DETERMINATION OF BACTERIAL RESISTANCE PROFILE OF CHRONIC WOUND PATHOGENS AND GENETIC CHARACTERISATION OF *Staphylococcus aureus* FROM PATIENTS AT MERU TEACHING AND REFERRAL HOSPITAL, MERU COUNTY, KENYA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted for an award of diploma or conferment of degree in any institution or University.

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Recommendation

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DEDICATION

This work is dedicated to my Husband Alfred Murithi and our sons Judah and Ezra for their support and encouragement during the writing of this research

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ABSTRACT

Chronic wound infections remain a major public health concern, particularly in low- and middle-income countries, where they are often complicated by polymicrobial colonization and the emergence of multidrug-resistant (MDR) pathogens. Effective management is challenged by incomplete pathogen identification, which hampers accurate diagnosis and treatment. This study aimed to determine the prevalence, bacterial profile, and antimicrobial resistance patterns of pathogens associated with chronic wound infections at Meru Teaching and Referral Hospital (MeTRH) in Kenya, and to provide genomic insights into their resistance and virulence determinants. A cross-sectional survey design was employed, in which 68 wound swab samples were collected from a stratified random sample of 293 patients over eight months, following consent. Data were analyzed using SPSS for epidemiological trends, R for visualization, and bioinformatics pipelines for sequencing data processing, assembly, annotation, and phylogenetic reconstruction to characterize antimicrobial resistance and virulence profiles. The findings indicate that the highest burden of chronic wounds occurred among patients aged 21–30 years (22%) and 51–60 years (19.2%). The predominant pathogens were *Staphylococcus aureus* (26.6%), *Pseudomonas aeruginosa* (13.2%), and *Escherichia coli* (10.3%). Clinically significant MDR organisms included *Klebsiella pneumoniae*, *Proteus hauseri*, *Morganella morganii*, *Acinetobacter baumannii*, and *Enterococcus faecalis*, while 20.5% of wounds showed no bacterial growth despite clinical evidence of infection. Notably, 50% *Staphylococcus aureus* isolates exhibited resistance to multiple antibiotics. Distinct susceptibility patterns were observed for *Morganella morganii* and *Proteus* spp, whereas coagulase-negative staphylococci displayed variable meropenem resistance reaching up to 100% in some cases. *Klebsiella* and *Pseudomonas* spp. were largely sensitive to meropenem, although resistance ranged from 0% to 11%, depending on the species. Whole-genome sequencing revealed frequent resistance determinants, including MecA (60%), blaZ (60%), aadD/aphA-3 (60%), mrsA (70%), ermB (30%), ermC (10%), ermA (20%), rpob_H481N (20%), and grlA_S80F; GrA_S84L (20%), reflecting co-occurrence of beta-lactam, MLS, sulfonamide/trimethoprim, tetracycline, aminoglycoside and quinolone resistance genes. Virulence markers such as aureolysin and toxin/exoenzyme genes (edinB, hlgA/B/C, lukPV, sak, scn, splA/B/E) were identified, alongside diverse spa types (t355-centered clusters), mosaic SCCmec elements, and varied MLST lineages (MLST/spa) were observed across *Staphylococcus aureus*. Plasmid replicons detected included Rep5a (n = 5), Rep16 (n = 4), Rep7a, Rep10, Rep9a, Rep9b, RepUS70, Rep21, Rep13, and Rep15, suggesting circulation of MRSA and MRSA-like lineages with mobile genetic elements. Clonal complexes CC30, CC121, CC45, and MLST 152 reflected both community- and hospital-associated strains, indicating dynamic *Staphylococcus aureus* populations shaped by clonal expansion and horizontal gene transfer. The findings highlight the substantial antimicrobial resistance burden in chronic wound infections, the importance of integrating advanced molecular diagnostics with traditional surveillance, and the need for antibiotic stewardship programs. Moreover, the genomic diversity and virulence architecture revealed here highlight the necessity for localized surveillance strategies and virulence-informed risk stratification to guide infection control and therapeutic interventions.

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LIST OF ABBREVIATIONS AND ACRONYMS

AM:	Antimicrobial
AMR:	Antimicrobial Resistance
ANOVA:	Analysis of Variance
Arc:	Arginine Catabolic Mobile Element
ARG:	Antibiotic Resistance Genes
AroE:	Aromatic Amino Acid Synthesis Enzyme
AST card:	Antimicrobial Susceptibility Testing card
ATCC12600:	American Type Culture Collection12600
AWaRE:	Access Watch Reserve
CAGR:	Compound Annual Growth Rate
CA-MRSA:	Community-Acquired Methicillin-Resistant *Staphylococcus aureus*
CC**:**	Clonal Complex
CDC:	Center for Disease Control and Prevention
CLSI:	Clinical and Laboratory Standards Institute
CONs:	Coagulase-negative staphylococci
DNA:	Deoxyribonucleic Acid
dNTP:	Deoxynucleotide Triphosphate
ECM:	Extracellular Matrix
EDTA:	Ethylenediaminetetraacetic Acid
ESBLs:	Extended-Spectrum Beta-Lactamases
FGF:	Fibroblast Growth Factor
HA-MRSA:	Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus*
KEMRI:	Kenya Medical Research Institute
KMA:	K-mer Alignment
KNH:	Kenyatta National Hospital
MAFFT:	Multiple Alignment using Fast Fourier Transform
MAR index:	Multiple Antibiotic Resistance Index
mecA:	Methicillin Resistance Gene A
MeTRH:	Meru Teaching and Referral Hospital
MGE:	Mobile Genetic Element
MIC:	Minimum Inhibitory Concentrations
MLS:	Macrolide-Lincosamide-Streptogramin

MLSB: Macrolide-Lincosamide-Streptogramin B Resistance

MLST: Multilocus Sequence Typing

MMPs: Matrix Metalloproteinases

MRSA: Methicillin-Resistant *Staphylococcus aureus*

MSA: Multiple Sequence Alignment

MSCRAMMs: Microbial Surface Components Recognizing Adhesive Matrix Molecules

MSL: Macrolide-Streptogramin-Lincosamide

MSR (A): Macrolide-Streptogramin Resistance Protein A

MSSA: Methicillin-Susceptible *Staphylococcus aureus*

MUSCLE: Multiple Sequence Comparison by Log-Expectation

NACOSTI: National Commission for Science, Technology and Innovation

NTP: Nucleotide Triphosphate

PBP: Penicillin-Binding Protein

PBP2a: Penicillin-Binding Protein 2a

PDGF: Platelet-Derived Growth Factor

PVL: Panton-Valentine Leukocidin

SCCmec: Staphylococcal Cassette Chromosome mec

SSSS: Staphylococcal Scalded Skin Syndrome

TGF-β: Transforming Growth Factor

TIMPs: Tissue Inhibitors of Metalloproteinases

TMP: Trimethoprim

VanA: Vancomycin Resistance Gene A

VEGF: Vascular Endothelial Growth Factor

VITEK2AST: VITEK 2 Automated System for Antimicrobial Susceptibility Testing

WGS: Whole genome sequence

WHO: World Health Organization

XDR: Extensively Drug-Resistant

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

One of the global challenges in health sector is the spreading pandemic of antimicrobial resistance (AMR) (George *et a.,* 2022). Chronic wounds constitute a worldwide silent epidemic (Lindholm *et al.,* 2016). A detailed analysis of chronic wounds in the U.S., particularly focusing on Medicare patients, found that approximately 15% of Medicare beneficiaries are affected by chronic wounds. This results in an annual cost to the U.S. healthcare system ranging from $28 billion to $31 billion (Nussbaum et al., 2018). Additionally, venous leg ulcers, a specific type of chronic wound, impact between 500,000 to 600,000 individuals annually in the U.S., significantly contributing to the overall burden of chronic wounds (Rice *et al*., 2014).

Accordingly, the global wound care market is estimated at $18.7 billion by 2027, with a compound annual growth rate (CAGR) of 6.6% for the period 2020 – 2027 (Wangoye *et al.,* 2022). In Africa, wound infections are among the major causes of hospital visits, contributing to 30 – 42% of hospital attendance as well as 9% mortality rate every year, though most are underreported (Bizuayew *et al.,* 2021). Wound treatment failure burdens healthcare due to extended stay in hospital and massive antibiotic therapy (Beyene *et al.,* 2023). Wound management through antibiotics remains the most convenient way (O'Meara *et al.,* 2000). However, inappropriate use of these drugs has led to the development of multidrug-resistant bacteria that are continually evolving, leading to new mutants (Baym *et al*., 2016).

There are several causes of antimicrobial resistance, including selective pressure, mutation and gene transfer. To address this, several measures are suggested to curb AMR, which include infection control and prevention, surveillance, and the improvement of antimicrobial application in both humans and animals (O'Neill *et al.*, 2016). Regardless of the kind of wound, their infections are related to morbidity and mortality of patients. More often, antibiotics are administered in situations where prompt management is required, while balancing the need for targeted therapy once more specific information, such as the infecting pathogen and its susceptibility, becomes available. However, detecting microbial species colonizing a wound and their

antimicrobial susceptibility allows for targeted treatment and reduces the cost of therapy (Puca *et al*., 2021).

The therapeutic choices are impaired by an insufficient understanding of wound microbiology, drug resistance profiles, genetic diversity among clones and antimicrobial resistance genes (Shettigar *et al.,* 2020). In order to improve the antimicrobial use, the local data on pathogens causing chronic wounds and antimicrobial resistance profile need to be available to those prescribing antibiotics for informed decision on the selection of antimicrobials (Hamilton *et al.,* 2018). The most common bacteria that may cause wound infection comprise of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Acinetobacter baumannii/haemolyticus* and *Streptococcus* (Ding *et al.,* 2022).

The pathogenic bacteria causing chronic wounds differ significantly in antimicrobial resistance patterns within a geographic area (Guan *et al.,* 2021). Therefore, findings from one region may not be representative of global pathogen diversity. Such cases have led to over-generalization, which is a major source of drug misuse in both community and hospital set up, a factor causing antibiotic resistance (Prestinaci *et al.,* 2015). Regional establishment of bacterial pathogens causing chronic wound infections as well as their antimicrobial susceptibility patterns is vital in preventing the spread of antibiotic-resistant microbes (Thuo, 2021).

Globally, *Staphylococcus aureus* is a medically important bacterial pathogen causing both community and hospital-acquired infections, including chronic wounds (Tong.at el., 2015). *Staphylococcus aureus* has evolved as a global health threat due to its resistance to β-lactam antibiotics (Chua *et al*., 2014). *Staphylococcus aureus* acquires methicillin resistance by insertion of the staphylococcal cassette chromosome (SCCmec), carrying the *mecA* gene, into the chromosome. This gene encodes an altered penicillin-binding protein, PBP-2a, which is not inhibited by existing β-lactam antibiotics (Pournajaf *et al.,* 2014). Hospital-acquired *Staphylococcus aureus* infection is associated with hospitalization and lengthy exposure to health care environment (Loewen *et al.,* 2017). On the other hand, the predisposing factors associated with community *Staphylococcus aureus* infections are diverse and intimately allied to social

determinants of health like regular skin-to-skin contact, contact of the wound, and poor sanitation (Carnicer *et al.*, 2006).

The genetic diversity of *Staphylococcus aureus* is mainly characterized by the sequential emergence of epidemic strains (Silva *et al.*, 2020). Efficient surveillance measures have demonstrated a capacity to reduce the incidence of *Staphylococcus aureus* in certain geographic areas. However, *Staphylococcus aureus* remains a substantial clinical challenge, characterized by elevated rates of morbidity and mortality (Kourtis *et al.,* 2019). The integrated perspective on human, animal, and environmental health has significantly advanced our understanding of *Staphylococcus aureus* epidemiology, recognizing the transmission of community strains of *Staphylococcus aureus* between livestock and humans (Correia *et al.*, 2019) Transmission of *Staphylococcus aureus* between livestock and humans has been reported in Europe, Asia, Australia and America (Hassan *et al,* .2014).

The molecular epidemiology of *Staphylococcus aureus* is mainly characterized by the successive emergence of regionally predominant strain types. Penicillin-resistant phage type 80 or type 81 of *Staphylococcus aureus* surged between 1953 and 1963. After originating in hospitals, it spread to communities in North America, the United Kingdom and Australia before inexplicably receding again (DeLeo *et al.,* 2011). With the emergence of drug resistance in *Staphylococcus aureus* in the 1960s, hospital acquired *Staphylococcus aureus* began to affect hospitals in North America, the United Kingdom, Australia and Japan while spreading in the Scandinavian countries to a lesser extent. Sporadic reports of drug resistance *Staphylococcus aureus* occurring without prior health-care contact began to appear in the 1980s and 1990s, just before the widespread emergence of regionally dominant community acquired resistance in *Staphylococcus aureus* strains (Turner *et al*., 2019).

A study on the prevalence of resistant *Staphylococcus aureus* in Africa indicated relatively high rates of resistance in Nigeria, Kenya, and Cameroon (21-30%) compared to lower rates in Tunisia, Malta, and Algeria (below 10%) (Wangai *et al.*, 2019). In Kenya, local prevalence rates of *Staphylococcus aureus* at Kenyatta National Hospital have been increasing since 2003, when a rate of 27.7% was reported. Molecular gene

typing has shown a significant presence of epidemic clones in Kenyan public and private hospitals, with notable differences between public institutions like KNH and private hospitals in Nairobi (Omuse et al., 2016; Omuse *et al.*, 2014).

A genomic characterization study of two community-acquired *Staphylococcus aureus* strains in Kenya identified sequence types including STs 5, 8, 22, 30, 88, 152, 239, 241, 789, and 4705, belonging to four main clonal complexes (5, 8, 22, and 30), indicating evolution within these complexes (Njenga et al., 2022). In Western Kenya, a study found that 0.8% of thirty *Staphylococcus aureus* isolates from 29 inpatients were methicillin-resistant *Staphylococcus aureus* (MRSA), while 10.3% were methicillin-susceptible *Staphylococcus aureus* (MSSA). Additionally, 20% of isolates were multidrug-resistant, with high phenotypic and genotypic resistance to penicillin-G (96.8%), trimethoprim (73.3%), and tetracycline (13.3%). Virulence genes such as Panton-Valentine leukocidin (*pvl*), toxic shock syndrome toxin-1 (*tsst*-1), and the *sasX* gene were present in 16.7%, 23.3%, and 3.3% of isolates, respectively. Phylogenetic analysis identified four major clonal complexes: CC152, CC8, CC80, and CC508 (Benear et al., 2022). This study aims to establish the genetic diversity of resistant *Staphylococcus aureus* isolates, revealing circulating sequence types and antimicrobial resistance patterns that may have spread between hospital and community settings. Understanding prevalent resistance genes will provide a foundation for analyzing evolutionary processes within resistant *Staphylococcus aureus* associated with chronic wounds.

1.2 Statement of the Problem

The escalating prevalence of antimicrobial resistance (AMR) presents substantial challenges in the management of chronic wound-associated bacterial infections, constituting a critical yet underrecognized public health concern with profound implications for patient outcomes and socioeconomic burden on affected individuals and their families. Geographic variability in antimicrobial resistance profiles underscores the inadequacy and potential inaccuracy of current AMR surveillance data, thereby facilitating inappropriate antimicrobial prescribing practices across both healthcare and community settings. The paucity of region-specific epidemiological data pertaining to bacterial etiological agents of chronic wound infections, including

antimicrobial susceptibility patterns, genetic heterogeneity of *Staphylococcus aureus* isolates, resistance determinants, virulence factor-encoding genes, and clonal lineages circulating within healthcare facilities and community environments, substantially impedes the implementation of evidence-based therapeutic interventions, surveillance of emerging resistant strains, and the formulation of novel strategies for antimicrobial resistance mitigation.

1.3 Objectives of the Study

1.3.1 Broad Objective

To establish bacterial resistance profile of chronic wound-associated pathogens and the genetic characterization of *Staphylococcus aureus* isolated from patients at Meru Teaching and Referral Hospital (MeTRH), Meru County, Kenya.

1.3.2 Specific Objectives

i. To characterize bacterial pathogens associated with chronic wound infections at MeTRH.
ii. To determine the antibiotic resistance profiles of the pathogenic bacteria isolated from chronic wounds at MeTRH.
iii. To identify antibiotic resistance determinants and virulence factors in *Staphylococcus aureus* isolates from chronic wounds at MeTRH
iv. To characterize genetic diversity of resistant *Staphylococcus aureus* isolates from chronic wounds at MeTRH
v. To determine the clonal complexes of selected *Staphylococcus aureus* isolates using multilocus sequence typing

1.4 Research Questions

i. What are the common pathogens found in chronic wounds among patients visiting MeTRH?
ii. What are the antibiotic resistance profiles of the pathogenic bacteria isolated from chronic wounds at MeTRH?
iii. What are the antibiotic resistance determinants and virulence factor encoding genes in *Staphylococcus aureus* isolates from chronic wounds at MeTRH?

iv. What is the extent of genetic diversity among *Staphylococcus aureus* isolates from chronic wounds at MeTRH?

v. What clonal complexes of *Staphylococcus aureus* isolates from chronic wounds are circulating within the population visiting MeTRI

1.5 Justification

Chronic wounds substantially affect overall quality of life and pose significant clinical, social, and economic challenges within healthcare systems. Despite ongoing efforts to raise awareness and enhance knowledge about antimicrobial resistance (AMR), there remains a paucity of local data to monitor the emergence and distribution of AMR across different geographical regions (Jones et al., 2018)

Understanding the diverse bacteria causing chronic wounds infection is crucial in comprehending their determinant on healing outcomes and preventing the spread of antibiotic-resistant bacteria causing chronic wound infections (Awour *et al*., 2023). Identifying antibiotic resistance genes within bacterial species like *Staphylococcus aureus* is imperative for evaluating disease development and understanding the correlation of the pathogens to antimicrobial resistance patterns (Awour *et al.,* 2023) The persistence and transmission of resistant *Staphylococcus aureus* in healthcare especially in chronic wound infections, despite the advancements in infection prevention and new antibiotics, underline the need for continuous surveillance and understanding of its epidemiology (Nik *et al*., 2023). Furthermore, monitoring developing trends of antimicrobial resistance and the genetic diversity of bacterial profiles is essential in making informed clinical decisions and addressing the challenges associated with chronic wound infections (Leonid *et al*., 2023). This study aims to provide essential data for guiding empirical treatment, management, and surveillance of antimicrobial resistance in both hospital and community settings through investigating the pathogenic bacteria and their susceptibility patterns and genetic diversity of *Staphylococcus aureus* isolated from chronic wound infections in Meru Teaching and Referral Hospital, Meru County, Kenya. This will enrich existing information to improve diagnosis and antimicrobial stewardship, offering locally specific, genomics-informed insights into chronic wound infections in Meru, Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Pathophysiological and Clinical Aspects of Chronic Wounds

A chronic wound is a wound that does not heal in an orderly set of stages (Hemostasis, Inflammation, Proliferation and Maturation) and in a predictable amount of time or one that does not heal within three months (Iqbal *et al.,* 2017). Chronic wounds often remain in the inflammatory stage for too long and may never heal or may take years (Bjarnsholt *et al.,* 2008). Undoubtedly, the incidence of pathogens in wound sites delays wound healing and might even incite septicemia (Puca *et al*., 2021).

