP121237	NA	NA

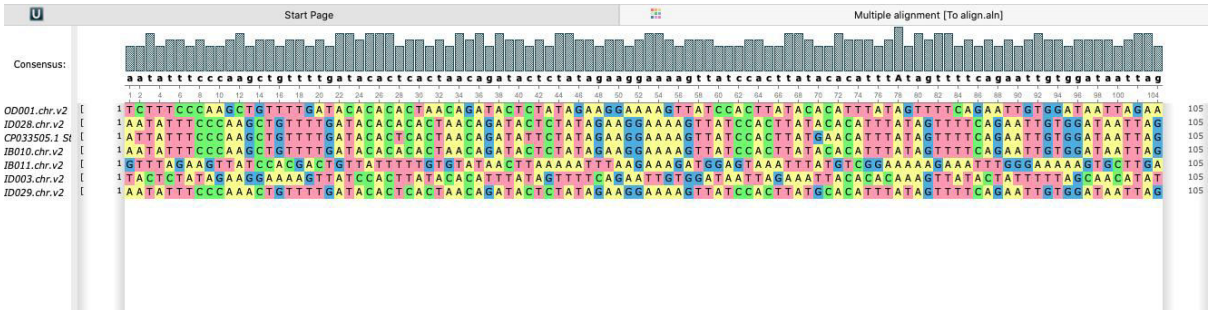
KEY: Chr- Chromosome, CDS: Total number of genes, Pls: Plasmid, NCBI: National Centre for Biotechnology Information.

The table shows sequence annotation summary with chromosome and plasmid annotation of predicted genes *versus* the actual number of protein-coding genes. The biggest chromosome was observed in ID029 isolate (2,652,936 bp) also bearing biggest number of genes (CDS)

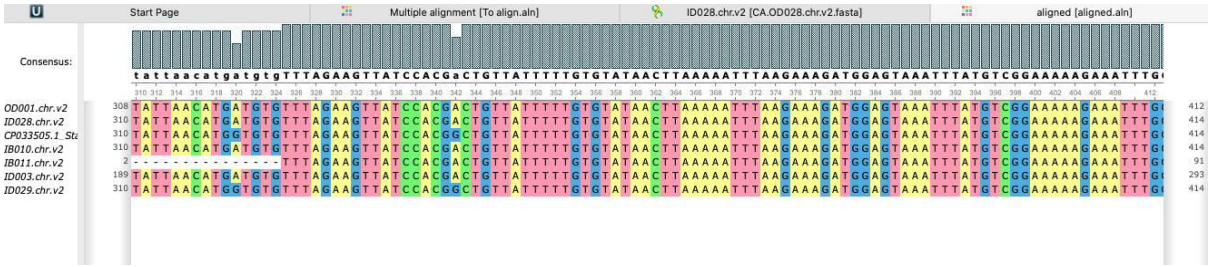
(2,860), while the smallest was IB011 isolate (2,398,889bp). The largest plasmid was carried in IB010 isolate (20814 bp) with biggest number of genes (23), as the smallest was in plasmid 2 of ID029 (10162 bp) which had 13 genes. NCBI accession numbers are both for respective chromosomes and plasmids.