In most instances, closed wounds resulting from blunt trauma are characterized by an intact external surface; however, subcutaneous bleeding and damage to underlying tissues—including muscle, internal organs, and bone—may still occur (Dogrul et al., 2020). Chronic wounds related to such trauma can extend beyond superficial skin layers, potentially involving the epidermis and dermis and, in more severe cases, reaching the fascia and deeper connective structures (Falanga et al., 2022). Most often, the colonization of the wound is polymicrobial, comprising of numerous microorganisms that are potentially pathogenic; any type of wound is at some risk of becoming infected. In the state where the infection fails to heal, the patient suffers increased trauma, there is rise on the treatment cost and basically the general wound management becomes more resource demanding. Wounds and other disruptions of the epithelial cells create a warm, wet, and nutrient-rich milieu that encourages growth and colonization of pathogenic agents (Leoni et al., 2015). Burn, surgical site, and traumatic wounds are the top common wounds that are prone to infection (Ding et al, 2022). with *Escherichia coli, Staphylococcus aureus, Klebsiella* spp., *Pseudomonas aeruginosa,* and *Acinetobacter* species being isolated as the most common bacterial strains that colonize wounds. Other microorganisms such as fungi, parasites, and viruses are also known to infect wounds (Qin *et al*., 2022).

2.1.1 Chronic Wound Infections and Associated Microbial Agents

Persistent microbial infections often aggravate chronic wounds by impeding healing and increasing the risk of systemic consequences. The fact that these wounds include polymicrobial communities rather than single-organism colonization is a major theme

in current knowledge. These interactions lead to the creation of biofilms, modified host responses, and chronic inflammation (Diban *et al*., 2023). Environmental and local wound circumstances, including tissue hypoxia, necrosis, and shear stresses, as well as host variables, including diabetes, vascular insufficiency, and immunosenescence, influence the shift from colonization to infection (Guo and Dipietro, 2010).

Pseudomonas aeruginosa and *Staphylococcus aureus* usually dominate bacterial populations in chronic wounds, with facultative anaerobes and anaerobic species co-occurring often. *Pseudomonas aeruginosa* aids in the formation of biofilms and the synthesis of virulence factors that prevent antibiotic penetration and epithelialization, whereas *Staphylococcus aureus,* including methicillin-resistant strains in many contexts is linked to elevated inflammation, protease activity, and tissue damage (Archer *et al*., 2011). Furthermore, anaerobes and Gram-negative Enterobacterales, including Bacteroides and Peptostreptococcus species, are frequently found in chronic wounds and are linked to patterns of resistance and synergistic biofilm resilience (Durand *et al.,* 2022).

Biofilms are a unifying concept in chronic wound microbiology. Microbial cells in biofilms are protected from host defenses and antimicrobials by an extracellular polymeric substance that they create on their own. This results in inadequate antibiotic responses and chronic inflammation. Even when planktonic bacteria seem susceptible in vitro, the biofilm mode of growth can maintain infection by changing gene expression, enhancing horizontal gene transfer, and fostering a cooperative metabolism (Sharma et al., 2023). The need for wound-bed debridement, moisture and maceration control, and therapies that target biofilm disruption in conjunction with systemic or local antimicrobials is thus highlighted by the fact that treatment strategies that do not address biofilms frequently result in disappointing clinical outcomes (Cavallo *et al.,* 2024).

Management is made more difficult by antimicrobial resistance. The advent of resistant Gram-negative bacteria and multidrug-resistant organisms, such as MRSA, limits the range of available treatments and calls for stewardship-informed regimens. Culture-guided therapy and local antibiograms are still crucial, with a focus on maximizing drug

distribution to the wound bed and reducing systemic exposure wherever feasible. To increase effectiveness while lowering the pressure of resistance selection, combination treatments or topical antibiotics with anti-biofilm activity are sometimes used (Bari *et al.*, 2023).

Although they are less frequent than bacterial pathogens, viral and fungal contributors can have an impact on the ecology of chronic wounds. For instance, in hyperglycemic or immunocompromised conditions, Candida species can become enriched in the wound microbiome and may work in concert with bacteria to worsen tissue damage and inflammation. Similar findings have been made regarding the presence of herpesviridae and viral reactivation in some chronic wounds; these findings may have importance for pain management and patient quality of life, but their implications for healing dynamics are unclear. In certain situations, these organisms might need specific antifungal or antiviral treatment (Cohen *et al*., 2022).

The course of persistent wound infections is influenced by host-microbe interactions. A pro-inflammatory environment that postpones re-epithelialization and the creation of granulation tissue can be sustained by dysregulated inflammatory responses, compromised neutrophil activity, and altered macrophage polarization. Through host-pathogen interaction and intermicrobial transmission, emerging data support the significance of the wound microbiome as a regulator of healing, indicating that therapeutic approaches should target both individual pathogens and microbial communities (Larouche *et al*., 2018)

2.1.2 Stages of Wound Healing and Chronic Wound Pathophysiology

In healthy individuals, wounds heal in a coordinated series of steps, including hemostasis, inflammation, proliferation, including the creation of granulation tissue and re-epithelialization, and remodeling (maturation). Cellular actors, signaling mediators, and tissue-level events are distinctive to each phase. The term "chronic wounds" refers to wounds that do not heal in a timely, orderly manner and frequently become stuck in one or more phases, usually a proliferative or prolonged inflammatory phase, because of systemic factors like diabetes, vascular disease, local factors such as infection, biofilm, necrotic tissue, or a combination of these factors. The dynamic interactions

between immune responses, cellular metabolism, extracellular matrix remodelling, and the wound microbiome are highlighted in recent research as being essential to the chronic phenotype and to chances for focused intervention (Scales *et al*., 2013).

Hemostasis is the stage of healing right after an injury, this process halts the bleeding and establishes a temporary healing scaffold. Growth factors such as PDGF and TGF-β and cytokines are released by platelet activation, which initiates inflammation and attracts immunological and reparative cells. Hemostasis is usually achieved in chronic wounds, although the initial stages may be complicated by ongoing bleeding or aberrant platelet function. The transition to proliferation requires a properly resolved inflammatory phase, but chronic wounds frequently exhibit maladaptive or prolonged inflammation with persistent neutrophil activity, elevated proteases like matrix metalloproteinases (MMPs), and a pro-inflammatory milieu that can degrade growth factors and extracellular matrix components, impeding the progression to proliferation and influencing downstream healing (Landén *et al.,* 2016).

Growth factors like VEGF, FGF, and TGF-β families coordinate the proliferative phase, which includes keratinocyte migration and proliferation to re-epithelialize the wound epidermis, fibroblast activity with extracellular matrix deposition most notably type III collagen initially, and angiogenesis to re-establish perfusion. Despite proper perfusion and moisture balance, chronic wounds frequently do not heal because of impaired keratinocyte migration, arrested or defective granulation tissue formation, and dysregulated ECM remodeling brought on by imbalanced MMPs and TIMPs (Widgerow *et al.,* 2013).

During the remodeling stage, Collagen I replaces collagen III, which also sees ECM reorganization, myofibroblast-mediated contraction, and gradual increases. Chronic wounds, on the other hand, may not remodel well because of ongoing proteolytic activity, persistent inflammation, and disrupted cell–matrix interactions, which can lead to scar tissue with changed mechanical properties or a wound bed that doesn't heal over time (Singh *et al.,* 2023).

2.1.3 Colonization and Infection in Chronic Wounds with Clinical and Pathological Distinctions

According to Lipsky *et al.* (2019), colonization is the existence of bacteria on the surface of the wound or inside the wound bed without resulting in tissue damage or a systemic inflammatory response. Although bacteria can be found during colonization and may even form biofilms, they do not cause tissue damage or significant host inflammation, and they usually do not advance to necrosis or impede healing beyond what is typical of chronic wounds (Sen *et al*., 2020). Contrarily, infection happens when bacteria infiltrate tissue and cause an inflammatory response in the host, with the resulting clinical indications being attributed to bacterial activity. Signs of infection include increased inflammation, discomfort, changes in exudate, malodor, or necrosis, as well as tissue invasion with cytotoxic consequences and enzymatic damage that interferes with normal wound healing (Labib & Winters, 2023). Systemic infections can occasionally manifest as fever, malaise, or abnormal laboratory results, especially in high-risk people (Cojocaru *et al*., 2011).

The clinical difference between infection and colonization is entrenched in standardized criteria, with the wound serving as the main site for evaluating local infection signs. Monika *et al. (*2022) highlight the importance of combining local wound indicators with systemic findings to make this distinction. These symptoms include erythema that extends past the edges of the incision, warmth around the site, edema or induration of surrounding tissue, increased pain or tenderness, and purulent, purulent with color changes, or foul-smelling discharge. In addition to delayed wound healing or a halt in development, such as a lack of granulation or re-epithelialization over an anticipated timeframe, the wound bed may be oily or friable with necrotic tissue (Falanga, 2005). Fever, chills, tachycardia, leukocytosis, or other nonspecific inflammatory indicators in laboratory testing are examples of systemic indications that raise concerns about infection (Hotchkiss *et al*., 1016). A wound may appear misleadingly "normal" while harboring resistant organisms and exhibiting slowed healing because biofilms can conceal infection by reducing local inflammatory signals (Peek *et al*., 2018). In contrast to simple colonization, persistent non-healing wounds with substantial bioburden and exudate, when treated with normal wound care, should raise suspicions of biofilm-associated infection (Jones & Kennedy, 2012).

2.1.4 Clinical and Diagnostic Assessment of Chronic Wounds

A comprehensive history that highlights comorbidities like diabetes or vascular disease, recent operations, antibiotic exposure, and wound duration is the first step in a stepwise clinical examination. The wound edge, bed, surrounding skin, signs of necrosis, discomfort, exudate quantity and quality, odor, and any bleeding propensity should all be evaluated during a focused physical examination (Grey *et al*., 2006). Systemic involvement, such as fever or sepsis symptoms, must be assessed, and risk factors for infection progression, such as immunosuppression or inadequate perfusion, must be taken into account.

When clinical symptoms point to infection or when healing is not advancing even with the right care, a wound culture is recommended (Jensen *et al*., 2021). Techniques for sampling include tissue biopsy for deeper infections or wound swabs, swab culture may be useful for surface colonization but may not be as instructive for invasive infections. Clinical symptoms should direct interpretation because quantitative thresholds for infection based on bacterial load are disputed and not widely accepted (Copeland *et al*., 2016)

2.1.5 Biofilm-Associated Infections in Chronic Wound and their Management

Biofilms are structured populations of bacteria that are protected from environmental stressors such as host defenses and antimicrobial agents by a matrix of extracellular polymeric substance (EPS), which the microorganisms produce on their own and cling to surfaces. Because biofilms can withstand routine wound care, remain for extended periods of time, and delay healing, they are especially problematic in chronic wounds. Polysaccharides, proteins, nucleic acids, and lipids make up the EPS matrix, which is a hydrated, viscoelastic scaffold that promotes nutrition exchange, cell-to-cell communication, and defense against immunological agents and shear stresses (Goswami *et al*., 2020). Bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus* species, and polymicrobial consortia are frequently found in biofilms in chronic wounds; interspecies interactions affect virulence and resilience (Percival *et al*., 2015).

According to Omar *et al.* (2017), chronic wounds, biofilm formation usually takes place in a multi-stage, sequential process. Bacterial adhesins and the surface characteristics of the wound bed, skin margins, and necrotic tissue propel the first attachment phase. Microenvironments inside the biofilm establish nutritional gradients and zones of reduced oxygen tension, promoting phenotypic variety and antibiotic tolerance. Irreversible attachment and maturation follow, during which time microcolonies proliferate and produce EPS. The biofilm's microenvironments produce zones of lower oxygen tension and nutritional gradients, which promote antibiotic tolerance and phenotypic diversity. Planktonic cells released during the last dispersion phase can spread to other locations or seed new wound regions. Host variables like decreased perfusion, hyperglycemia, malnourishment, immunological dysfunction, and wound environment factors like moisture, pH, exudate burden, and necrotic tissue presence all affect the formation of biofilms. These dynamics lead to a never-ending cycle of infection and compromised tissue healing (Burgess *et al*., 2021).

Biofilms interfere with healing in a number of ways and change the host response. They generate persistent, low-grade inflammation that impedes the natural course of healing, alter inflammatory signals, and protect germs from phagocytosis and neutrophil activity. Antibiotic penetration and effectiveness are decreased by the EPS matrix and physiological heterogeneity inside biofilms, frequently necessitating greater doses, longer courses, or combination therapies. Additionally, biofilms can promote horizontal gene transfer, which helps the wound microbiome develop and sustain antibiotic resistance. According to clinical research, biofilms are linked to greater discomfort, delayed wound closure, odor, persistent exudate, and a higher likelihood of re-interventions and, in extreme situations, amputations (Versey *et al.*, 2021)

In clinical practice, biofilm detection and identification are still difficult. Standard wound swabs might not accurately capture the biofilm matrix, and conventional culture-based techniques frequently fall short in differentiating colonization from biofilm-associated infection. Though these are mostly research tools and not yet common in many clinical settings, emerging approaches include assays targeting EPS components, molecular techniques to detect biofilm-specific genes, and confocal laser scanning microscopy and sophisticated imaging modalities (Mayer *et al*., 2016).

Clinical suspicion of biofilm involvement is raised by persistent non-healing despite proper wound care, excessive inflammation, persistent purulence or malodor, and recurrent infection despite antibiotic therapy. The wound management involves optimizing the wound environment (moisture balance, perfusion, and oxygenation), using topical and systemic treatments having antibiofilm action, and mechanically breaking up the biofilm through extensive and frequently repeated debridement (Alves *et al*., 2021)

2.1.6 Pathophysiological Mechanisms of Biofilm-Mediated Impairment in Chronic Wound Healing

First, bacteria are protected from innate immune responses by biofilms. The EPS matrix reduces bacterial clearance by acting as a physical barrier that prevents neutrophils and macrophages from phagocytosing bacteria. Additionally, the altered gene expression of biofilm-associated bacteria dampens pro-inflammatory signaling and skews the immune response away from an effective acute response and toward a chronic, low-grade inflammatory milieu (Montanari *et al*., 2021). This tenacity impedes prompt clearance and prolongs wound inflammation, both of which interfere with the natural healing process.

Second, biofilms cause healing to stop by altering matrix remodeling and cytokine networks. Bacteria in biofilms emit a range of virulence factors and enzymes (such as nucleases and proteases) that break down growth factors and extracellular matrix components, prevent keratinocyte migration, and prevent re-epithelialization. Chronic, low-level inflammatory signaling can change the wound environment from a regenerative to a degradative state by upregulating matrix metalloproteinases (MMPs) and downregulating tissue inhibitors of metalloproteinases (TIMPs). Despite proper perfusion and moisture balance, this shows up clinically as prolonged necrotic tissue, increased exudate, and delayed closure (Ramírez-Larrota & Eckhard, 2022)

Third, biofilms change the local microenvironment to make bacteria more resilient to antibiotics. Different metabolic states within biofilms produce oxygen and food gradients, and subpopulations that are dormant or slow-growing are less vulnerable to antibiotics that target cells that are actively dividing. Additionally, the EPS matrix limits the diffusion of antibiotics, and horizontal gene transfer in biofilms speeds up the

propagation of resistance determinants, making pharmacologic eradication more difficult. Higher antibiotic exposures, combination tactics, and non-antibiotic measures are all required by these factors taken together (Uruén *et al*., 2020).

Fourth, through modified macrophage morphologies and immunological tolerance, biofilms influence host-pathogen interactions. Although beneficial for wound healing, biofilm-associated bacteria can cause macrophages to polarize toward an M2-like, tissue-remodeling phenotype, which may allow for prolonged infection by reducing microbial death. Neutrophils may continue to be recruited but exhibit functional impairment at the same time, releasing neutrophil extracellular traps (NETs) that can capture germs but also cause collateral inflammation and tissue damage. According to Li M *et al*. (2021), this contradiction promotes an environment in which inflammation endures without efficient microbial clearance, postponing recovery.

Finally, Biofilms serve as repositories for chronic infections. Following apparent clinical recovery, residual biofilm microcolonies may reseed the infection, increasing the risk of relapse and treatment duration. Recurrent bacterial burden undermines sustained wound closure and raises the likelihood of complications like osteomyelitis in open wounds by maintaining a cycle of inflammation and tissue damage (Attinger *et al.*, 2012)

2.1.7 The Etiological Factors Contributing to Chronic Wound Development

Complex and multiple etiologies that obstruct the natural healing process are frequently the cause of chronic wounds. Vascular insufficiency, especially peripheral arterial disease (PAD), is a major contributing factor. In peripheral arterial disease (PAD), ischemia occurs, particularly in the lower extremities, and it interferes with the transport of oxygen and nutrients necessary for tissue regeneration. (Zemaitis *et al*., 2023). Diabetes mellitus plays a major role in the development of chronic wounds. Hyperglycemia delays healing and increases susceptibility to infections, particularly in diabetic foot ulcers, by producing neuropathy, microvascular damage, and compromised immunological responses (Akkus *et al*., 2022). Infection makes wound care more difficult because it prolongs inflammation and causes further tissue damage, especially when it involves bacteria that form biofilms.

Pressure ulcers are brought on by mechanical forces including pressure and friction, particularly on bony prominences, which cause tissue necrosis and prolonged ischemia (Kumar & Patel, 2024). Tissue regeneration is hampered by nutritional inadequacies, such as insufficient consumption of proteins, vitamin C, and zinc, which also affect collagen synthesis and immunological function (Liu *et al*., 2023). Further complicating healing processes are factors like obesity, venous insufficiency, immobility, and chronic comorbidities, which foster an environment that is favorable for the development and persistence of venous and other ulcers (Fernandez & Lee, 2022). For chronic wounds to be effectively managed and prevented, it is imperative to comprehend how these factors interact.

2.2 Bacterial Pathogens Associated with Chronic Wound Infections

A wide variety of bacterial pathogens are usually responsible for chronic wound infections, which are frequently made worse by the development of biofilms that increase the bacteria's resistance to immunological reactions and medications. Methicillin-resistant strains of *Staphylococcus aureus* (MRSA), one of the most prevalent pathogens, are infamous for their virulence factors and antibiotic resistance, which make infections challenging to treat (Bhattacharya *et al.*, 2015). Another common pathogen, especially in damp settings, is *Pseudomonas aeruginosa*, which forms biofilms and produces toxins that prevent wounds from healing. It usually coexists with other bacteria, which makes infections much more difficult to treat (Diggle *et al.*, 2020).

Additionally, implicated are *Enterococcus faecalis* and *Enterococcus faecium*, particularly in hospital-acquired chronic wounds where care is complicated by their antibiotic resistance. Furthermore, it is becoming more common to find gram-negative bacteria in chronic wounds, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, which frequently show signs of multidrug resistance (Said et al., 2025).

Because it shields bacteria from drugs and immunological reactions, biofilm formation plays a crucial role in the recalcitrance and persistence of chronic wound infections, delaying the healing process (Bjarnsholt *et al*., 2020). Effective management, which includes targeted antibiotic medication and stringent infection control measures,

requires an understanding of the pathogenic processes and resistance profiles of these bacteria.

2.2.1 The epidemiology and Clinical Significance of *Pseudomonas aeruginosa*

A common isolate from chronic wounds, such as diabetic foot ulcers, venous leg ulcers, pressure injuries, and burn wounds, is the Gram-negative, opportunistic bacterium *Pseudomonas aeruginosa*. Its diverse virulence repertoire, capacity to flourish in harsh wound conditions (such as hypoxia and oxidative stress), strong biofilm production, and inherent and adaptive antimicrobial resistance all contribute to its effect on healing. Immune defenses frequently successfully eradicate *Pseudomonas aeruginosa* in healthy skin or in acute wounds; in chronic wounds, however, a combination of a niche that promotes biofilm formation, a persistent bioburden, and a dysregulated immune response leads to prolonged inflammation and delayed re-epithelialization. Recent research highlights how *Pseudomonas aeruginosa* and the wound microbiome, including polymicrobial communities, interact dynamically to modify virulence and affect the effectiveness of treatment (Touati *et al.*, 2025).

According to Tuon *et al*. (2022), Chronic *Pseudomonas aeruginosa* infections are largely caused by biofilm development and antibiotic tolerance. In wound beds, the organism easily forms biofilms and produces an extracellular polymeric material that prevents antibiotic penetration and protects bacteria from being cleared by neutrophils and macrophages. Different metabolic states are displayed by subpopulations within these biofilms, which leads to antibiotic tolerance as opposed to complete resistance. Chronicity and treatment response are shaped by quorum-sensing networks (las and rhl), which coordinate the production of virulence factors and biofilm formation (Mitra *et al.*, 2024). A persistent inflammatory environment that postpones re-epithelialization is maintained by pyocyanin, elastases, rhamnolipids, and other released substances that harm host tissues, interfere with keratinocyte function, and alter inflammatory signaling (Ruffin *et al.,* 2019).

Treatment is made more difficult by biofilm-associated adaptive resistance and intrinsic resistance mechanisms (efflux pumps, low outer membrane permeability), which frequently call for combination therapies and non-antibiotic tactics to break up biofilms

and lessen bioburden (Khan *et al.*, 2021). A dynamic niche that supports *Pseudomonas aeruginosa* persistence and can change virulence and treatment response is created by the hypoxia and moisture conditions of the wound environment as well as interactions with co-colonizing species such as *Staphylococcus aureus* (Sahu *et al.*, 2021).

Clinically, an integrated approach is necessary to treat *Pseudomonas aeruginosa* in chronic wounds. When available, supplementary assessments can help guide strategy because accurate identification is crucial, but culture findings might not accurately indicate biofilm activity (Reynolds *et al.,* 2020). In order to prevent niche specialization for *Pseudomonas aeruginosa,* debridement and wound-bed optimization are still fundamental, involving routine removal of necrotic tissue and techniques to maintain a suitable moisture balance (Harries *et al.*, 2020). Culture and susceptibilities should direct antimicrobial therapy, keeping in mind that bacteria linked to biofilms would need longer or more intense exposures, as well as possible combination regimens. Antiseptics that work against *Pseudomonas aeruginosa* biofilms, topical antibiofilm agents, and dressings.

2.2.2 The Epidemiology and Clinical Significance of *Escherichia coli* in Chronic Wounds

A prevalent Gram-negative bacterium that has been isolated from persistent wound infections, *Escherichia coli* frequently contributes to a polymicrobial milieu that hinders healing (Liu *et al.,* 2023). Both endogenous intestinal flora and foreign contamination from the perineal area or environment are reflected in its presence in wounds. *Escherichia coli* rates in chronic wounds are epidemiologically variable by community and context, but they continuously appear as a frequent isolate with *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Bessa *et al.,* 2015). The organism's relevance in chronicity and delayed healing is further supported by the fact that its capacity to form biofilms correlates with persistence and resistance to common wound care therapies.

High levels of resistance to widely used oral and parenteral treatments are often seen in *Escherichia coli* from chronic wounds. Ciprofloxacin resistance is well-established and, according to multiple investigations, varies by region and local prescribing practices, approaching or surpassing 70–80% (Kale *et al.*, 2021; Johnson *et al.*, 2020).

A significant issue in many areas is the formation of extended-spectrum beta-lactamases (ESBLs), which reduces the effectiveness of cephalosporins and penicillins and, in extreme situations, calls for the use of carbapenems, albeit with caution (Paterson, 2018; Cantón & Coque, 2006). There have also been reports of resistance to amoxicillin-clavulanate and ampicillin-sulbactam, highlighting the necessity of earlier, culture-guided treatment for chronic wounds (Muteeb *et al.,* 2022). The diversity of resistance patterns highlights how crucial local antibiograms are for guiding empirical decisions while awaiting culture findings (Centers for Disease Control and Prevention, 2023).

Escherichia coli strains isolated from wounds frequently carry virulence characteristics that increase tissue invasion and immune evasion, even though mucosal and systemic *Escherichia coli* pathotypes are well defined. Chronicity can be exacerbated by genes that encode adhesins, toxins, and iron acquisition mechanisms, which can promote biofilm formation and persistence in the wound bed (Pakbin *et al.,* 2021). Certain strains of *Escherichia coli* linked to wounds have chromosomal islands and virulence plasmids that could affect clinical outcomes by influencing virulence characteristics and interactions with co-infecting organisms (Kikuchi *et al.,* 2019). Finding high-risk clones and adjusting interventions appropriately can be facilitated by combining virulence profiling and resistance data (Luo *et al.,* 2021).

With the presence of multiple sequence types (STs) and phylogroups circulating in clinical settings, genomic approaches such as whole-genome sequencing (WGS) have demonstrated substantial diversity among wound-associated *Escherichia coli* isolates. (Pitkänen *et al.,* 2020). However, in low- and middle-income nations, where resources restrict frequent WGS and thorough surveillance, epidemiology in many chronic-wound groups is still poorly defined (Van Boeckel *et al.,* 2019). Increasing genomic surveillance can help detect dominant resistant lineages, shed light on transmission networks, and guide specific infection-control strategies (Didelot *et al.,* 2020).

Given varying virulence and resistance, empirical treatment for chronic wounds should strike a compromise between local resistance patterns and coverage for Gram-negative organisms, particularly those that may produce ESBL. De-escalation to targeted drugs

should come after prompt culture and susceptibility testing to improve therapy. To reduce the selection pressure that leads to resistance, antibiotic stewardship, infection-control procedures, wound debridement, and wound environment optimization are still essential (Bassetti *et al.*, 2023; World Health Organization, 2022).

2.2.3 The Epidemiology and Clinical Significance of *Corynebacterium striatum* in Chronic Wound Infection

It is becoming more widely acknowledged that *Corynebacterium striatum* is a wound-associated pathogen, especially in nosocomial and chronic conditions. Previously thought to be a skin commensal, it is now linked to a number of illnesses, such as soft-tissue infections, wound colonization, and infections connected to devices. *Corynebacterium striatum* often inhabits polymicrobial communities in chronic wounds, which hinders healing and may act as a reservoir for resistance determinants. Several surveillance data show increasing isolation rates in long-term care institutions and hospital-associated wounds, while their frequency varies by location and healthcare exposure (Lorre *et al.*, 2020; Zhang *et al.*, 2022).

Because of its strong resistance to antibiotics, *Corynebacterium striatum* is difficult to treat in chronic wounds. Reports usually mention varying susceptibility to vancomycin, linezolid, and daptomycin, as well as resistance to regularly used beta-lactams (including penicillins) and macrolides. Treatment conundrums in situations with little susceptibility data have been defined by the emergence of multidrug resistance (MDR). When host defenses are weakened, the organism's acquired and innate resistance mechanisms such as efflux pumps and target modification help to ensure persistent colonization and eventual invasion. These trends highlight how crucial it is to identify species-level infections and conduct thorough susceptibility testing in order to direct treatment, especially in cases of complex wounds or when empirical therapy is unsuccessful (Collins *et al.*, 2021; Fernández-Romero *et al.*, 2020).

Numerous virulence characteristics of *Corynebacterium striatum* may make it easier for the bacteria to colonize and infect wounds. Surface-associated proteins facilitate biofilm development and adherence to extracellular matrix, allowing for persistence in the wound bed and resistance to antimicrobials and host defenses. Establishment in the wound microenvironment may be facilitated by enzymatic systems and released

substances that cause tissue damage and inflammation. Genomic studies have begun to identify virulence-associated loci that correlate with invasive potential, antibiotic resistance, and clonal adaptability in hospital settings, despite the fact that these loci are not as well defined as major pathogens (Keller et al., 2019; Chen et al., 2021).

With the growing use of whole-genome sequencing for epidemic investigations and Matrix assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF MS) for quick identification, the molecular epidemiology of *C. striatum* is continually evolving. Though rigorous surveillance in chronic wounds is still lacking, especially in low- and middle-income countries, comparative genomics has started to uncover a variety of clonal lineages and shared resistance determinants across geographies. Increasing WGS-based surveillance would help detect dominant MDR clones, shed light on transmission networks, and guide stewardship and targeted infection-control measures (Khan et al., 2020; Li et al., 2021).

Robust susceptibility testing and precise species identification should be the cornerstones of management. Given the possibility of MDR, local antibiograms should serve as a guide for empirical therapy, which should be modified in response to culture findings. Depending on susceptibility data, treatment options may include medicines with dependable activity, such as vancomycin or, in more severe situations, linezolid. Standard wound care procedures, such as debridement, wound environment optimization, and infection control measures, are still crucial in addition to antimicrobial therapy. In order to maintain treatment options for infections that are difficult to treat and to stop the development of new resistance, antibiotic stewardship is essential (Hodges et al., 2020; WHO, 2022).

2.2.4 The Epidemiology and Clinical Significance of Coagulase-Negative *Staphylococci* in Chronic Wounds

Coagulase-negative *Staphylococci* (CoNS) are a broad category of skin commensals that become significant pathogens in chronic wounds, especially in hospitalized and immunocompromised patients. This bacterium can cause biofilm formation, delayed healing, and device-related infections. They are commonly recovered from wound specimens, frequently as a component of polymicrobial communities. Among the most often found CoNS in wound microbiology are species including *Staphylococcus*

hominis, *Staphylococcus epidermidis*, and *Staphylococcus lugdunensis*. Several surveillance studies suggest that CoNS is frequently isolated from chronic wounds, occasionally replacing more conventionally virulent organisms in some wound cohorts. However, the incidence of CoNS varies depending on the context and sampling technique (Eriksson *et el*,.2022).

The innate and acquired resistance properties of CoNS are noteworthy. In wound environments, methicillin-resistant CoNS (MR-CoNS, which includes methicillin-resistant Staphylococcus epidermidis (MRSE) and MRSE-like strains are prevalent and make treatment more difficult. Beta-lactam resistance is common, and reports of resistance to aminoglycosides, macrolides, and quinolones are growing. Notably, in polymicrobial settings, CoNS can act as repositories of resistance genes, such as *mecA* and other SCCmec components, which can be passed on to more aggressive *Staphylococcus* species. The need for culture-guided therapy and de-escalation based on susceptibility results is highlighted by the cautious assumption that chronic wounds are resistant to numerous medication classes, even if resistance patterns vary greatly by region (Tamma *et al.*, 2012).

Although CoNS were once thought to be low-virulence commensals, several species have virulence factors that promote wound infection. One important virulence tactic is the creation of biofilms, which increase resistance to host defenses and antimicrobials by allowing persistence on wound surfaces and prosthetic devices. Secreted enzymes, immunological evasion strategies, and surface adhesins, microbial surface components that recognize adhesive matrix molecules, or microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)are additional variables. Crucially, under specific situations, certain CoNS species, like *Staphylococcus lugdunensis*, can be more pathogenic than *S. aureus*, exhibiting aggressive tissue invasion and the generation of toxins (Kloos & Bannerman, 2014; Frank, 2020).

The population structure and resistance factors of several CoNS species are starting to be revealed by genomic techniques (MLST, spa typing, SCCmec characterisation, and whole-genome sequencing). However, there is still a lack of rigorous genetic surveillance for chronic wounds, particularly in low- and middle-income nations. By shedding light on virulence repertoires, resistance gene pools, and clonal dissemination,

expanding WGS-based surveillance can help guide infection-control and stewardship tactics (Waldron *et al.*, 2013; Baines *et al*., 2018).

Because methicillin resistance is so likely, managing CoNS infections in wounds necessitates precise species-level identification and thorough susceptibility testing. When there is a high level of clinical suspicion, empirical therapy should take into account local resistance patterns and favor medicines that are effective against MR-CoNS. Depending on susceptibility, vancomycin, linezolid, and daptomycin may be used to treat severe infections. The basics of wound care, such as debridement, biofilm drainage, moisture control, and wound environment optimization, are crucial in addition to antimicrobial therapy. To avoid resistance selection and maintain treatment alternatives, antimicrobial stewardship is still essential (Hersh et al., 2020; World Health Organization, 2022).

2.2.5 The Epidemiology and Clinical Significance of *Proteus* Species in Chronic Wounds

Gram-negative enteric bacteria called *Proteus* species are typically associated with urinary tract infections, but they are also increasingly being found in chronic wound infections, frequently in polymicrobial communities. The most commonly implicated *Proteus* species in wounds is *P. mirabilis*, which is followed by *P. vulgaris* and *P. penneri*. *Proteus* species have the potential to cause biofilm formation, local inflammation, and delayed healing in chronic wounds, especially in hospital or long-term care settings where previous exposure to antibiotics and medical devices are frequent risk factors. *Proteus* isolation from non-healing wounds is still a clinically significant signal in several surveillance cohorts, despite variations in geography and facility (Kwaku *et al*., 2018; Chen *et al*., 2020).

Different resistance patterns displayed by *Proteus* species make empirical treatment more challenging. Resistance to ampicillin, first-generation cephalosporins, and occasionally fluoroquinolones are common issues. The creation of ESBL and AmpC beta-lactamases can result in higher-level resistance, which reduces the effectiveness of many beta-lactam medicines and calls for the use of carbapenems or other targeted alternatives depending on susceptibility data. To guide empirical decisions in wounds, regional antibiograms and local stewardship initiatives are crucial. Culture-directed

therapy should be given priority in order to prevent needless broad-spectrum use (Cantón & Coque, 2006; Paterson, 2018; Wichmann et al., 2021).

Swarming motility, urease activity, fimbriae for adhesion, and secretion systems that regulate host responses are some of the virulence characteristics of *Proteus* species that facilitate wound colonization and infection. In the wound bed, flagella and pili aid in surface exploration and persistence, while urease adds to alkaline environments that can promote biofilm formation and tissue injury. In chronic wounds, biofilm development is a key pathogenic tactic that permits antibiotic tolerance and host defense evasion (Giacomazzi et al., 2019; Clements et al., 2016).

Proteus isolates from clinical sources, such as wounds, have shown significant genetic variety according to genomic techniques (MLST, whole-genome sequencing). However, there is little comprehensive genetic surveillance for *Proteus* species in chronic wounds, particularly in low- and middle-income environments. Increasing WGS-based surveillance can reveal virulence profiles that are associated with clinical outcomes, track resistant clones, and shed light on transmission networks (Didelot et al., 2020; Barrett et al., 2022).