Appendix XI: Sequence alignment chart

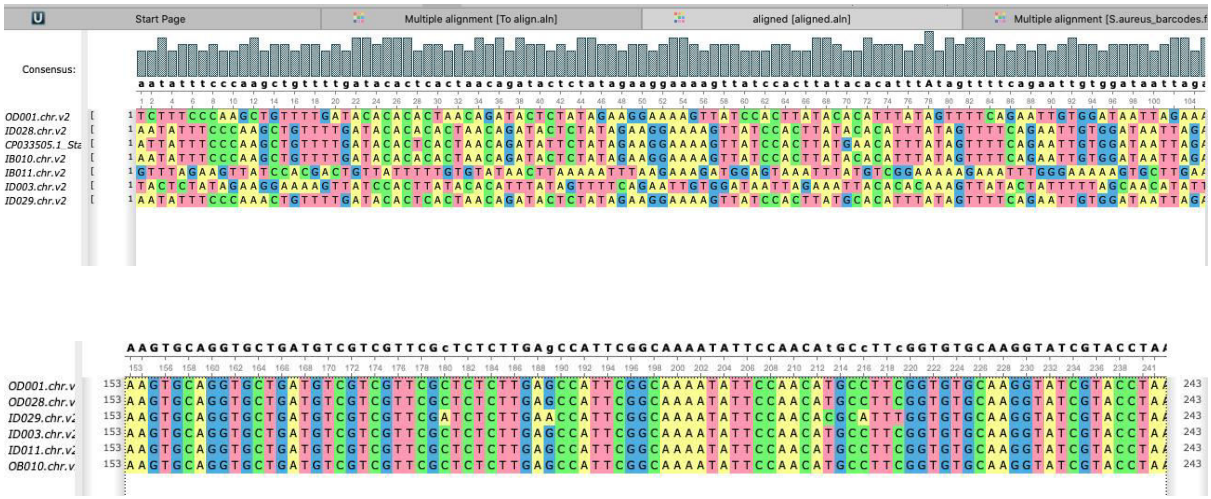
a. Study sequences before alignment



b. Aligned sequences with gaps



c. Aligned sequences with gaps removed to distinguish among the expected and unexpected variability for each aligned sequence.



Appendix XII: Genes covered by genetic islands of *Staphylococcus aureus*

Strain	GI	Location	CDS
***S. aureus* ATCC BAA-39 [CP033505]**			
	GI #1	54000..68099	MaoC domain protein, rev[54251..54680]
			Glycerophosphoryl diester phosphodiesterase, rev[54776..55520]
			Hydroxymethylglutaryl-CoA synthase, rev[56275..56698]
			Replication initiation protein, topoisomerase, rev[56714..57515]
			plasmid recombination enzyme type 3, rev[58156..59398]
			Tetracycline resistance, MFS efflux pump -> Tet(K), rev[59583..60963]
			hypothetical protein, dir[61202..61616]
			Organomercurial lyase, rev[61810..62461]
			Mercuric ion reductase, rev[62542..64186]
			hypothetical protein, rev[64243..64582]
			hypothetical protein, rev[64755..65397]
			hypothetical protein, rev[65426..65747]
			Transcriptional regulator, MerR family, rev[65763..66171]
			pyridine nucleotide-disulfide oxidoreductase, rev[66470..67817]
	GI #2	438000..452099	Exotoxin 15, dir[439967..440669]
			Hypothetical protein, dir[440690..442193]
			hypothetical protein, rev[442300..442609]
	GI #3	598000..608099	adhesin SdrD, dir[601018..604729]
	GI #5	1918100..1930099	Epoxyqueuosine reductase, rev[1918385..1919513]
			Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1919664..1920393]
			ABC transporter/substrate-binding protein/ABC transporter,permease protein, rev[1920379..1921570]
			Mobile element protein, dir[1922115..1923435]
			Membrane protein, rev[1923595..1924657]
	GI #6	2018100..2030099	Phage lysin, N-acetylmuramoyl-L-alanine amidase, rev[2018430..2018727]
			Staphylokinase, rev[2019417..2019909]
			Phage lysin, N-acetylmuramoyl-L-alanine amidase, rev[2020099..2020855]
			Phage holin, rev[2020866..2021121]
			Phage protein, rev[2021651..2022026]
			Phage protein, rev[2022081..2022369]
			Structural protein, phage associated, rev[2022559..2026342]
			Phage protein, rev[2026357..2027848]
	GI #7	2042000..2050099	Phage protein, rev[2044050..2044257]
			Dimeric dUTPase, rev[2044293..2044830]
			Phage protein, rev[2044822..2045071]
			hypothetical protein, rev[2045063..2045348]
			hypothetical protein, rev[2045344..2045794]
			Phage protein, rev[2045790..2045985]
			hypothetical protein, rev[2045981..2046368]
			Phage protein, rev[2046381..2046624]
			Phage protein, rev[2046627..2046996]
			Phage Holliday junction resolvase, rev[2047008..2047413]
			Phage protein, rev[2047421..2047640]