Robust susceptibility testing and precise species identification are essential for management. Local resistance patterns should serve as a guide for empirical therapy, which should be modified based on culture findings. Options could include cephalosporins with ESBL coverage, beta-lactams that are active against *Proteus*, fluoroquinolones for susceptible infections, or carbapenems for MDR patients, depending on susceptibility. Standard wound care procedures, such as debridement, moisture control, anti-biofilm techniques, and wound environment optimization, are crucial in addition to antimicrobial therapy. To stop the emergence of new resistance, antibiotic stewardship is essential (Harriman et al., 2021; WHO, 2022).

2.2.6 The Epidemiology and Clinical Significance of *Klebsiella pneumoniae* in Chronic Wound Infection

A well-known pathogen in a number of illnesses, such as pressure ulcers, diabetic and chronic foot ulcers, and wounds connected to medical devices, is *Klebsiella pneumoniae*. Particularly in hospitalized or long-term care patients, *Klebsiella*

pneumoniae frequently contributes to polymicrobial biofilms in chronic wounds, which can cause delayed healing, elevated inflammation, and recurring infections. Numerous surveillance studies show that *Klebsiella pneumoniae* is a common gram-negative wound pathogen, with a higher risk in patients who have previously been exposed to antibiotics, have indwelling devices, or have compromised immune systems (Podschun & Ullmann, 1990; van Acker et al., 2020). The prevalence varies by geography and care setting.

Extended-spectrum beta-lactamases (ESBLs), carbapenemases, *Klebsiella pneumoniae* carbapenemases, New Delhi metallo-beta-lactamases, oxacillinase-48 family enzymes, including PAMs such as KPC (Klebsiella pneumoniae carbapenemase), NDM (New Delhi metallo-β-lactamase), and OXA-48, and plasmid-mediated resistance determinants are among the significant inherent and acquired resistance that *Klebsiella pneumoniae* exhibits. The emergence of extensively drug-resistant (XDR) and multidrug-resistant (MDR) phenotypes in isolates linked to wounds has complicated empirical treatment and raised the demand for local antibiograms and quick susceptibility testing. Mobile genetic elements, biofilm-associated tolerance, and high-risk clones (such as different STs connected to ESBL or carbapenemase synthesis) are frequently linked to resistance. Culture data should inform treatment choices, taking into account combination therapy and stewardship to protect last-line medications (Pitout & Nordmann, 2015; Noordman *et al*., 2019).

A variety of virulence factors carried by *Klebsiella pneumoniae* aid in the colonization, invasion, and biofilm formation of wounds. Capsule polysaccharide (anti-phagocytic), siderophore systems (enterobactin, aerobactin), adhesion fimbriae, and secretion systems that engage with host immunity are noteworthy characteristics. Although they are more frequently linked to liver abscesses, hypervirulent *Klebsiella pneumoniae* (hvKP) strains, which are distinguished by increased siderophore synthesis and virulence factor expression, can also arise in wound situations and indicate more severe illness in vulnerable patients. Persistence and treatment difficulties are exacerbated by the development of biofilm on wound surfaces and devices (Kremer *et al.*, 2017; Shon *et al*., 2013).

Different lineages and resistance factors among *Klebsiella pneumoniae* isolate linked to wounds have been revealed by whole-genome sequencing. The proliferation of ESBL and carbapenemase-producing strains in communities and healthcare settings is highlighted by surveillance. Despite advancements, many locations still lack systematic genetic surveillance that focuses exclusively on chronic wounds. In addition to improving knowledge of virulence repertoires, resistance gene flow, and clonal propagation, expanded WGS-based surveillance would also help guide infection-control and treatment plans (Wyres *et al*., 2019; Martin *et al*., 2020).

Robust susceptibility testing and precise identification are essential for management. Local resistance patterns should serve as a guide for empirical therapy, which should be rapidly modified in response to culture results due to the high probability of ESBL formation and carbapenemase activity in certain circumstances. Depending on susceptibility, treatment options may include carbapenems for many ESBL producers and ceftazidime-avibactam or polymyxin-based regimens for organisms that produce carbapenemase. Standard wound care procedures, such as proper debridement, moisture control, biofilm disruption, and device removal when practical, are essential in addition to antimicrobials. To prevent the spread of resistance and maintain treatment alternatives, stewardship is crucial (López-Carrasco et al., 2020; WHO, 2022).

2.2.7 The Epidemiology and Clinical Significance of *Morganella Morganii* in Chronic Wound Infection

A Gram-negative member of the order Enterobacterales, *Morganella morganii* is becoming more well acknowledged as a wound pathogen, especially in burns, device-associated infections, and polymicrobial chronic wounds. Even though *Morganella morganii* is less frequent than important wound infections like *Pseudomonas* or *Staphylococcus* species, it can nonetheless lead to biofilm formation and chronic infection, particularly in hospitalized or immunocompromised patients who have previously been exposed to antibiotics or have had antibiotic instrumentation. Reports of *Morganella morganii* in wound care cohorts highlight its potential clinical impact and significance to antibiotic stewardship, despite geographic and facility diversity (Krogh *et al*., 2015; Kaye *et al*., 2019).

The susceptibility to Morganella morganii varies. It can contain resistance determinants like beta-lactamases, especially AmpC-type enzymes, and, in some situations, ESBLs. It also naturally resists several conventional drugs. Some clones have been reported to be resistant to trimethoprim-sulfamethoxazole (TMP-SMX) and fluoroquinolones. Concern for MDR phenotypes is raised by the ability of the organism to acquire resistance plasmids, which may restrict empirical alternatives. Similar to other Enterobacterales, prompt culture data and local antibiograms inform treatment choices. Broader-spectrum antibiotics should be saved for infections that are proven to be resistant, and de-escalation should be done as quickly as feasible (Kuhnert *et al*., 2016; Tanti *et al.*, 2020).

Compared to more frequently researched pathogens, there is less genomic data available for *Morganella morganii* in wounds. Clinical isolates exhibit genetic diversity, resistance determinants, and potential virulence repertoires that are progressively uncovered through whole-genome sequencing. Coordinated sequencing initiatives would enhance the tracking of virulence traits, resistance gene content, and clonal dissemination that influence clinical outcomes, particularly given the ongoing lack of systematic surveillance in chronic wound infections. (Didelot *et al.*, 2020; Sahlstrom *et al.*, 2021).

Given the possibility of both acquired and innate resistance, management should be directed by susceptibility testing and culture. Once susceptibilities are known, empirical treatment should be improved to account for local resistance patterns. Beta-lactams that are active against *Morganella morganii* (such as certain cephalosporins or piperacillin-tazobactam, depending on susceptibility) and carbapenems for resistant patients are examples of possible treatment choices. Debridement, decontamination, moisture management, and biofilm disruption are all crucial adjunctive wound care techniques (Harris *et al*., 2018; WHO, 2022).

2.2.8 The Epidemiology and Clinical Significance of *Acinetobacter baumannii* in Chronic Wound Infection

Acinetobacter baumannii is a common nosocomial pathogen that is being found in more and more chronic and non-healing wounds, including as burns, diabetic foot ulcers, pressure ulcers, and infections linked to medical devices. Particularly in patients who

are extremely ill, immunocompromised, or have received extensive treatment, *Acinetobacter baumannii f*requently contributes to polymicrobial biofilms in wound situations, which can cause delayed healing, ongoing inflammation, and recurrent infections. Its clinical significance across care settings is influenced by its resistance to hospital surroundings, capacity to withstand desiccation, and correlation with prior antibiotic exposure and invasive devices (Peleg *et al*,. 2008; Fournier & Richet, 2006).

Multidrug resistance in *Acinetobacter baumannii* is well established, and its mechanisms of resistance include both acquired and innate factors. Resistance to aminoglycosides, fluoroquinolones, cephalosporins, and carbapenems (via OXA-type carbapenemases and other mechanisms) are frequent problems. More and more wound isolates are exhibiting MDR and XDR phenotypes, which reduces the number of available treatments and emphasizes the value of local antibiograms and quick susceptibility testing in directing treatment. According to stewardship principles and susceptibility data, treatment frequently involves combination regimens that may involve polymyxins (e.g., colistin), tigecycline, sulbactam-containing treatments, and newer medicines when available (Pennisi *et al*., 2008; Zavascki *et al.*, 2010; Higgins *et al.*, 2019).

Numerous virulence factors that support colonization, biofilm formation, and immune evasion are present in *Acinetobacter baumannii*. Iron acquisition systems (e.g., siderophores), several secretion systems, outer membrane proteins and capsules that improve evasion, and strong biofilm-forming abilities on abiotic surfaces and injured tissue are important components. According to Cerezales *et al*. (2014) and Higgins *et a*l. (2020), these characteristics promote tolerance to antibiotics, resistance to host defenses, and persistence in the wound bed.

Based on whole-genome sequencing, *Acinetobactor baumannii* lineages exhibit significant genetic diversity, with worldwide lineages linked to hospital outbreaks and carbapenem resistance. The global proliferation of high-risk clones in healthcare contexts, especially wound care facilities, is demonstrated via surveillance. Despite advancements, many locations still lack systematic genetic surveillance tailored to chronic wounds, making it difficult to precisely follow the evolution of resistance and

transmission. Increased genomic surveillance would help guide infection-control measures and enhance knowledge of clonal spread (Stratford *et al*., 2013; Zarrilli *et al*., 2013).

Given the possibility of widespread resistance, management calls for prompt, precise species identification and susceptibility testing. Local resistance patterns should provide as a guide for empirical therapy, which should be improved upon based on culture findings. Minocycline, doxycycline, tigecycline, sulbactam-based regimens, cephalosporins that are active against *A. baumannii*, and combination therapy are among the available treatments for susceptible strains. Colistin (polymyxin E) or other last-line medications may be used in MDR/XDR instances, with careful toxicity monitoring. Antimicrobial therapy must be used in conjunction with aggressive wound care techniques, such as debridement, moisture balancing, decontamination, and biofilm destruction. To stop the spread and maintain the remaining treatment choices, stewardship and infection-control measures are essential (Doi *et al.,* 2015; Evans & Bonomo, 2013; World Health Organization, 2022).

2.2.9 The Epidemiology and Clinical Significance of *Enterococcus faecalis* in Chronic Wounds

Enterococcus faecalis is detected across diverse chronic wound environments and frequently constitutes a component of polymicrobial wound infections. Its intrinsic resistance to multiple therapies, together with the emergence of vancomycin-resistant enterococci (VRE) in certain regions, underpins its significant clinical impact. These factors can complicate treatment and potentially impede wound healing (Arias & Murray, 2012; Bleiberg et al., 2019). Enterococcus faecalis contributes to increased bioburden and prolonged infection in wounds, particularly in the presence of biofilms and co-infections (Bleiberg et al., 2019; Harbarth et al., 2014).

On wound beds and medical equipment, *Enterococcus faecalis* easily creates strong biofilms that improve resistance to antibiotics and host defenses. With phenotypic heterogeneity and decreased antibiotic resistance within biofilms, biofilm-associated persistence is a major contributor to chronicity in wounds (Fernandez *et al*., 2021). Ongoing infection and inflammation are supported by the ability of the organism to move inside wound matrices and remain on surfaces (Arias and Murray, 2012).

While resistance factors, such as aminoglycoside resistance and, in certain situations, vancomycin resistance, restrict alternatives, *Enterococcus faecalis* is typically still responsive to a wide range of conventional treatments (Arias and Murray, 2012). Agents having anti-biofilm and anti-gram-positive action are frequently needed for treatment of chronic wounds. For resistant Enterococcus infections, such as VRE, linezolid and daptomycin are frequently used; for susceptible isolates, susceptible beta-lactams and glycopeptides may still be useful. Antimicrobial therapy can be enhanced by combination regimens and local wound-management techniques (debridement, moisture control, and non-antibiotic dressings). In chronic wounds, tailored therapy is guided by rapid culture and susceptibility testing in conjunction with antimicrobial stewardship (Fernandez *et al.,* 2021; Bleiberg *et al*., 2019).

2.2.10 Clinical Significance of *Staphylococcus aureus* in Chronic Wound Infection

Globally, *Staphylococcus aureus* continues to be the most prevalent pathogen linked to persistent wound infections. Numerous biological, anatomical, and environmental variables contribute to its prevalence by facilitating its efficient colonization and persistence in wound tissues. The exceptional capacity of *Staphylococcus aureus* to produce biofilms is a key factor in its domination. According to Gao *et al*. (2023), biofilms are intricate bacterial colonies that are protected from host immune responses and drugs by an extracellular polymeric material. Because of its ability to build biofilms, *Staphylococcus aureus* can create chronic infections in the wound bed, which makes eradication challenging and increases the risk of chronicity.

Toxins, enzymes, and surface proteins are only a few of the many virulence factors that *S. aureus* produces and which aid in invasion, immune evasion, and tissue damage. For instance, immunoglobulins' Fc region is bound by surface protein A, which hinders opsonization and phagocytosis (Li *et al.,* 2022). *S. aureus* can successfully elude human defenses thanks to these processes, particularly in the weakened environment of a chronic wound.

The pathogen is more common in chronic wounds because of its adaptability. Due in part to its metabolic adaptability, it can endure in hostile situations, including low oxygen levels and nutrient-deficient settings typical of non-healing wounds (Martinez

& Rivera, 2023). Furthermore, the prevalence of methicillin-resistant *Staphylococcus aureus (*MRSA) in chronic wound infections has increased due to treatment complications and persistent colonization caused by the rise of antibiotic resistance (Chamberlain and Luby, 2022).

2.2.10.1 Epidemiology of *Staphylococcus aureus:* Global Context with Africa and Kenya Insights

One of the most frequent causes of bacterial infections around the globe, especially those affecting the skin and soft tissues, such as chronic wounds, is *Staphylococcus aureus*. Although studies consistently demonstrate significant transmission rates and infection frequencies across different locales, its prevalence varies globally. Gao et al. (2023) estimate that *Staphylococcus aureus* colonizes the skin and nasal passages of 20–30% of healthy people worldwide. It is a leading cause of infections linked to the community and healthcare, and the rise of multidrug-resistant strains such as MRSA is making treatment and control extremely difficult worldwide (van *et al*., 2016). *Staphylococcus aureus* represents a significant pathogen in Africa, where it is responsible for invasive infections and wound complications, challenges compounded by insufficient healthcare infrastructure and antimicrobial misuse. According to recent surveillance reports, a significant percentage of skin infections are caused by *Staphylococcus aureus*, especially in hospitalized and immunocompromised individuals. Although it varies throughout the continent, MRSA incidence is on the rise, particularly in hospital settings where resistant strains make infection control procedures more difficult. Additionally, 20% to 40% of healthy persons carry *Staphylococcus aureus*, which affects infection risk and transmission dynamics in African communities and healthcare facilities (Cheung *et al*, 2021).

Based on Kenya's epidemiology, *Staphylococcus aureus* is a significant cause of infections acquired in hospitals and the community, with high prevalence rates reported in different regions. Due to variables including antibiotic abuse and inadequate infection control methods, resistant bacteria like MRSA are more common in urban areas. Detailed knowledge of its distribution and resistance patterns is hampered by issues including insufficient laboratory capacity and surveillance (Mogoi *et al*., 2024).

2.2.10.2 Hospital-Acquired *Staphylococcus aureus* in Chronic Wounds, Epidemiology

Because of its high level of antibiotic resistance, hospital-acquired *Staphylococcus. aureus* (HA-SA), especially methicillin-resistant *Staphylococcus aureus* (MRSA), is a serious concern in healthcare settings. Patients who have undergone invasive surgeries, extended hospital stays, or weakened immune systems are more likely to get HA-SA infections. These strains are more virulent, biofilm-forming, and multidrug-resistant, which makes treatment more challenging and results in chronic, challenging-to-cure wound infections (Gao *et al*., 2023). In chronic wound management, HA-SA colonization is associated with increased morbidity, impaired wound healing, and elevated healthcare costs.

Staphylococcus aureus infections obtained in hospitals or other healthcare facilities are referred to as HA-SA. These infections usually manifest 48 hours or more after admission, or within a specified window following exposure at the facility. Because methicillin-resistant S. aureus (MRSA) is linked to more severe infections, fewer treatment choices, and higher healthcare expenditures, it has traditionally dominated discussions of HA-SA (Isigi *et al*., 2023). However, patient demographics, infection control strategies, and regional patterns of antibiotic use all have an impact on the changing epidemiology of HA-SA. Methicillin-susceptible S. aureus (MSSA) and other resistance phenotypes are increasingly being detected by surveillance programs in addition to MRSA, underscoring the persistent burden of HA-SA in various clinical settings (An *et al*,.2023).

Transmission dynamics in healthcare settings are multifactorial, where transmission networks are facilitated by contaminated hands of healthcare personnel, recurring contaminated surfaces, and direct contact with colonized or infected patients. Despite education and regulatory mandates, hand cleanliness is still a fundamental component of infection control; however, adherence gaps still exist (Armstrong *et al*., 2023). Transmission can be sustained by environmental reservoirs, like as doorknobs, bedrails, and medical equipment, especially in high-acuity units where invasive operations and patient turnover are frequent. Control efforts are further complicated by the role of asymptomatic carriage in patients and staff. This highlights the importance of targeted

decontamination measures in situations where outbreaks occur frequently, while also taking resistance and microbiome disruption issues into account (Dancer *et al*., 2024).

According to Beceiro *et al* (2016), relevant virulence variables for HA-SA combine its ability to invade tissues, colonize, and elude host defenses. While immune evasion strategies allow survival inside phagocytes and inflammatory conditions common in hospitalized patients, surface adhesins, poisons, and enzymes aid in adherence to and invasion of host tissues. A feature of HA-SA in clinical practice, biofilm development on indwelling devices and wound beds substantially impairs antibiotic penetration and leads to recurrent or chronic infections. Clinical outcomes are influenced by the interaction between virulence factors and antibiotic resistance; in certain cohorts, resistant strains are linked to longer hospital admissions, greater expenses, and higher fatality rates (Almatroudi *et al*., 2025)

The healthcare-associated *Staphylococcus aureus* exhibits diverse antimicrobial resistance phenotypes, including MRSA, MSSA, and vancomycin-intermediate or vancomycin-resistant isolates under specific clinical conditions (Doron et al., 2011). The evolution of these resistance mechanisms complicates empirical therapeutic approaches, necessitating broad-spectrum antimicrobial coverage pending definitive susceptibility determination. Implementation of antimicrobial stewardship programs incorporating rapid diagnostic methodologies, systematic de-escalation protocols, and evidence-based treatment algorithms is essential to optimize clinical outcomes while mitigating the emergence of additional resistance mechanisms (Khadse et al., 2023).

According to Majumder et al (.2020, Bloodstream infections, surgical site infections, pneumonia, infections linked to devices, and infections of the skin and soft tissues are all clinically caused by HA-SA. Compared to community-acquired S. aureus infections, hospitalized patients with HA-SA infections experience significant morbidity, longer hospital stays, and higher medical expenses. The type of infection, the patient's age, comorbidities, and immune condition, as well as the promptness of effective antimicrobial therapy, all affect the outcome. Prognostic factors include early detection and source control, as well as suitable antibiotic selection based on local susceptibility patterns.

Strategies for HA-SA prevention and control take a multifaceted approach. Active surveillance cultures in outbreak situations, environmental cleaning, rigorous adherence to hand hygiene and contact precautions, and antimicrobial stewardship to reduce needless exposure to broad-spectrum agents are essential elements. Although risks like disruption of the normal microbiota and resistance must be considered, bundle-based interventions, such as routine decolonization in certain high-risk populations and chlorhexidine body baths for at-risk patients, have shown reductions in HA-SA transmission and infection rates in some settings (Haque et al., 2020). Behavioural therapies are supplemented by engineering controls, such as device management guidelines, isolation facilities, and optimal patient location. Timely implementation of customized treatment and prompt diagnostic testing enhances patient outcomes and may reduce hospital stays (Haleem *et al*., 2021).

Addressing gaps in prevention and therapy is the goal of emerging medicines and research avenues. Although there are still difficulties in clinical translation, new vaccines or immunotherapies that target important virulence factors, host-pathogen interaction modulators, and anti-biofilm medicines are being researched. Targeted therapies can be informed by more accurate tracking of transmission networks and resistance determinants made possible by advances in genomics and real-time surveillance. Long-term reduction of the HA-SA burden may also be possible with methods to prevent biofilm formation on devices and in wounds as well as with improved antibiotic stewardship (Oliveira *et al.*, 2024).

2.2.10.3 Community-Acquired *Staphylococcus aureus* in Chronic Wound Epidemiology

Community-acquired *Staphylococcus aureus* (CA-SA) refers to *Staphylococcus aureus* infections acquired outside of healthcare settings, or soon after community exposure, including both MSSA and MRSA strains that may become implicated in chronic wound infections. In individuals with risk factors such as diabetes, peripheral vascular disease, obesity, smoking, and compromised immunological function, CA-SA is especially linked to delayed healing, elevated inflammation, and recurrent infections in the context of chronic wounds. With geographical differences in MRSA incidence and MSSA virulence determinants that affect clinical outcomes and treatment

decisions, CA-SA may represent a wider diversity of virulence and resistance profiles than hospital-associated strains (Abebe *et al*,.2023).

In many different geographical locations, CA-SA continues to be a prevalent etiologic organism in chronic wounds, such as pressure injuries, diabetic foot ulcers, and venous leg ulcers. Regional variations have been observed in the prevalence of CA-MRSA, which frequently correlates with local public health initiatives, social determinants of health, and community antibiotic usage trends (Ramirez *et al*., 2019). A significant percentage of wound isolates in many populations are caused by CA-SA, with MSSA accounting for a higher fraction in areas where MRSA prevalence is lower. According to surveillance data, wounds linked to CA-SA often contain virulence elements that promote colonization and biofilm development, making removal and recovery more difficult (Bashabsheh *et al*., 2024).

In chronic wounds, CA-SA uses a variety of virulence factors that encourage adhesion, invasion, and persistence. While released enzymes and toxins contribute to inflammation and tissue damage, surface adhesins (MSCRAMMs) allow binding to extracellular matrix components and injured tissue. Antimicrobial resistance and pathogenicity in community strains can interact to affect how well a treatment works, with combination therapy frequently being needed for infections linked to biofilms (Paharik *et al*., 2016).

2.2.10.4 Hospital-Acquired Infections Versus Community-Acquired Infections: *Staphylococcus aureus* in Chronic Wound

In wound care, the determination between HA-SA and CA-SA is crucial. Treatment failures and recurring infections are caused by the widespread antibiotic resistance and biofilm formation of HA-SA, particularly MRSA. Despite frequently being more susceptible to antibiotics, CA-SA has demonstrated an increasing trend of resistance, making it more difficult to distinguish between the two groups and making empirical therapy decisions more difficult (Gao *et al.,* 2023). To avoid chronicity and enhance healing results, their distinct resistance profiles and virulence characteristics require customized antimicrobial approaches. Effective infection control and management of chronic wound infections require an understanding of the distinctions between

community-acquired and hospital-acquired *Staphylococcus aureus*. To stop the emergence of resistant bacteria and enhance therapeutic results, surveillance and prudent antibiotic usage are essential (Tsouklidis *et al*., 2020).

2.2.11 Overview of Bacteria Isolation and Susceptibility Testing

According to Bayot et al. (2024), Antimicrobial susceptibility testing (AST) and bacterial isolation are essential laboratory techniques for identifying bacterial pathogens, determining how sensitive they are to antibiotics, and directing efficient treatment. To get a pure culture for identification and additional testing, isolation entails cultivating bacteria from a patient sample, frequently prior to antibiotic use. Zones of inhibition or minimum inhibitory concentrations (MICs) are then measured using AST techniques, for as the broth/agar dilution or disk diffusion procedures, to evaluate how well various antibiotics work on the isolated bacteria. The findings assist clinicians in choosing the best antibiotic, stopping the spread of bacterial resistance, and keeping an eye on resistance patterns (Gajic *et a*l., 2022).

2.2.11.1 Outline of Bacterial Isolation and Identification

Wound cultures are performed when an infection is suspected through visual observation. The culture is used to confirm the original diagnosis, recognize species of resident microorganisms, and determine effective antibiotics to treat the wound. A culture helps to determine whether a wound has become infected, which type(s) of bacteria are causing the infection, and which antibiotic would best treat the infection and help heal the wound (Syal et al., 2017).

A culture is performed by collecting a sample of fluid, cells or tissue from the wound and placing it on or in appropriate nutrient media. The media encourages the growth of bacteria that may be present, allowing for further testing and identification (Belanger, and Hancock, 2021). The type of culture medium used for isolation of bacteria pathogens from chronic wounds include Blood Agar, MacConkey and Chocolate agar (Nahid.*et al.*, 2021).

2.2.11.1.1 MacConkey agar

MacConkey agar is the first solid differential media to be formulated which was developed in the 20th century by Alfred T. MacConkey agar is both selective and differential medium that only grows gram-negative bacterial species, differentiating the gram-negative organisms based on their lactose metabolism. Fermentation of lactose produces organic acids such as lactic acid, which decreases the pH of the agar. The PH indicator in the media turns pink under acidic conditions, Lactose-fermenting-gram-negatives forms pink colonies while non-lactose fermenters form off-white opaque colonies. Table 1, illustrates some characteristics of specific bacteria in MacConkey agar. Lactose-fermenters, species still show varying rate of growth which is also a way to further differentiate organisms in this medium. Some species also forms capsules appearing different from others (Jung *et al*., 2022).

Table 1: Some Characteristics of Bacteria on MacConkey Agar

Organisms	Growth results
Klebsiella	Mucoid, pink colonies
Proteus	Colorless colonies, swarming growth
Pseudomonas	Irregular, colorless to pink colonies
Escherichia coli	Pink to rose-red colonies (may be surrounded by a zone of precipitated bile)

Source: Annor *et al*. (2023)

2.2.11.1.2 Blood Agar

Blood agar is a differential and rich complex medium that contains 5% sheep red blood cells. This medium test the ability of an organism to produce hemolysins, enzymes that lyse red blood cells. The type of hemolysis by the hemolysins helps differentiate members of the genera *Staphylococcus*, *Streptococcus*, and *Enterococcus* (Gordon & Baron, 2005). Beta-hemolysis is complete hemolysis characterized by a clear zone surrounding the colonies. This is demonstrated in *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus agalactiae* (Cowan, 2008). Alpha-hemolysis is partial hemolysis characterized by a green, opaque zone surrounding the colonies. Bacteria indicated by this characteristic include *Streptococcus pneumoniae* and *Streptococcus mitis* (Murray, Rosenthal, & Pfaller, 2016). In gamma-hemolysis, there is no occurrence of hemolysis. There are no notable zones around the colonies, and this is seen in *Staphylococcus epidermidis* (Perry & Fischetti, 2017).

2.2.11.1.3 Chocolate blood agar

Chocolate blood agar is a nonselective, enriched growth medium used for isolation of pathogenic bacteria. It is a variant of the blood agar, containing red blood cells that have been lysed by slowly heating to 80°C (Casino *et al.,* 2023). It is recommended for use in the isolation and cultivation of fastidious aerobic and anaerobic bacteria like *Bacteroides, Clostridium,* and *Streptococcus* that may be active at deeper levels of the dermis. These anaerobic bacteria are responsible for many devastating infections resulting in gangrene. The aerobic bacteria are identified with superficial epidermal layers but may also be involved in infective processes and include *Staphylococcus epidermis, Corynebacteria,* and *Propionibacteria* (Posnett.*et al.,* 2009).

2.2.11.1.4 Bacterial Identification Methods

Gram stain is regularly performed to help determine the type of bacteria present and provide rapid result to the healthcare practitioner. The staining characteristics help determine the other tests needed for further identification of bacteria pathogen (Belanger, and Hancock, 2021). Gram positive bacteria are identified using biochemical tests such as the catalase test, coagulase test, starch hydrolysis test, and nitrate test, while Gram-negative bacteria are identified using biochemical tests such as the oxidase test, Citrate, Ornithine, lysine, urease test, indole test, sulfur test, and methyl red/Voges-Proskauer test. (Kohlerschmidt *et al*., 2021). The automated systems, like VITEK 2, perform bacterial identification and antibiotic susceptibility testing using biochemical reactions and nutrient usage of the microorganism to make the identification (Ling *et al*., 2003)

2.3 Antibiotic Resistance Profiling from Chronic Wound Infection

Antibacterial drugs, also known as antibiotics, are drugs that either kill bacteria or inhibit the growth of bacteria. They're mainly used to treat systemic infections involving the whole body rather than a localized area bacterial infection (Kohanski. *et al.,* 2010). Antibiotics can be classified by: spectrum of activity, type of action, chemical structure, mechanism of action, and selection and use of Access, Watch, Reserve (AWaRE) antibiotics (Yonga *et al*., 2024).

There are several methods available for testing antibiotic suitability to treat a patient's infection or for determination of the efficacy of a new antibiotic compound, MIC is the lowest concentration of an antimicrobial drug that inhibits the growth of a microorganism and it can be determined using broth dilution, agar dilution or antimicrobial gradient methods (Kowalska *et al*., 2021). Broth dilution is a technique in which ampules holding identical volumes of broth with antimicrobial solution in incrementally increasing concentrations are inoculated with a known number of bacteria. This method is intended mainly for the testing of pure cultures of aerobic bacteria that are easily grown by overnight incubation on agar and which grow well in Mueller-Hinton broth with minimal supplementation (Wiegand *et al.,* 2008).

Similarly, agar dilution method is another MIC determination method where addition of different concentrations of antibiotics into agar medium samples that are poured into Petri dishes. Once the plates are dry, up to 60ul incubated on one plate following determination of the MIC by classifying the lowest antimicrobial concentration that completely inhibits visible microbial growth (Balouiri *et al*., 2015). Agar dilution is appropriate to test multiple microbial pathogens against a single compound. It is recommended for fastidious organisms like anaerobes and *Helicobacter* species (Gajic *et al*., 2022).

Antimicrobial Gradient Methods use specialized test strips or plates where a gradient of antibiotic concentrations is pre-diffused into the agar. Bacterial cultures are applied to the surface, and the MIC is determined by observing growth inhibition along the gradient (Kowalska *et al.,* 2021). Disk diffusion method is a technique where up to 12 small paper disks impregnated with specified concentrations of antimicrobial compounds are used (Bauer *et al.,* 1966). The antimicrobial agents sets-up concentration gradients when diffusing into the culture media. During incubation, bacterial growth occurs across the agar surface except within zones of inhibition surrounding antimicrobial disks, where drug concentrations exceed the minimum inhibitory threshold (Arena et al., 2015). While this method remains widely employed in routine clinical microbiology, its utility is constrained by the limited availability of disks for non-conventional or experimental antimicrobial agents (Plackett et al., 2020).

Antibiotic sensitivity testing plays a vital role in the management of infectious diseases by guiding the selection of effective antimicrobial agents. Among the various methods available, the VITEK 2 system has gained widespread adoption in clinical microbiology laboratories due to its automation, speed, and reliability. The VITEK 2 system by bioMérieux automates both bacterial identification and antimicrobial susceptibility testing (AST), providing rapid results that are critical for timely patient management (Reller et al., 2018). This system utilizes specialized cards containing multiple antibiotics, inoculated with a bacterial suspension. The system then monitors bacterial growth in the presence of these antibiotics, interpreting the results through growth patterns to determine susceptibility or resistance (Rasher et al., 2020). The detection of minimum inhibitory concentrations (MICs) allows for precise interpretation based on established clinical guidelines such as those from the Clinical and Laboratory Standards Institute (CLSI, 2022).

One of the main advantages of the VITEK 2 system is its ability to deliver results within a short turnaround time—generally between 6 and 18 hours—compared to traditional manual methods. This rapid processing improves clinical decision-making by enabling prompt initiation or adjustment of antimicrobial therapy. Additionally, the standardization inherent in the automation process reduces human error and enhances reproducibility, which is important for consistent patient care. The system is a preferred option for many institutions due to its automatic interpretation algorithms and user-friendly interface, which further streamline workflows in busy laboratories (Rasher et al., 2020). Furthermore, recent updates to the system include expanded antibiotic panels that are tailored to detect resistance mechanisms such as those seen in carbapenem-resistant Enterobacteriaceae, reflecting ongoing efforts to combat antimicrobial resistance (Rasher et al., 2020).

Despite its many advantages, the VITEK 2 system does have limitations. Some studies highlight occasional discrepancies in susceptibility results, particularly for certain drugs or bacterial species, emphasizing the importance of confirmatory testing in some cases (Rasher et al., 2020). The accuracy of the system is also dependent on the continual updating of its database, which may lag behind emerging resistance trends if not maintained regularly. Moreover, the system is limited to bacteria included in its testing

panels, and it may not be suitable for all clinical isolates. Overall, the VITEK 2 system is considered a reliable and efficient tool, but it must be complemented with clinical judgment and phenotypic confirmation when necessary (Reller et al., 2018).

2.3.1 Overview of Antibiotic Resistance Profiles of Chronic Wound Pathogens

Antimicrobial resistance is a growing global health problem that occurs when microorganisms evolve mechanisms to resist the effects of antimicrobial drugs (Salam. et al., 2023). Although the development of AMR is a naturally occurring process, overuse and misuse of antimicrobial drugs has led to the evolution and spread of bacterial resistance to most of the routinely used antibiotics (Lushniak et al., 2014). Chronic wounds harbor a diverse array of bacterial species, frequently existing as polymicrobial communities (Dowdell et al., 2008). Several key pathogens consistently emerge with the most frequent being *Staphylococcus aureus,* which is a gram-positive bacterium, a frequent skin colonizer, and a major cause of chronic wound infections (Percival et al., 2015).

2.3.2 Mechanism of Antimicrobial Resistance in Chronic Wounds

Bacteria have evolved mechanisms of drug resistance to avoid killing by antimicrobial molecules, a process that has likely occurred over millions of years of evolution. Table 2 displays some of the resistance mechanism for specific antibiotics while Table 3 is showing some of antibiotic resistance genes, antibiotic class and their resistance mechanisms.

Table 2: Showing Some Resistance Mechanism of Specific Antibiotics

Resistance mechanism	Antibiotic
Efflux	Fluoroquinolones, Aminooglycosides, Tetracycline, Beta – Lactams and Macrolides
Immunity and Bypass	Tetracyclines, Trimethoprime, Sulfonamides and Vancomycin
Target Modification	Fluoroquinolones, Vancomycin, Macrolides, Rifamycins, Penicillinis and Aminoglycosides
Inactivating Enzymes	Beta–lactams, Aminoglycosides, Macrolides and Rifamycins

Source: Wright et al., 2010

Table 3: Some Antibiotic Resistance Genes

Antibiotic Resistance Gene	Antibiotic Class	Mechanism of Resistance	References
mecA	Beta-lactams	Encodes penicillin-binding protein 2a (PBP2a), which has a low affinity for beta-lactams, leading to methicillin resistance	Katayama *et al*., (2000)
blaZ	Beta-lactams	Produces beta-lactamase, an enzyme that hydrolyzes the beta-lactam ring, rendering the antibiotic ineffective	Becker *et al*., (2014)
Antibiotic Resistance Gene	Antibiotic Class	Mechanism of Resistance	References
aac(6')-Ie-aph(2'')-Ia	Aminoglycosides	Encodes bifunctional enzyme that modifies aminoglycosides, leading to resistance	Adhikari *et al*., (2014)
aph(3')-IIIa	Aminoglycosides	Phosphorylates aminoglycosides, inactivating them	Ploy *et al*., (2000)
Antibiotic Resistance Gene	Antibiotic Class	Mechanism of Resistance	References
vanA	Glycopeptides	Alters the target of glycopeptides, reducing binding affinity and leading to vancomycin resistance	Perichon *et al*., (2009)
ermA/ermC	Macrolides	Methylates 23S rRNA, preventing macrolide binding and causing resistance	Leclercq, (2002)
msrA/msrB	Macrolides, Streptogramins B	Encodes an efflux pump that expels the antibiotic from the cell	Ross *et al*., (1990)
tetK/tetM	Tetracyclines	Encodes an efflux pump (tetK) or ribosomal protection protein (tetM), both leading to tetracycline resistance	Chopra & Roberts, (2001)

2.3.2 Multiple Antibiotic Resistance Index

The Multiple Antibiotic Resistance (MAR) index is a widely used tool for quantifying the overall burden of resistance in bacterial isolates. By condensing resistance data into a single numerical value, it enables rapid comparisons across strains, sources, or

sampling periods, making it especially valuable in epidemiological and surveillance studies. Higher MAR values typically reflect exposure to environments with greater antibiotic selection pressure and an increased likelihood of multidrug resistance (Tang *et al*., 2023).