			Phage protein, rev[2047646..2048540] Single-stranded DNA-binding protein, phage associated, rev[2048569..2049040] Metallo-beta-lactamase superfamily domain protein in prophage, rev[2049040..2049526]
	GI #8	2210000..2226099	FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[2211318..2216919] Phosphoglucosamine mutase / FemD, factor involved in methicillin resistance, rev[2217196..2218552] putative secreted protein, rev[2218578..2219511] Diadenylate cyclase spyDAC, Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[2219512..2220322] Arginase, rev[2220510..2221419] Mobile element protein, rev[2221570..2222890]
	GI #10	2540000..2556099	Virulence-associated cell-wall-anchored protein SasG, rev[2541229..2545345] Transcriptional regulator SarT, rev[2545649..2546006] Transcriptional regulator SarU (accessory regulator U), dir[2546329..2547073] UTP--glucose-1-phosphate uridylyltransferase, rev[2547283..2547769] Fibronectin binding protein FnbB, rev[2548328..2551193] Fibronectin binding protein FnbA, rev[2551873..2554963]
ID029			
	GI #2	544000..552099	Adhesin of unknown specificity SdrC, dir[544008..545769] Adhesin of unknown specificity SdrC, dir[545765..546677] Adhesin of unknown specificity SdrD, dir[547064..548219] Adhesin of unknown specificity SdrD, dir[548206..550165] Adhesin of unknown specificity SdrE, similar to bone sialoprotein-binding protein Bbp, dir[550196..550682]
	GI #4	1814100..1826099	Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1814802..1815531] ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1815517..1816411] ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1816512..1816710] Mobile element protein, dir[1817241..1818561] Membrane protein, rev[1818714..1819776]
	GI #5	1914000..1924099	Staphylokinase, rev[1914609..1915101] Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1915291..1916047] Phage holin, rev[1916058..1916313] Phage protein, rev[1916524..1916659] hypothetical protein, rev[1916693..1916831] Structural protein, phage associated, rev[1916845..1919593] Phage protein, rev[1919608..1920580] Phage tail length tape-measure protein T, rev[1920803..1921364] Phage protein, rev[1921466..1921670]

		Hypothetical protein, SAV0881 homolog [SA bacteriophages 11, Mu50B], rev[1921710..1921890] Hypothetical protein, SAV0877 homolog [SA bacteriophages 11, Mu50B], rev[1921886..1922093] Hypothetical protein, PVL orf52 homolog [SA bacteriophages 11, Mu50B], rev[1922089..1922392] Hypothetical protein, SAB1734c homolog [SA bacteriophages 11, Mu50B], rev[1922384..1922567] hypothetical protein, rev[1922670..1922811] Hypothetical protein, PVL orf51 homolog [SA bacteriophages 11, Mu50B], rev[1922824..1923067] Phage Holliday junction resolvase, rev[1923084..1923489] Phage protein, rev[1923497..1923635]
GI #6	2074000..2092099	FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[2075284..2077102] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[2077104..2078829] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[2078971..2080714] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[2080809..2082036] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[2082242..2082737] Phosphoglucosamine mutase (EC 5.4.2.10) @ FemD, factor involved in methicillin resistance, rev[2083013..2084369] Uncharacterized secreted protein associated with spyDAC, rev[2084395..2085328] Diadenylate cyclase spyDAC Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[2085329..2086139] Arginase (EC 3.5.3.1), rev[2086327..2087236] Mobile element protein, rev[2087387..2088695]
GI #8	2404000..2414099	Virulence-associated cell-wall-anchored protein SasG (LPXTG motif), binding to squamous nasal epithelial cells, rev[2405270..2409386] Transcriptional regulator SarT (Staphylococcal accessory regulator T), rev[2409690..2410047] Transcriptional regulator SarU (Staphylococcal accessory regulator U), dir[2410370..2411114] UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9), rev[2411384..2411837] UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9), rev[2411944..2412250] Fibronectin binding protein FnbA, rev[2412429..2412897] Fibronectin binding protein FnbA, rev[2412906..2413035] Fibronectin binding protein FnbB, rev[2413264..2413774] Fibronectin binding protein FnbB, rev[2413880..2414072]
***S. aureus* ID003**		
GI #1	506000..516099	Deoxyguanosine kinase (EC 2.7.1.113), rev[506399..506912] tRNA-specific adenosine-34 deaminase (EC 3.5.4.33), dir[506978..507449] Hydrolase, HAD superfamily, dir[507595..508465] Predicted flavoprotein, dir[508485..509052]