Several studies have demonstrated the utility of MAR in both clinical and environmental contexts. For instance, it has been applied to monitor resistance trends in hospital pathogens, foodborne bacteria, and waterborne isolates, thereby helping to trace potential sources of contamination and guide antibiotic stewardship strategies (Wanja *et al*., 2020). The index also complements categorical definitions such as multidrug resistance (MDR), as it provides a continuous measure of resistance burden, allowing for finer resolution in detecting trends over time or between populations (Mori *et al*., 2025).

Despite its usefulness, interpretation of MAR values requires caution, as results can be influenced by the number and choice of antibiotics included in testing. Broader antibiotic panels may inflate MAR values even when clinical resistance remains moderate, highlighting the need for context-specific benchmarks (Sohaili *et al*., 2024). Nevertheless, MAR remains a simple yet powerful indicator for resistance surveillance, risk assessment, and policy development.

2.4 Resistance Determinants and Virulence Factors in *Staphylococcus aureus*

Whole-genome sequencing (WGS) has transformed the study of *Staphylococcus aureus* by enabling precise identification of resistance genes, alleles, and mobile genetic elements (MGEs), as well as the genomic environments in which they occur. Unlike conventional phenotypic methods, WGS captures the true genetic composition of a strain, providing a high-resolution platform for surveillance, outbreak investigation, and informed therapeutic choices (Quainoo *et al*., 2017). Resistance determinants in *Staphylococcus aureus* include β-lactamases, efflux pumps, and methyltransferases, which can exist in allelic variants influencing the level and spectrum of resistance. Many of these genes are carried on MGEs such as plasmids, integrons, and transposons, facilitating horizontal gene transfer and the co-selection of multiple resistance traits. In addition to acquired genes, chromosomal mutations in loci such as gyrA/gyrB, rpoB,

and fusA1 contribute significantly to antimicrobial resistance (Partridge *et al*., 2018). Whole genome sequencing (WGS) is particularly useful in reconstructing the structure of MGEs and mapping co-occurring determinants, thereby offering insights into the dynamics of resistance evolution.

Virulence in *Staphylococcus aureus* is mediated by a diverse array of genetic factors. Toxin genes, including those encoding enterotoxins, Panton-Valentine leukocidin, and toxic shock syndrome toxin, are central to pathogenicity. Adhesins and surface proteins such as protein A, fibronectin-binding proteins, clumping factors, and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) play critical roles in colonization and immune system evasion. Additional immune evasion strategies involve genes such as scn, sak, and chp, while regulatory systems, most notably the agr quorum-sensing network, govern the expression of many virulence determinants in response to environmental conditions (Jenul *et al*., 2019). Importantly, WGS facilitates the study of these virulence factors in their genomic context, including their association with resistance determinants on phages, plasmids, or pathogenicity islands, thereby elucidating their potential for mobility and horizontal transfer.

Beyond cataloguing genetic elements, WGS supports phylogenomic and SNP-based analyses, which are invaluable for identifying transmission pathways, resolving outbreak-related strains, and situating isolates within larger epidemiological frameworks (Su *et al*., 2019). Integrating transcriptomic data with WGS further enhances interpretation, as gene presence alone does not always predict expression or virulence potential. Regulatory networks such as agr and stress-response pathways ultimately shape the phenotypic outcome of virulence gene carriage. The WGS provides a comprehensive approach to understanding resistance and virulence in *Staphylococcus aureus*. By linking genetic determinants to evolutionary dynamics, it not only advances basic scientific understanding but also informs clinical management, antibiotic stewardship, and the design of targeted infection control strategies (Vashisht *et al*., 2023).

2.4.1 Genomic Characterization of *Staphylococcus aureus* using Illumina Sequencing

Next-generation sequencing (NGS) has revolutionized the genetic characterization of *Staphylococcus aureus*, providing high-resolution insights into antimicrobial resistance (AMR), virulence factors, and clonal diversity. Among available platforms, Illumina sequencing has become the most widely adopted due to its accuracy, throughput, and cost-effectiveness, enabling comprehensive analyses that extend beyond the scope of traditional molecular methods (Garg et al., 2021; Satam et al., 2023).

Whole-genome sequencing (WGS) with Illumina data allows systematic identification of AMR determinants, including mecA in methicillin resistance, plasmid-borne beta-lactamases, efflux pumps, and resistance-conferring target modifications. Mobile genetic elements (MGEs), such as SCCmec cassettes, plasmids, and pathogenicity islands, can also be reconstructed, providing insights into horizontal gene transfer and the co-localization of resistance and virulence traits (Partridge et al., 2018; Schmidt et al., 2020). Parallel to resistance profiling, Illumina sequencing facilitates the detection of virulence-associated genes. These include toxins (e.g., Panton–Valentine leukocidin, enterotoxins, TSST-1), immune evasion genes (scn, sak, chp), and adhesion factors such as fibronectin-binding proteins and clumping factors, which contribute to host colonization and immune modulation (Jenul et al., 2019). The genomic co-occurrence of AMR and virulence factors is of particular concern, as it can enhance the pathogenic potential of circulating lineages.

Illumina data also supports in silico typing approaches, including multilocus sequence typing (MLST), spa typing, and SCCmec characterization, which enable clonal complex assignment and epidemiological tracking of methicillin-resistant *Staphylococcus aureus* (MRSA) across healthcare and community settings (Enright et al., 2000; Silva et al., 2015). The integration of typing data into global frameworks is facilitated by platforms such as Pathogen Watch (Harris et al., 2019).

A critical strength of Illumina-based WGS is the availability of standardized bioinformatics pipelines and curated databases. Resistance determinants can be identified using ResFinder, CARD, and PointFinder, while virulence factors are routinely screened through the Virulence Factor Database (VFDB) (Zankari et al.,

2012; Jia et al., 2017; Chen et al., 2016; Zhao et al., 2017). These resources enhance reproducibility and enable cross-study comparisons.

Nonetheless, Illumina sequencing has limitations, particularly in resolving repetitive elements and fully reconstructing MGEs such as SCCmec or large plasmids. To address this, hybrid approaches that combine Illumina short reads with long-read platforms (e.g., Oxford Nanopore, PacBio) are increasingly employed, yielding more complete assemblies and improved resolution of resistance and virulence contexts (Rang et al., 2021; Zhang et al., 2022).

Taken together, Illumina sequencing has become a cornerstone of S. aureus research, underpinning surveillance, outbreak investigation, and antimicrobial stewardship. Its integration with long-read sequencing and functional validation strategies represents a promising direction to fully resolve the genomic architecture and clinical implications of resistance and virulence in this globally important pathogen.

2.4.2 Antibiotic Resistance Genes in *Staphylococcus aureus*

Notable resistance genes found in *Staphylococcus aureus* are; Methicillin-resistant determinant (*mecA*) gene that confers resistance to the bacteria against the bactericidal effect of methicillin, oxacillin, and the other penicillin family members (Kapoor et al., 2017). These antibiotics are β-lactam antibiotics, which bind the bacterial transpeptidases/Penicillin Binding Proteins and bar the complete formation of the bacterial cell wall (Cox, 2015). Coding for the formation of alternative PBP2a, whose β- lactam antibiotics have low affinity for them as they lack the receptors for such antibiotics. In this case, β-lactam antibiotics fail to bind with them and prevent transpeptidation process. (Lade et al., 2023).

The *vanA* gene confers resistance to glycopeptide antibiotics such as vancomycin and teicoplanin by altering the synthesis of peptidoglycan precursors in bacterial cell walls. Normally, these antibiotics inhibit bacterial cell wall synthesis by binding to the terminal D-alanyl-D-alanine (D-Ala-D-Ala) residues of peptidoglycan precursors, preventing the necessary cross-linking for cell wall integrity and leading to bacterial cell death (Perichon et al., 2009). The *vanA* gene encodes enzymes that modify these

precursors by substituting D-alanine with D-lactate (D-Lac). This modification is accomplished through the action of the *VanH* dehydrogenase, which reduces pyruvate to D-Lac, and the *VanA* ligase, which links D-Ala to D-Lac to form the D-Ala-D-Lac dipeptide (Arthur et al., 1996).

Once incorporated into the peptidoglycan precursor, the D-Ala-D-Lac modification significantly reduces the binding affinity of glycopeptide antibiotics. This is due to the loss of a critical hydrogen bond that is present when these antibiotics bind to the D-Ala-D-Ala dipeptide, thus preventing the antibiotics from effectively inhibiting cell wall synthesis (Katayama et al., 2000). Consequently, bacteria with the *vanA* gene can continue synthesizing their cell walls and survive in the presence of glycopeptide antibiotics, leading to antibiotic resistance (Perichon et al., 2009). The D-Ala-D-Ala target residues are synthesized intracellularly as a dipeptide by a D-Ala:D-Ala ligase and are added to UDP-N-acetylmuramyl- L-Ala-g-D-Glu-L-Lys by an adding enzyme (Aqib et al., 2022).

The *qac* gene confers resistance against quaternary ammonium compounds and cationic biocides. The genes are plasmid-encoded capable of transferring to another susceptible bacterium. *qacA* and *qacB* genes are similar but 7 base pairs are different in their entire gene. *qac* genes encode for the formation of multidrug efflux pumps in the bacterial membranes. These efflux pumps are membrane proteins that export quaternary ammonium compounds. Different *qac* genes encode for different types of *qac* efflux pumps. Though, the roles and modes of action of all the pumps are almost the same (McMurry et al., 1998).

The *NorA* gene is an antibiotic resistance gene that codes for efflux pump conferring resistance against quinolones and fluoroquinolones (Ferreira et al., 2022). Erythromycin ribosomal methylase (*erm*) gene conferrers resistance to bacteria against MLS including macrolide such as erythromycin, azithromycin, clarithromycin, lincosamides such as clindamycin, lincomycin and streptogramins such as pristinamycin, virginiamycin. *erm* genes confer resistance against MSL antibiotics by modifying the target site, the bacterial ribosome (Lina et al., 1999).

The *mph* (A) gene confers resistance to macrolide antibiotics in *Staphylococcus aureus* and other bacterial species by encoding a macrolide phosphotransferase enzyme. This enzyme inactivates macrolides through phosphorylation, rendering them ineffective. Macrolides, such as erythromycin, clarithromycin, and azithromycin, function by binding to the 50S ribosomal subunit of bacterial cells, thereby inhibiting protein synthesis and effectively halting bacterial growth. The binding occurs at the peptidyl transferase center of the ribosome, preventing the elongation of the protein chain (Leclercq, 2002).

The *mph* (A) gene produces an enzyme that modifies macrolides by adding a phosphate group to the antibiotic molecule. This phosphorylation occurs at 2'-hydroxyl group of the desosamine sugar moiety in the macrolide structure, which is crucial for its binding to the ribosome. By adding a phosphate group, the enzyme sterically hinders the macrolide from binding to the ribosomal subunit, thus nullifying its inhibitory effect on protein synthesis (Noguchi et al., 2000). This resistance mechanism is particularly vital because it can be transferred horizontally among bacteria through plasmids, leading to the spread of resistance within bacterial populations.

The *msr*(A) gene is an antibiotic-resistant gene conferring bacterium resistance against macrolides found on mobile elements of the Staphylococcal chromosome and plasmid and protects the bacteria from erythromycin and streptogramin-B and also offers efflux-based resistance to *Staphylococcus* spp. and other bacteria (Miklasińska et al., 2021). The synthesis of macrolide 2′-phosphotransferase I which inactivates erythromycin, is inducible by erythromycin. The expression of high-level resistance to erythromycin requires the *mph* (A) and *mrx* genes, which encode *mph* (A) and an unidentified protein (Noguchi et al., 2000). The resistance genes can be identified through molecular techniques like PCR, DNA microarray method, and probe detection method. Whole genome sequencing identifies the causative variants and novel genome assembly detecting single nucleotide variants, insertions/deletions, copy number changes, and large structural variants (Chaplin et al., 2021).

2.4.2.1 The Dual Threat of *Staphylococcus aureus*: Integrating Resistance and Virulence Determinants

Staphylococcus aureus possesses a diverse repertoire of antimicrobial resistance determinants that underpin its success as both a commensal and pathogen. The most clinically significant are mecA and mecC, which encode the alternative penicillin-binding protein PBP2a and confer methicillin resistance, defining MRSA (Chambers, 2001; Ito et al., 2019). Additional resistance arises from genes such as blaZ (beta-lactamase production), as well as determinants conferring resistance to tetracyclines, aminoglycosides, macrolides, and fluoroquinolones via efflux, enzymatic inactivation, or target modification. The genetic background of MRSA is further shaped by SCCmec elements, whose structural diversity distinguishes hospital-associated from community-associated lineages (Kurosawa et al., 2012). Importantly, many of these determinants are mobilized by plasmids, transposons, and other MGEs, enabling clonal expansion and inter-strain dissemination of resistance within healthcare environments (Lindsay, 2010).

Parallel to resistance, *Staphylococcus aureus* expresses a wide spectrum of virulence factors that mediate colonization, immune evasion, and host damage. Key toxins include alpha-hemolysin (Hla), leukocidins (including PVL), enterotoxins, and superantigens, which contribute to invasive disease and toxic shock syndromes (Dinges et al., 2000; Diep & Chambers, 2008). Virulence is further enhanced by surface adhesins such as clumping factors, fibronectin-binding proteins, and protein A, which facilitate biofilm formation and persistence (Kobayashi & DeLeo, 2013).

Recent studies emphasize the interplay between resistance and virulence, noting that certain MRSA lineages simultaneously harbour multidrug resistance and potent virulence determinants, thereby complicating treatment and infection control (Tso et al., 2010). Surveillance efforts, therefore, integrate SCCmec typing with virulence gene profiling to track high-risk clones and anticipate clinical challenges (Liu et al., 2019). This dual focus has revealed significant heterogeneity across patient populations and geographic settings, highlighting the dynamic evolution of *Staphylococcus aureus* resistance–virulence networks (Chambers & DeLeo, 2009).

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2.4.2.2 Staphylococcal Cassette Chromosome Mec Elements in *Staphylococcus aureus*

Methicillin resistance in *Staphylococcus aureus* is conferred by the staphylococcal cassette chromosome mec (SCCmec), a crucial mobile genetic element that serves as the defining marker of MRSA (Kong et al., 2019). The SCCmec diversity reflects the evolutionary adaptation of *Staphylococcus aureus* across clinical and community contexts, making its structural variability central to both molecular epidemiology and antimicrobial resistance research (Zhou et al., 2020).

Staphylococcal chromosomal cassette mec elements are classified based on the mec gene complex, ccr recombinase complex, and joining (J) regions. Historically, six major types (I–VI) were described, but current classification recognizes at least 13 types (I–XIII) and multiple subtypes (Oliveira & de Lencastre, 2020; Zhang et al., 2023). Distinct structural characteristics underpin their epidemiological associations: smaller elements such as Type IV are frequently linked to community-acquired MRSA (CA-MRSA), supporting their efficient transfer, while larger elements such as Types II and III predominate in hospital-associated MRSA (HA-MRSA), often harboring additional resistance determinants (Liu et al., 2021; Kumar et al., 2023).

Recent studies highlight the plasticity of SCCmec. Novel variants and hybrid configurations, including emerging Type VIII and Type V (5C2), have been reported across diverse regions, often displaying atypical gene arrangements (Clarke et al., 2023). In parallel, cryptic or untypeable SCCmec elements have been identified, underscoring the ongoing genetic flux of these cassettes and the limitations of current typing schemes (Chen et al., 2022). This dynamic nature complicates molecular surveillance and suggests that SCCmec evolution remains an active process shaped by horizontal gene transfer.

From a clinical perspective, SCCmec type distribution carries important therapeutic implications. Although CA-MRSA strains typically exhibit more limited antimicrobial resistance profiles compared to HA-MRSA, their capacity for rapid acquisition of additional resistance determinants presents concerns regarding the emergence of multidrug-resistant phenotypes (Zhou et al., 2020). Conversely, HA-MRSA, with its

larger SCCmec elements, presents an immediate challenge due to the accumulation of multiple resistance genes and limited treatment options (Liyanage et al., 2022). Comparative summaries of SCCmec structures (Table 4) highlight these differences and provide a framework for understanding the evolutionary and epidemiological dynamics of MRSA.

Table 4: Key SCCmec Elements

Type	mec gene complex	ccr gene complex	Characteristic	Typically Associated With
i	mec complex A	ccrA1B1	Large, multidrug resistance	HA-MRSA
ii	mec complex A	ccrA2B2	Large, multidrug resistance	HA-MRSA
iii	mec complex A	ccrA3B3	Largest, multi-resistance	HA-MRSA
iv	mec complex B	ccrA2B2	Smaller, less resistance	CA-MRSA
v	mec complex C2	ccrC	Less resistance, mobile	CA-MRSA / community variants
viii	mec complex B	ccrC	Emerging variant	Various settings

Source: Center for Disease Control and Prevention (CDC). (2023)

2.4.2.3 The Staphylococcal Cassette Chromosome Mec Elements based on Gene Content and Structure in *Staphylococcus aureus*

A complex mobile genetic element essential to methicillin resistance in *Staphylococcus aureus* is the staphylococcal cassette chromosome mec (SCCmec). Variations in gene content and structural organization, specifically the mec gene complex, ccr gene complex, and the upstream and downstream junction regions are crucial to its classification (Zhou et al., 2020).