		Transcription termination factor Rho, dir[509595..510111]
		Adhesin of unknown specificity SdrD, dir[510262..511402]
		Adhesin of unknown specificity SdrD, dir[511401..513558]
		Adhesin of unknown specificity SdrC, dir[513554..513971]
GI #3	1734100..1746099	tRNA (cytidine(34)-2'-O)-methyltransferase (EC 2.1.1.207), rev[1734780..1735239]
		Epoxyqueuosine reductase (EC 1.17.99.6) QueG, rev[1735243..1736371]
		Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1736522..1737251]
		ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1737237..1738428]
		Membrane protein, rev[1739034..1740096]
GI #4	1828100..1838099	Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1828759..1829056]
		Staphylokinase, rev[1829746..1830238]
		Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1830428..1831184]
		Phage holin, rev[1831195..1831450]
		Phage protein, rev[1831661..1831796]
		Phage protein, rev[1831912..1832074]
		Phage minor structural protein (ACLAME 95), rev[1832192..1832378]
		Structural protein, phage associated, rev[1832559..1834950]
		Phage tail length tape-measure protein T, rev[1834965..1836738]
		Phage tail length tape-measure protein T, rev[1836754..1836958]
		Phage protein, rev[1836980..1837181]
		Hypothetical protein, SAV0881 homolog [SA bacteriophages 11, Mu50B], rev[1837240..1837390]
		Hypothetical protein, SAV0877 homolog [SA bacteriophages 11, Mu50B], rev[1837386..1837593]
GI #5	1990100..2000099	FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1990260..1991229]
		FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1991188..1991422]
		FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1991429..1991903]
		FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1991899..1993192]
		Phosphoglucosamine mutase (EC 5.4.2.10) @ FemD, factor involved in methicillin resistance, rev[1993469..1994825]
		Uncharacterized secreted protein associated with spyDAC, rev[1994851..1995784]
		Diadenylate cyclase spyDAC
		Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[1995785..1996595]
		Arginase (EC 3.5.3.1), rev[1996783..1997692]
GI #7	2296000..2306099	hypothetical protein, dir[2297262..2297529]
		hypothetical protein, dir[2297672..2297981]
		UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9),

		rev[2298042..2298495] UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9), rev[2298602..2298908] Fibronectin binding protein FnbA, rev[2299087..2299297] Fibronectin binding protein FnbB, rev[2299278..2301243] Fibronectin binding protein FnbB, rev[2301245..2301743] FIG01108157: hypothetical protein, rev[2301774..2302026] Fibronectin binding protein FnbB, rev[2302423..2302633] hypothetical protein, dir[2302661..2302922] Fibronectin binding protein FnbA, rev[2303096..2303927] Fibronectin binding protein FnbA, rev[2303889..2304468] hypothetical protein, rev[2304602..2304803]
***S. aureus* IB010**		
GI #3	1696100..1708099	Hypothetical protein SAV1852, rev[1696799..1697195] Hypothetical protein SAV1853, rev[1697700..1697850] Hypothetical protein SAV1854, rev[1697874..1698474] tRNA (cytidine(34)-2'-O)-methyltransferase (EC 2.1.1.207), rev[1698521..1698953] Epoxyqueuosine reductase (EC 1.17.99.6) QueG, rev[1698957..1700085] Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1700229..1700958] ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1700944..1702135] Membrane protein, rev[1702471..1703533]
GI #4	1796000..1802099	Phage tail length tape-measure protein T, rev[1796704..1798474] Phage tail length tape-measure protein T, rev[1798490..1798673] Phage protein, rev[1798696..1798897] Hypothetical protein, SAV0881 homolog [SA bacteriophages 11, Mu50B], rev[1798896..1799052] Hypothetical protein, SAV0877 homolog [SA bacteriophages 11, Mu50B], rev[1799048..1799255] Dimeric dUTPase (EC 3.6.1.23), rev[1799291..1799828] Hypothetical protein, SAB1734c homolog [SA bacteriophages 11, Mu50B], rev[1799820..1800033] Hypothetical protein, Lmo2313 homolog [Bacteriophage A118], rev[1800029..1800479] Hypothetical protein, PVL orf51 homolog [SA bacteriophages 11, Mu50B], rev[1800549..1800792] Hypothetical protein, PVL orf50 homolog [SA bacteriophages 11, Mu50B], rev[1800795..1801164] Phage Holliday junction resolvase, rev[1801176..1801581] Phage protein, rev[1801589..1801808]
GI #5	1962100..1974099	FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1963506..1964454] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1964461..1964674] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1964946..1965948] FmtB (Mrp) protein involved in methicillin resistance and

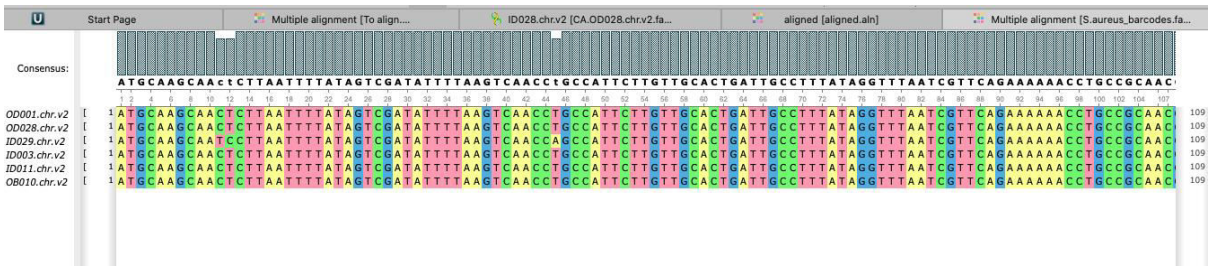
		cell wall biosynthesis, rev[1966009..1966141] Phosphoglucosamine mutase (EC 5.4.2.10) @ FemD, factor involved in methicillin resistance, rev[1966335..1967691] Uncharacterized secreted protein associated with spyDAC, rev[1967717..1968650] Diadenylate cyclase spyDAC Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[1968651..1969461] Arginase (EC 3.5.3.1), rev[1969649..1970558]
***S. aureus* IB011**		
GI #1	478000..488099	tRNA-specific adenosine-34 deaminase (EC 3.5.4.33), dir[478041..478512] Hydrolase, HAD superfamily, dir[478658..479519] Predicted flavoprotein, dir[479534..480101] Antiadhesin Pls, binding to squamous nasal epithelial cells, dir[480417..480870] Adhesin of unknown specificity SdrD, dir[480998..482144] Adhesin of unknown specificity SdrD, dir[482143..484045] Adhesin of unknown specificity SdrE, similar to bone sialoprotein-binding protein Bbp, dir[484349..487700]
GI #3	1636100..1646099	Hypothetical protein SAV1853, rev[1636543..1636693] Hypothetical protein SAV1854, rev[1636717..1637317] tRNA (cytidine(34)-2'-O)-methyltransferase (EC 2.1.1.207), rev[1637364..1637670] Epoxyqueuosine reductase (EC 1.17.99.6) QueG, rev[1637674..1638814] Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1638910..1639639] ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1639625..1640900] Membrane protein, rev[1640985..1642047]
GI #4	1724100..1736099	Aspartate aminotransferase (EC 2.6.1.1), dir[1724114..1725383] Beta-hemolysin, dir[1725417..1725618] Phage protein, dir[1726547..1726739] Involved in expression of fibrinogen binding protein, phage associated, rev[1726791..1727142] Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1727594..1727891] Staphylokinase, rev[1728346..1728838] Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1729028..1729784] Phage holin, rev[1729795..1730050] Phage protein, rev[1730261..1730396] Structural protein, phage associated, rev[1730430..1733244] Phage protein, rev[1733259..1734429] Phage tail length tape-measure protein T, rev[1734419..1735010] Hypothetical protein, SAV0881 homolog [SA bacteriophages 11, Mu50B], rev[1735228..1735378] Hypothetical protein, SAV0877 homolog [SA bacteriophages 11, Mu50B], rev[1735374..1735581]