The mec gene complex, comprising mecA, mecI, and mecR1, is a core component commonly located within SCCmec elements. (Zhou et al., 2020). The penicillin-binding protein (PBP2a), which is encoded by the *mecA* gene, provides resistance to β-lactam antibiotics. *MecR1* encodes a sensor-transducer that regulates *mecI* repression in response to the presence of β-lactam, whereas the *mecI* gene functions as a repressor of *mecA* expression (Oliveira & de Lencastre, 2020). Gene organization and regulatory

elements are used to classify the mec gene complex into several categories (A, B, C, etc.).

The ccr gene complex, which is next to the mec complex and mostly consists of *ccrA* and *ccrB*, encodes site-specific recombinases that give SCCmec its mobility (Kong et al., 2019). The sequencing and mobility potential of variations like *ccrA2, ccrB2*, *ccrA3*, and *ccrC* vary, which affects how resistance determinants spread.

Structural Variability and SCCmec Types: J (joining) regions, which frequently contain extra resistance genes, transposons, or insertion sequences like IS1272, further differentiate SCCmec elements. These areas allow auxiliary genes to be acquired or lost, which adds to SCCmec's genetic flexibility (Zhou et al., 2020). Different varieties range in size and composition; Type III is larger and frequently multidrug resistant, whereas Type IV is smaller and lacking numerous resistance genes.

Ongoing genetic evolution is reflected in recent structural variants including IVa(2B), V, and VIII, some of which contain novel ccr gene complexes or hybrid arrangements. The pathogenicity and resistance profiles of *S. aureus* strains may be affected by these variations (Clarke et al., 2023).

Variations in both gene content and structure have an impact on clinical resistance patterns as well as epidemiology. Larger SCCmec types are related with hospital-associated strains and multi-resistance, but smaller, more mobile types (like IVa) are common in community strains and promote quick spread (Kong et al., 2019).

2.4.2.4 Regulatory Genes within the Staphylococcal Cassette Chromosome Mec in *Staphylococcus aureus*

In order to preserve bacterial resources, a group of genes closely regulates the *mecA* gene, which codes for the low-affinity PBP2a enzyme that causes β-lactam resistance. This ensures that resistance is expressed only when necessary (Zhou et al., 2020). *MecI*, *mecR1*, and in certain variations, *mecR2 a*re among these genes. *MecI*: Represses *mecA* by attaching itself to its promoter region and blocking expression in non-stressful circumstances. According to Oliveira and de Lencastre (2020), its activity guarantees

that the bacteria do not needlessly suffer the fitness penalty linked to high-level resistance.

The expression of mecA in MRSA is tightly controlled by a regulatory system composed of the repressor MecI and the sensor-transducer MecR1. In the absence of β-lactam antibiotics, MecI attaches itself to the mec operator and prevents the transcription of MecA. The transmembrane sensor-regulator MecR1 responds to the presence of β-lactam antibiotics by undergoing autocleavage or structural changes, which initiates its protease activity (Kong et al., 2019). Activated MecR1 alleviates transcriptional repression by cleaving MecI, which permits mecA expression and the ensuing synthesis of PBP2a. This inducible mechanism allows MRSA to rapidly develop methicillin resistance when exposed to β-lactam drugs.

Some SCCmec types have *mecR2*, which further modifies the regulatory network and affects expression levels and resistance stability (Clarke et al., 2023). Control of SCCmec Mobility: Recombinases that facilitate SCCmec excision and integration into the *S. aureus* chromosome are encoded by the ccr gene complex, which is made up of the *ccrA* and *ccrB* genes. Although they play a major role in movement, their modulation is also essential for limiting the spread of SCCmec (Kong et al., 2019). The effectiveness of SCCmec elements' transfer between strains is influenced by variations in *ccr* gene activity.

On extra regulatory and accessory genes, SCCmec may contain additional resistance genes, insertion sequences (like IS1272), and regulatory elements that work together to control gene expression, stability, and horizontal transmission. In a variety of ecological niches, these components enable adaptive responses in response to environmental stresses (Zhou et al., 2020).

Clinical Significance of Regulatory Elements: MRSA strains' capacity to adapt to antimicrobial environments and their phenotypic resistance levels are influenced by regulatory genes that govern the production of resistance determinants. Treatment may become more difficult if *mecI* or *mecR1* mutations or changes cause constitutive *mecA*

expression, which can result in high-level resistance even in the absence of antibiotic pressure (Oliveira & de Lencastre, 2020).

2.4.3 Virulence Factors in *Staphylococcus aureus*

Staphylococcus aureus expresses many virulence factors including the pore-forming toxins like hemolysins, leukotoxins and enzymes such as nucleases, staphylokinase, coagulases, epidermolytic toxins, and superantigens (SAgs) (Lacey et al., 2010). Some of the virulence factors involved in wound progression are shown in Appendix III. Panton–Valentine leukocidin (PVL) encoded by two genes, *luk-S-PV*, and *luk-F-PVL* is an extracellular protein with dermonecrotic and leucocidal functions. It has cytotoxic activity against mammalian neutrophils, monocytes, and macrophages. The toxin can be produced both by community-acquired *Staphylococcus aureus* PVL-positive strains usually cause surgical site infections (SSTIs) they are also associated with severe infections, including septic arthritis, bacteremia, necrotizing pneumonia, and purpura fulminans (Shallcross et al., 2013).

Exfoliative toxins (ETs) are serine proteases that recognize and hydrolyze desmosome proteins in the skin playing a role in host colonization and the invasion of injured mucosa and skin. ETs are causative agents for localized epidermal infections and generalized disease staphylococcal scalded skin syndrome (SSSS). Serotypes ETA, ETB, and ETD are also associated with septic shock and may increase the severity of the infection (Yamaguchi et al., 2002). Toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins (SEs), contribute to the development of serious infections due to the activation of enormous numbers of T lymphocytes. TSST-1 is an exotoxin produced by approximately 5–25% of *Staphylococcus aureus* strains (Fraser, and Proft, 2008).

Specific clonal lineages and virulence factors such as TSST-1, leukocidins, enterotoxins, and exfoliatins play a significant role in determining wound outcomes (Shettigar et al., 2020). *Staphylococcus aureus* strains from chronic wounds were more often resistant to antibiotics compared to strains from blood. Strains from chronic wounds had a significantly higher prevalence of resistance to certain aminoglycoside antibiotics (Budzyńska et al., 2021). A table of *Staphylococcus aureus* virulence factors involved in wound progression is indicated in Appendix III.

2.4.3.1 Toxin-Encoding genes in *Staphylococcus aureus*

With virulence determinants frequently present on chromosomes, plasmids, or mobile elements that can transfer between strains and species, toxin genes in chronic wounds affect persistence, tissue damage, and host response (Liu, Chen, & Wang, 2019). The expression of toxin genes, including cytolysins and proteases, is regulated in the wound environment, which is typified by hypoxia, necrotic tissue, biofilm formation, and persistent inflammation, in order to facilitate survival and immune evasion (Mavor, Le, & Bassler, 2018). Targeted therapy and infection control can be guided by molecular detection of toxin genes by PCR, qPCR, or sequencing, which offers actionable insights into pathogen aggressiveness (Lakić, Novak, & Petrovic, 2020) The generation of toxins in biofilms leads to antibiotic resistance and chronicity, underscoring the necessity of virulence-regulating techniques in addition to traditional antimicrobials (Fraser, Patel, & Green, 2017

2.4.3.2 Exoenzyme Gene in *Staphylococcus aureus*

A variety of exoenzymes that *Staphylococcus aureus* possesses greatly enhance its pathogenicity. Among these, *aur*, which genes for aureolysin, has a variety of functions as a zinc-dependent protease that breaks down immunological molecules like antimicrobial peptides and complement components, reducing immune responses and encouraging tissue invasion (Hsu et al., 2022). Aureolysin increases proteolytic activity at the infection site by activating more proenzymes. The serine protease-like (Spl) enzymes, *SplA*, *SplB*, and *SplE*, are a gene family that complements aureolysin and has a variety of roles in tissue destruction and immune evasion. *SplB* leads to tissue necrosis by breaking down immunoglobulins and complement factors, which weakens host defenses, while *SplA* cleaves host proteins, allowing immunological escape and colonization (Clarke et al., 2023).

Although its exact roles are still being studied, *SplE* seems to help in immune regulation and nutrition uptake (Miller et al., 2022). Global regulatory systems like agr frequently manage the co-regulation of these enzymes, coordinating their synthesis throughout several stages of infection. *S. aureus* is able to infiltrate tissues, elude immunological responses, and create long-lasting infections due to the cooperation of the aur and spl proteases. According to recent genomic research, these protease genes play a crucial

role in pathogenicity because strains with many copies or variations of these genes are linked to more serious or persistent infections (Hsu et al., 2022; Miller et al., 2022). According to recent genomic investigations, strains of these protease genes that have many copies or variations are typically linked to infections that are severe, invasive, or chronic (Clarke et al., 2023). Global systems like agr regulate these enzymes, enabling the bacteria to synchronize the expression of proteases with the stages of infection progression.

2.4.3.3. Host Immune Evasion (Hostimm Gene) *Staphylococcus aureus*

Staphylococcus aureus secretes immuno-modulatory genes such Sak (staphylokinase) and Scn (staphylococcal complement inhibitor) as part of a complex attempt to weaken host immune responses. In order to help bacteria, survive and persist during infection, these virulence factors are essential for avoiding host immune responses.

Sak is the gene for staphylokinase, an extracellular protein that is released and acts as an activator of plasminogen. It changes host plasminogen into plasmin, a broad-spectrum protease that breaks down immunological mediators, fibrin clots, and extracellular matrix elements. This action aids *S. aureus* in escaping immunological trapping inside fibrin matrices and promotes bacterial dispersion through tissues (Li et al., 2023). By breaking down cytokines and chemokines, *Sak* may also weaken host immune responses by lowering leukocyte migration to infection locations (Hsu et al., 2022).

Staphylococcal Complement Inhibitor, or Scn, is an essential part of *S. aureus'* immune evasion toolbox because it inhibits complement. It directly prevents complement-mediated bacterial death by binding to human C5b-9 complexes and preventing the membrane attack complex (MAC) from forming (Marraffini et al., 2019). By doing this, *S. aureus* prevents phagocytosis and opsonization, which helps the germs survive inside the host. As a member of the immune evasion cluster (IEC), the *scn* gene is frequently found in close proximity to other immune-modulatory genes, underscoring its significance in chronic infections (Rooijakkers et al., 2020).

The ability of *Staphylococcus aureus* to evade the immune system is improved by the cohabitation of the *sak* and *scn* genes; *sak* facilitates bacterial spread by dissolving

fibrin barriers, while *scn* inhibits complement activation and phagocytosis. Particularly in bloodstream and deep tissue infections, their tightly controlled coordinated expression throughout infection offers a benefit for long-term colonization (Li et al., 2023; Hsu et al., 2022).

The significance of these immune evasion genes for virulence has been highlighted by recent genomic studies that have revealed that strains carrying numerous copies or variations of these genes are typically more invasive and linked to poor clinical outcomes (Rooijakkers et al., 2020).

2.4.4 Spa Typing in *Staphylococcus aureus*

The polymorphic X region of the *spa* gene, which codes for staphylococcal protein A, is sequenced to perform spa typing, a highly selective molecular typing technique. Spa typing is a useful tool for epidemiological research, outbreak tracking, and comprehending the dynamics of *S. aureus* transmission because of the heterogeneity in the amount and sequence of repeats within this region, which allows for precise strain classification (Harmsen et al., 2019). Several short tandem repeats found in the spa gene can differ from isolate to isolate, resulting in a variety of spa types, including t355, t318, t84, t13194, t37, t1476, t62, and t2036. Methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) strains are frequently linked to t355, which is one of them that is commonly seen in clinical and community settings (Harmsen et al., 2019).

The epidemiological relevance of Spa Type t355 is demonstrated by its notable prevalence across multiple geographic regions and its association with nosocomial outbreaks and community-acquired infections (Xie et al., 2021). The fact that several isolates have spa type t355 indicates that clonal spread may occur in medical facilities or the general public. Although less prevalent than t355, other Spa Types including t318, t84, and t37 are linked to certain lineages or outbreaks and may have distinct resistance or pathogenicity profiles (Harmsen et al., 2019). Rarer kinds like t13194, t1476, and t2036 have been found, which suggests that *Staphylococcus aureus* populations continue to evolve and exhibit genetic variety.

Monitoring spa types yields information about the prevalence of pathogens, patterns of strain transmission, and the effectiveness of infection management strategies. For instance, the frequency of spa type t355 in various contexts underscores its part in persistent transmission and the significance of ongoing molecular monitoring for infection control measures. Table 5 displays some spaTypes and their significance

Table 5: Some Spa Types and their Significance

Spa Type	Significance / Associations	References
t355	Both MRSA and MSSA share this trait; it is linked to outbreaks and community dissemination.	Harmsen et al., 2019; Xie et al., 2021
t318, t84, t37	less common, connected to particular clones or breakouts	Harmsen et al., 2019
t13194, t1476, t62, t2036	Infrequent, suggesting continuous genetic diversity	Harmsen et al., 2019

2.5 Genetic Diversity of Resistant *Staphylococcus aureus* Isolates

The genetic diversity of resistant *Staphylococcus aureus* reflects a complex interplay of clonal evolution, horizontal gene transfer, and selective pressures from antimicrobial use. This diversity is structured into distinct clonal complexes (CCs) and sequence types (STs), many of which display host specificity, for example, lineages adapted to humans, livestock, or poultry, and distinct repertoires of antimicrobial resistance (AMR) determinants such as mecA, blaZ, and tetracycline resistance genes (Chambers, 2001; Ito et al., 2019). Characterizing this diversity is critical for understanding the epidemiology, persistence, and clinical impact of resistant *Staphylococcus aureus.*

Early studies used pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to reveal the global clonal population structure of MRSA, showing the dominance of a few successful lineages such as CC5, CC8, CC22, and CC398 (Enright et al., 2000; Kwon et al., 2014). These clonal complexes are associated with different SCCmec elements and resistance phenotypes, linking genetic diversity to both ecological niche and clinical outcome. More recent approaches, including spa typing, core-genome MLST (cgMLST), and whole-genome SNP-based phylogenetics, have provided greater resolution, enabling outbreak tracking and the detection of emerging clones with novel resistance and virulence gene combinations (Thomson et al., 2017; Adame et al., 2020).

The population structure of S. aureus consists of a conserved core genome, largely shaped by point mutations and recombination, and a highly variable accessory genome composed of mobile genetic elements (MGEs) such as plasmids, prophages, transposons, and pathogenicity islands (Fitzgerald et al., 2016; Al-Jabri & Al-Mebairik, 2023). Horizontal acquisition of MGEs drives much of the observed genetic heterogeneity, contributing to differences in virulence, resistance profiles, and host adaptation across lineages (Lebeurre et al., 2019). For example, livestock-associated CC398 demonstrates tetracycline resistance and reduced human virulence gene content, whereas community-associated MRSA often couples SCCmec IV with Panton-Valentine leukocidin genes, reflecting distinct evolutionary trajectories (Edwards et al., 2016; Liu et al., 2019).

2.5.1 Clonal Assignment and eBURST.

The BURST and goeBURST algorithms, applied to MLST data, remain foundational for identifying clonal complexes and inferring evolutionary descent among STs (Heritage et al., 2015). These tools enabled the recognition of globally disseminated MRSA lineages and the reconstruction of their diversification. However, reliance on seven housekeeping genes limits resolution, especially for recent outbreaks, leading to increasing reliance on cgMLST and SNP-based methods (Bosi et al., 2016).

2.5.2 Population Structure and Genetic Variation

Comparative analyses demonstrate that SNP-based phylogenies and cgMLST outperform classical MLST for distinguishing closely related isolates and uncovering microevolutionary dynamics (Méric et al., 2018; Stegger et al., 2020). These high-resolution approaches are critical for outbreak investigation and for tracing the spread of resistant clones across healthcare and community settings. At a broader scale, population structure analyses reveal that S. aureus diversity is not randomly distributed, but shaped by selective pressures such as antibiotic use, immune evasion, and host adaptation (Adame et al., 2020).

2.5.3 Phylogenomics

Phylogenetic methods have advanced substantially with the integration of whole-genome sequencing. Maximum likelihood and Bayesian approaches, implemented in

tools such as RAxML, IQ-TREE, and BEAST, allow robust reconstruction of *Staphylococcus aureus* evolutionary histories, including divergence times, ancestral host transitions, and the emergence of resistance traits (Nguyen et al., 2015; Sukumaran & Holder, 2023). Whole-genome data have revealed frequent recombination events, rapid clonal expansions, and recurrent acquisition of AMR determinants, underscoring the evolutionary plasticity of the species (Guindon et al., 2021).

Single-nucleotide polymorphisms (SNPs) play a central role in high-resolution genotyping, outbreak tracing, and linking specific mutations to phenotypic traits such as resistance or virulence (Méric et al., 2018; Li et al., 2019). Notably, SNP-based approaches have demonstrated that resistance can arise both through horizontal gene transfer and through recurrent mutations in conserved targets such as gyrA and rpoB (Stegger et al., 2020). These findings highlight the complementary roles of vertical and horizontal evolution in shaping S. aureus populations.

2.6 Clonal Complexes of *Staphylococcus aureus*: Insights from Multilocus Sequence Typing

Clonal complexes (CCs) are groups of related bacterial strains that share a common ancestor, often defined through multilocus sequence typing (MLST). Understanding CCs is central to bacterial population genetics, as they reveal patterns of evolution, transmission, and epidemiology. For pathogens such as *Escherichia coli*, *Neisseria meningitidis*, and *Staphylococcus aureus*, CCs provide a framework for monitoring epidemic lineages and tracking the spread of antimicrobial resistance (Maiden et al., 1998).