GI #5	1882100..1892099	FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1882643..1883243] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1883374..1884886] Phosphoglucosamine mutase (EC 5.4.2.10) @ FemD, factor involved in methicillin resistance, rev[1885108..1886464] Uncharacterized secreted protein associated with spyDAC, rev[1886490..1887423] Diadenylate cyclase spyDAC Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[1887424..1888234] Arginase (EC 3.5.3.1), rev[1888422..1889331]
***S. aureus* OD001**		
GI #2	500000..510099	tRNA-specific adenosine-34 deaminase (EC 3.5.4.33), dir[500269..500740] Hydrolase, HAD superfamily, dir[500886..501759] Predicted flavoprotein, dir[501777..502344] Adhesin of unknown specificity SdrD, dir[502726..503872] Adhesin of unknown specificity SdrD, dir[503855..505829] Adhesin of unknown specificity SdrD, dir[506176..506680] Adhesin of unknown specificity SdrE, similar to bone sialoprotein-binding protein Bbp, dir[507072..510099]
GI #4	1700100..1712099	Hypothetical protein SAV1853, rev[1700906..1701056] Hypothetical protein SAV1854, rev[1701080..1701680] tRNA (cytidine(34)-2'-O)-methyltransferase (EC 2.1.1.207), rev[1701808..1702261] Epoxyqueuosine reductase (EC 1.17.99.6) QueG, rev[1702265..1703393] Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1703544..1704273] ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1704259..1705450] Membrane protein, rev[1706005..1707067]
GI #5	1800000..1806099	Phage protein, rev[1800904..1801876] Phage tail length tape-measure protein T, rev[1802033..1802579] Phage protein, rev[1802592..1802811] Hypothetical protein, SAV0881 homolog [SA bacteriophages 11, Mu50B], rev[1802865..1803015] Hypothetical protein, SAV0877 homolog [SA bacteriophages 11, Mu50B], rev[1803011..1803218] Dimeric dUTPase (EC 3.6.1.23), rev[1803254..1803653] Hypothetical protein, PVL orf52 homolog [SA bacteriophages 11, Mu50B], rev[1803775..1804027] Hypothetical protein, Lmo2313 homolog [Bacteriophage A118], rev[1804297..1804747] Hypothetical protein, PVL orf51 homolog [SA bacteriophages 11, Mu50B], rev[1804847..1805090] Hypothetical protein, PVL orf50 homolog [SA bacteriophages 11, Mu50B], rev[1805093..1805462] Phage Holliday junction resolvase, rev[1805474..1805879]
GI #6	1952100..1964099	Mannitol-1-phosphate 5-dehydrogenase (EC 1.1.1.17),

		dir[1952400..1953507] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1953825..1954038] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1954051..1954423] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1954554..1954830] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1954978..1955842] FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1955919..1956084] Phosphoglucosamine mutase (EC 5.4.2.10) @ FemD, factor involved in methicillin resistance, rev[1956360..1957716] Uncharacterized secreted protein associated with spyDAC, rev[1957742..1958675] Diadenylate cyclase spyDAC Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[1958676..1959486] Arginase (EC 3.5.3.1), rev[1959674..1960583]
***S. aureus* OD028**		
GI #2	492000..502099	Deoxyguanosine kinase (EC 2.7.1.113), rev[492188..492701] tRNA-specific adenosine-34 deaminase (EC 3.5.4.33), dir[492767..493238] Hydrolase, HAD superfamily, dir[493384..494224] Predicted flavoprotein, dir[494244..494811] Antiadhesin Pls, binding to squamous nasal epithelial cells, dir[495090..495582] Adhesin of unknown specificity SdrD, dir[495714..496857] Adhesin of unknown specificity SdrD, dir[496831..498838] Adhesin of unknown specificity SdrE, similar to bone sialoprotein-binding protein Bbp, dir[498942..499428]
GI #4	1694100..1706099	Hypothetical protein SAV1852, rev[1694484..1694880] Hypothetical protein SAV1853, rev[1695402..1695552] Hypothetical protein SAV1854, rev[1695576..1696176] tRNA (cytidine(34)-2'-O)-methyltransferase (EC 2.1.1.207), rev[1696299..1696764] Epoxyqueuosine reductase (EC 1.17.99.6) QueG, rev[1696768..1697896] Glutamine ABC transporter, ATP-binding protein GlnQ, rev[1698046..1698775] ABC transporter, substrate-binding protein (cluster 3, basic aa/glutamine/opines) / ABC transporter, permease protein (cluster 3, basic aa/glutamine/opines), rev[1698761..1699856] Membrane protein, rev[1700444..1701506]
GI #5	1788100..1798099	Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1788630..1788927] Staphylokinase, rev[1789617..1790109] Phage lysin, N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28), rev[1790299..1791055] Phage holin, rev[1791066..1791321] Phage protein, rev[1791532..1791667] Phage protein, rev[1791824..1791935]

		Structural protein, phage associated, rev[1792054..1794793] Phage protein, rev[1794808..1795780] Phage tail length tape-measure protein T, rev[1795956..1796517] Phage tail length tape-measure protein T, rev[1796510..1796714] Phage protein, rev[1796740..1796941] Hypothetical protein, SAV0881 homolog [SA bacteriophages 11, Mu50B], rev[1796995..1797145] Hypothetical protein, SAV0877 homolog [SA bacteriophages 11, Mu50B], rev[1797141..1797348] Dimeric dUTPase (EC 3.6.1.23), rev[1797384..1797921]
GI #6	1946100..1958099	FmtB (Mrp) protein involved in methicillin resistance and cell wall biosynthesis, rev[1946879..1949648] Phosphoglucosamine mutase (EC 5.4.2.10) @ FemD, factor involved in methicillin resistance, rev[1949924..1951280] Uncharacterized secreted protein associated with spyDAC, rev[1951306..1952239] Diadenylate cyclase spyDAC Bacterial checkpoint controller DisA with nucleotide-binding domain, rev[1952240..1953050] Arginase (EC 3.5.3.1), rev[1953238..1954147]

Appendix XIII. DNA barcoding alignment with UniproUGENE software of the six isolate sequences.



Appendix XIV: Multi-drug resistance chart for *S. aureus*

	CLM	PEN	OXA	GEN	CEF	TET	ERY	AMX	CFZ	CAZ	CXM	AMP	TMP	CRO	CEF	Level of Resistance
OD0001	S	R	R	S	S	R	I	R	R	R	R	R	S	R	R	10
OD0002	R	R	S	R	R	R	R	S	R	S	R	S	R	S	S	9
ID0003	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	15
ID0009	S	R	S	S	S	R	S	S	R	S	R	R	R	S	S	6
IB0111	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	15
ID0016	S	R	S	S	S	R	I	S	R	S	R	R	R	S	R	7
ID0018	I	R	S	S	S	S	I	R	R	R	R	R	S	S	R	7
ID0025	I	R	S	S	S	R	I	S	R	S	R	R	R	S	R	7
ID0026	S	R	S	S	S	S	S	R	R	S	R	R	R	S	S	6
OD0028	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	15
ID0	R	R	R	R	R	R	I	R	R	I	R	R	R	R	S	12

29																
ID035	I	R	S	S	R	R	I	S	R	S	R	R	R	S	S	7
OD036	R	R	S	R	R	R	R	R	S	S	S	R	S	S	S	8
ID037	S	S	S	S	S	S	S	R	R	R	R	R	S	S	R	6
ID040	R	R	S	R	R	R	R	R	R	S	R	R	R	S	R	12
ID041	R	R	S	R	R	S	R	S	R	S	R	R	S	S	S	11
ID042	S	R	S	R	R	R	R	R	R	S	R	R	R	S	R	11
ID043	I	R	S	R	S	R	R	R	R	S	R	R	S	S	S	8
ID044	R	R	S	R	R	R	R	R	R	S	R	R	S	S	R	11
ID045	R	R	S	R	R	R	R	S	R	R	R	R	R	S	S	11
OD053	S	S	S	S	S	S	S	R	R	R	R	R	S	S	R	6
OD061	S	S	S	S	S	S	S	R	R	S	R	R	R	S	S	5
IB	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	14

010																
OD069	I	R	S	R	S	R	R	R	R	S	R	R	S	S	S	8
ID070	S	S	R	S	R	S	S	R	R	S	R	R	R	R	R	9

*R- Resistant, S- Sensitive, I- Intermediate

Ampicillin (AMP), Amoxicillin (AMX), Ceftazidime (CAZ), Cephalothin (CEF), Cefalexin (CFX Cephazolin), (CFZ), Clindamycin (CLM), Ceftriaxone (CRO), Cefuroxime (CXM), Erythromycin (ERY)**,** Gentamicin (GEN), Oxacillin (OXA), Penicillin (PEN), Tetracycline (TET), Trimethopan (TMP), Trimethoprim (TMP)

Appendix XV. Chromosome encoded AMR genes

Gene	OD001	ID003	IB011	OD028	ID029	IB010
PC1 beta-lactamase (blaZ)	0	0	0	0	0	0
***S. aureus* LmrS**	1	1	1	1	1	1
***S. aureus* gyrA conferring resistance to fluoroquinolones**	0	0	0	0	2	0
***S. aureus* murA with mutation conferring resistance to fosfomycin**	1	1	1	1	0	1
***Staphylococcus aureus* norA**	0	0	0	0	1	0
***Staphylococcus aureus* parC conferring resistance to fluoroquinolones**	0	0	0	0	1	0
arlR	0	1	1	1	1	1
mepA	0	0	0	0	1	0
mepR	1	1	1	1	1	4
mgrA	0	1	1	0	0	1
norC	2	2	2	2	4	2
sdrM	1	1	1	1	1	1
sepA	1	1	1	1	1	1
vanT gene in vanG cluster	1	1	1	1	1	1
***Staphylococcus aureus* GlpT with mutation conferring resistance to fosfomycin**	0	0	0	0	0	3

Appendix XVI: Various drug resistant genes and the mechanisms they encode for both isolates of ST152 and ST8.

A. Various drug resistant genes and the mechanisms they encode for both isolates of ST152 and ST8					
NO.	**Drug Resistant Gene**	**Mechanism of action encoded**	**Resistance Against Common Drug Class**	**Genes Encoded in ST152 Isolates**	
				Hospital acquired (HA) (ID003, IB010, IB011)	**Community acquired (CA) (OD001, OD028)**
1	***S. aureus* LmrS**	antibiotic efflux	macrolide antibiotic; aminoglycoside antibiotic; oxazolidinone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic.	All	All
2	***S. aureus* murA with mutation conferring resistance to fosfomycin**	Antibiotic target alteration.	phosphonic acid antibiotic	All	All
3	**arlR**	antibiotic efflux	fluoroquinolone antibiotic; disinfecting agents ;antiseptics.	All	Only OD028
4	**mepR**	antibiotic efflux	glycylcycline; tetracycline antibiotic	All	All
5	**mgrA**	antibiotic efflux	cephalosporin; penam ;fluoroquinolone antibiotic;; peptide antibiotic tetracycline antibiotic;; disinfecting agents and antiseptics.	All	None
6	**norC**	antibiotic efflux	disinfecting agents and antiseptics fluoroquinolone antibiotic;	All	All
7	**sdrM**	antibiotic efflux	disinfecting agents and antiseptics. Fluoroquinolone antibiotic;	All	All

8	**sepA**	antibiotic efflux	disinfecting agents; antiseptics	All	All
9	**vanT mutational gene in vanG cluster**	antibiotic target alteration	glycopeptide antibiotic	All	All
10	***Staphylococcus aureus* GlpT with mutation conferring resistance to fosfomycin**	antibiotic target alteration	phosphonic acid antibiotic	Only IB010	None

B. Mutations in genes coding for antibiotic resistance

NO.	Drug Resistant Gene in ST8	Mechanism of action encoded	Resistance Against Common Drug Class
1	*mepR*	antibiotic efflux	glycylcycline; tetracycline antibiotic
2	*norC*	antibiotic efflux	disinfecting agents and antiseptics fluoroquinolone antibiotic;
3	*arlR*	antibiotic efflux	fluoroquinolone antibiotic; disinfecting agents ;antiseptics.
4	*sepA*	antibiotic efflux	disinfecting agents; antiseptics
5	*LmrS*	antibiotic efflux	macrolide antibiotic; aminoglycoside antibiotic; oxazolidinone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic.
6	*norA*	antibiotic efflux	fluoroquinolone based antibiotics
7	*mepA*	antibiotic efflux	glycylcycline; tetracycline antibiotic
8	*sdrM*	antibiotic efflux	disinfecting agents and antiseptics.fluoroquinolone antibiotic;