While conventional MLST has been the cornerstone of CC determination, recent advances in whole-genome sequencing (WGS) provide much higher resolution in reconstructing evolutionary relationships. Studies have shown that CCs are frequently associated with specific virulence determinants, resistance traits, and ecological niches. For example, CC8 and CC5 in S. aureus are strongly linked to healthcare-associated infections (Hussain et al., 2022), while the international spread of CC11 in N. meningitidis has been responsible for multiple invasive meningococcal outbreaks (Smith et al., 2023). However, recombination and horizontal gene transfer can blur CC

boundaries, emphasizing the importance of genomic approaches that integrate tools such as eBURST, BAPS, and core-genome MLST for precise lineage assignment (Johnson et al., 2024). Together, these developments demonstrate that CCs remain an indispensable unit of analysis for linking bacterial evolution with clinical and epidemiological outcomes, particularly when combined with WGS-based surveillance.

2.6.1 The Clonal Complex of *Staphylococcus aureus*

In *Staphylococcus aureus*, CCs represent the major evolutionary lineages within the global population and are closely associated with pathogenicity, host adaptation, and resistance profiles. Certain CCs are geographically widespread and clinically dominant. For instance, CC8 includes numerous MRSA lineages, notably USA300, a leading cause of community-associated infections in North America (Kong et al., 2020). CC30 and CC45 are also widely distributed, with strong associations with both hospital- and community-acquired infections (Zhang et al., 2021). By contrast, CC398 is primarily livestock-associated, while CC5 includes hospital-adapted strains, underlining the ecological versatility of S. aureus. These examples illustrate how clonal complex assignment not only facilitates tracking of epidemic strains but also provides insight into virulence patterns and informs targeted interventions (Kong et al., 2020).

2.6.2 Global and Regional Distribution of *Staphylococcus aureus* Clonal Complexes

Clonal complexes are defined as sequence types (STs) that share four or more identical alleles with a central genotype, unless they match another central genotype more closely (Maiden et al., 1998). Multilocus sequence typing (MLST) data curated in international databases have revealed the global distribution of several dominant CCs, including CC1, CC5, CC8, CC22, CC30, CC45, CC59, CC80, and CC239 (Monecke et al., 2023). While many of these lineages are globally disseminated, others exhibit regional restriction.

In Africa, genotypic data remain limited but growing evidence indicates circulation of major CCs such as CC5, CC7, CC21, CC30, CC121, and CC152 (Ibrahim et al., 2023). In Kenya specifically, reported sequence types include ST5, ST8, ST22, ST30, ST88, ST152, ST239, ST241, ST789, and ST4705, belonging largely to CC5, CC8, CC22, and CC30 (Njenga et al., 2022). These findings highlight both the presence of globally

dominant clones and region-specific diversification within the Kenyan *Staphylococcus aureus* population.

2.6.3 Clonal Complexity of *Staphylococcus aureus* in Kenya

Kenya demonstrates substantial clonal diversity of *Staphylococcus aureus* in both community and healthcare settings. Widely recognized global lineages such as ST239 and ST5 coexist with locally emerging strains, contributing to the complexity of the circulating population. Factors including widespread antibiotic misuse, high population mobility, and limited infection-control measures are believed to drive clonal diversification and the emergence of novel resistance variants (Nyasinga et al., 2021; Njiru et al., 2020). Understanding these clonal patterns is critical for designing tailored infection-control strategies and curbing the spread of resistant lineages.

Several molecular methods have been employed for lineage characterization, including pulsed-field gel electrophoresis (PFGE), ribotyping, and PCR-based fingerprinting. However, MLST and increasingly WGS provide the most robust means of clonal assignment. MLST, in particular, has been instrumental in defining the ancestral phylogeny of *Staphylococcus aureus* populations (Maiden et al., 1998; Jolley & Maiden, 2020). Table 6 illustrates the general overview of approaches for genomic characterization. The method relies on sequencing seven housekeeping genes—arcC, aroE, glpF, gmk, pta, tpi, and yqiL—to generate unique allelic profiles that are then assigned to sequence types. STs sharing ≥5 alleles are grouped into CCs, while those sharing all seven are considered clones. Data are deposited into a global database that enables international comparison and epidemiological linkage (Dingle et al., 2020).

Because MLST targets housekeeping genes that evolve slowly and are largely independent of mobile genetic elements, it offers a stable phylogenetic framework for tracing long-term population structure. At the same time, integration with WGS-based approaches provides higher discriminatory power for outbreak investigations, making it possible to track microevolutionary changes in Kenyan *Staphylococcus aureus* lineages with unprecedented resolution (Schmidt et al., 2019; Jolley et al., 2018).

Table 6: Overview of Approaches for Genomic Characterization of *Staphylococcus aureus*

Step	Tool/Approach	Purpose	Kenya-specific Relevance
Sequencing	Illumina Next-Generation Sequencing (NGS)	Generate high-throughput short reads	Provides cost-effective genomic data in Kenyan hospital-based studies
Quality Control & Assembly	Standard bioinformatics pipelines (FastQC, SPAdes, etc.)	Ensure clean, reliable assemblies	Critical for accurate local resistance/virulence gene detection
Annotation	Prokka, RAST, etc.	Identify coding sequences and genomic features	Supports comparison with Kenyan isolates for public health
Resistance Gene Identification	CARD, ResFinder	Detect antimicrobial resistance determinants	Key for monitoring multidrug resistance in Kenyan hospitals
Virulence Gene Profiling	VFDB, PathogenFinder	Identify virulence-associated genes	Explains epidemiology of severe infections in Kenyan settings
Clonal Complex Assignment	MLST, cgMLST, SNP phylogenies	Classify isolates into clonal lineages	Tracks dominant Kenyan *S. aureus* clones
Phylogenetic Analysis	RAxML, IQ-TREE	Establish evolutionary relationships	Helps trace transmission in Kenyan health facilities

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Site

The study was conducted at Meru Teaching and Referral Hospital laboratory, located at approximately 0.05° S latitude and 37.65° E longitude in Meru County, Kenya. The hospital is situated in the southeastern part of Meru County, as illustrated in Figure 1. The county borders Isiolo County to the north and northeast, Tharaka Nithi County to the south, Nyeri County to the southwest, and Laikipia County to the west. Meru County has an estimated population of 1,456,000. The average temperature in Meru County typically ranges from 15°C to 25°C (59°F to 77°F), which can influence microbial evolution and resistance patterns.

Despite well-documented regional variability in *Staphylococcus aureus* epidemiology, comprehensive genomic data for isolates from Meru County and the greater eastern region remain noticeably missing, particularly with regard to strains associated with persistent wound infections. A genome-wide characterization study would facilitate the development of diagnostic tools and treatment strategies specific to a region by elucidating local lineage diversity, virulence factor profiles, and antibiotic resistance patterns. Furthermore, long-term tracking of pathogen evolution would be possible with this type of genomic surveillance, providing crucial data to direct clinical care practices and public health campaigns.

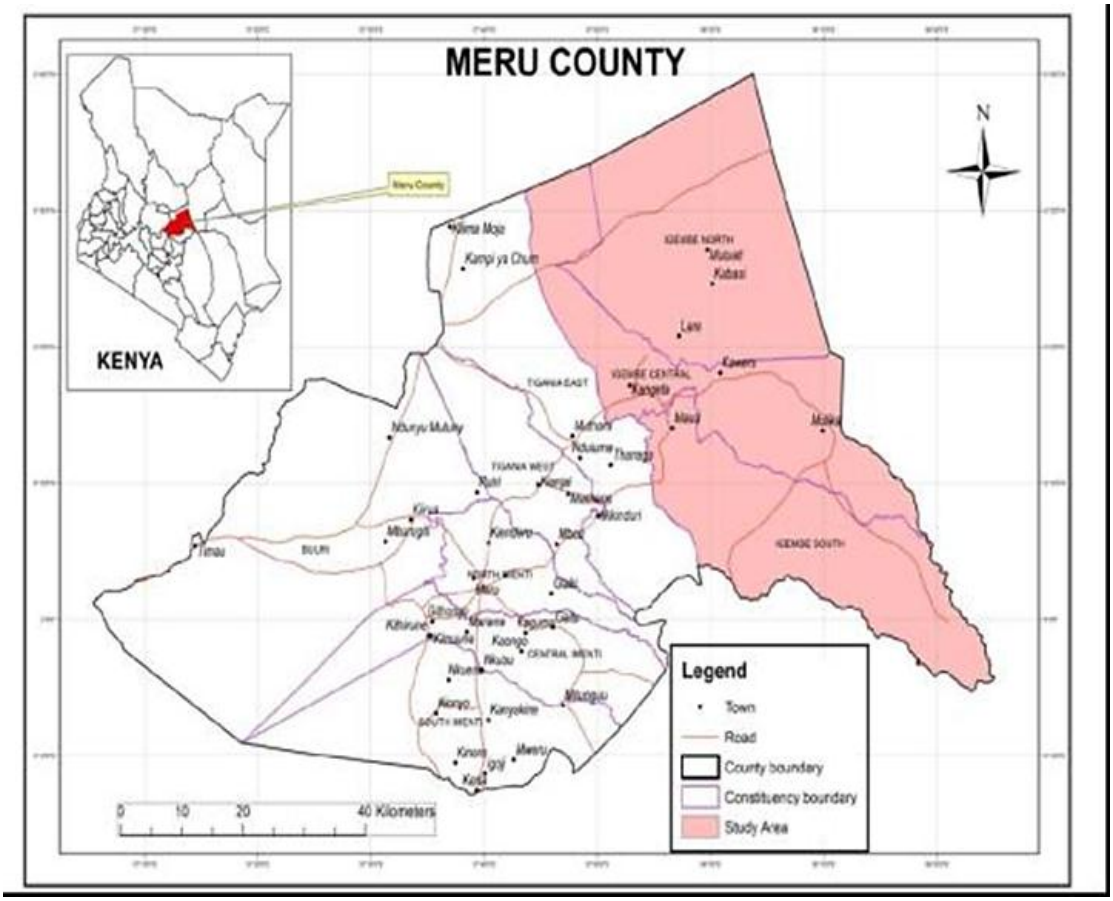

Figure 1: Map of Meru County

3.2 Study Design

This study employed a cross-sectional survey design, which was appropriate for capturing the prevalence and epidemiological patterns of *Staphylococcus aureus* infections and associated risk factors at a single point in time among patients with chronic wound infections at MeTRH. In parallel, a laboratory-based experimental design was applied to isolate, characterize, and genomically profile *S. aureus* strains. This combined approach allowed for linking patient-level epidemiological data with detailed microbiological and genomic analyses, thereby providing a comprehensive understanding of resistance patterns, virulence factors, and clonal diversity.

3.3 Target Population

The study included patients seeking medical treatment at Meru Teaching and Referral Hospital who had been diagnosed with chronic wound infections, and who consented to participate in the study. As per the patient records of Meru Teaching and Referral Hospital for 2023, a population of 293 individuals in four months' time, or roughly 73

patients per month, are anticipated to have chronic wound infections. Hence, the estimated target population was 293

3.4 Sample Determination and Sampling Procedure

3.4.1 Sample Determination

The sample size was calculated using Nassiuma's (2000) formula:

$$n = \frac{NC^2}{C^2 + (N-1)e^2}$$

where,

n = sample size

N = population size (293 in this study)

C = coefficient of variation (usually between 21% ≤ C ≤ 30%), 23.5% in this study

e = standard error (usually between 2% ≤ e ≤ 5%), 0.025 in this study

The coefficient of variation (CC) was fixed at 24%, following Nassiuma (2000), as it provides an optimal balance between sample precision and feasibility within the recommended 21%–30% range.

A 2.5% margin of error was used as it is the conventional level that balances precision with feasibility in sample surveys.

$$n = \frac{293 * 0.235^2}{0.235^2 + (293-1) = 0.235^2} = 68.03$$

Therefore, a sample of 68 patients was used in this study

To determine the sample size for each stratum (Table 7), the following formula was used:

$$n_s = \frac{\text{stratum size}}{Population\ size}\ x\ sample\ size$$

Table 7: Sampling Frame and Proportional Allocation of Study Participants

Stratum	Population size	Sample size
Patient Medical Ward	25	6
Surgical Ward	30	7
Burn Unit:	7	2
Postnatal Ward	40	9
Wound Clinic:	70	16
Diabetic Clinic	30	7
Outpatient Consultation Rooms	91	21
Total	293	68

Source: Self

3.4.2 Sampling Technique

Those who met the eligibility requirements for chronic wound infections were recruited for the study using a convenience sample technique. The selection of easily available patients with chronic wounds who presented at the participating healthcare facilities during the study period was made possible by this non-probability sampling technique.

3.4.2.1 Inclusion Criteria

Patients who freely agreed to participate in the study were recruited from all age groups and genders who presented with chronic wounds of various durations and etiologies. In addition to patient assent, when necessary, parents or legal guardians provided written informed permission for individuals under the age of 18. As long as the wound satisfied the clinical criteria for chronicity, there were no limitations on the type, anatomical location, or length of the wound.

3.4.2.2 Exclusion Criteria

Patients who were unable or unwilling to give their informed consent for participation were not allowed to continue in the trial. Furthermore, because the study was exclusively focused on the microbiology of chronic wound infections rather than acute wound contamination, participants who presented with acute wounds—including traumatic injuries from accidents and recent burn wounds—were not eligible for recruitment.

3.5 Data Collection Procedures

This study collected both clinical and laboratory data to characterize chronic wound infections caused by *Staphylococcus aureus*. Data collection was organized into three main domains: (i) clinical and demographic data from patients, (ii) microbiological and phenotypic data from wound swabs, and (iii) genomic data generated from whole-genome sequencing (WGS) of selected isolates.

3.5.1 Clinical Data Collection

Through a methodical assessment of presenting signs and symptoms in compliance with recognized diagnostic criteria, the clinical diagnosis of chronic wound infections was established. A standardized data collection form was used to methodically record

patient demographic data and pertinent clinical features, such as the etiology, duration, anatomical location, and infection parameters of the wound.

3.5.2 Laboratory Data Collection

3.5.2.1 Sample Collection

Two laboratory officers and one clinical officer, who had undergone training in consent administration and aseptic collection techniques, were responsible for sample collection. After informed consent was obtained, wound swabs were collected aseptically following the laboratory standard operating procedures of the laboratory. Each wound was superficially cleansed with physiologic saline to reduce surface contaminants. Using sterile, pre-moistened swabs, samples were collected by rotating the swab across a 1 cm^2 wound area in a zig-zag motion from the centre toward the periphery. Swabs were then immediately transported to the microbiology laboratory for culture and analysis.

3.5.2.2 Characterization of Bacterial Pathogens Associated with Chronic Wound Infections

Bacterial pathogens in chronic wound infections are described by culture-based isolation on both selective and non-selective media, identification, and antibiotic susceptibility testing using the VITEK 2 automated system. Using turbidimetric analysis and miniature biochemical panels, this platform provides rapid species-level identification and minimum inhibitory concentration (MIC) determination for common wound pathogens, including Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus species, and Enterobacteriaceae. This enables the development of clinically significant antimicrobial resistance profiles that can guide therapeutic interventions.

3.5.2.2.1 Bacterial Isolation and Culture

To reduce loss of viability, wound specimens obtained with sterile swabs were processed as soon as they arrived at the microbiology lab. Three selective and differential culture media MacConkey agar for Gram-negative bacterial differentiation, blood agar for hemolytic activity evaluation, and chocolate agar for picky organisms—were methodically inoculated with each swab. For 24 hours, inoculated plates were incubated aerobically at 37°C in a controlled atmosphere. Bacterial growth was

assessed after incubation, and colonies were first described using morphological characteristics such as size, shape, color, texture, and hemolytic patterns on blood agar.

3.5.2.2.2 Biochemical Identification

The automated Vitek 2 Compact system (bioMérieux, France) was used for conclusive biochemical analysis after colony morphology-based presumed identification. Subculture was used to obtain pure isolates, which were then processed in accordance with manufacturer guidelines. Gram-positive (GP) or Gram-negative (GN) organism-specific Vitek 2 identification cards were injected with bacterial samples that had been standardized to 0.5 McFarland turbidity. By comparing with the Vitek 2 database, the system produced species-level identification using colorimetric and fluorometric biochemical processes. While isolates with lower confidence scores were subjected to alternate identification techniques or repeat testing, identification findings with confidence levels ≥95% were accepted.

3.5.2.3 Determination of Antibiotic Resistance Profiles

An automated phenotypic technique for identifying antibiotic resistance profiles is the VITEK 2 system. It measures growth inhibition in microwell cards with particular antimicrobial dosages using standardized bacterial inocula from pure cultures. The platform generates minimum inhibitory concentration (MIC) values and categorical interpretations (susceptible, intermediate, and resistant) based on species-specific clinical breakpoints established by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The system provides fast turnaround times, supports a wide range of pathogen groups, and facilitates laboratory information system (LIS) integration, despite the possibility of occasional discrepancies for specific antimicrobial-organism combinations and the requirement to maintain up-to-date, validated breakpoint databases.

3.5.2.3.1 Antimicrobial Susceptibility Testing

The automated Vitek 2 system was used to conduct antimicrobial susceptibility testing in compliance with the recommendations set out by the Clinical and Laboratory Standards Institute (CLSI). Vitek 2 antimicrobial susceptibility testing (AST) cards,

which are intended for either Gram-positive (AST-GP) or Gram-negative (AST-GN) organisms, were used to test verified isolates after species identification. A panel of therapeutically relevant antimicrobial drugs at different concentrations was included on each card in order to calculate the minimum inhibitory concentration (MIC) values. The AST cards were injected with bacterial suspensions that were prepared to meet 0.5 McFarland turbidity criteria. During an incubation period, the system tracked the kinetics of bacterial growth in the presence of varying antibiotic concentrations, producing MIC values that were automatically classified as susceptible, intermediate, or resistant according to the most recent CLSI breakpoints.

3.5.2.3.2 Antimicrobial Agents Tested

The antimicrobial panel was chosen to reflect the main medication classesbeta-lactams, aminoglycosides, fluoroquinolones, glycopeptides, macrolides, tetracyclines, and folate pathway inhibitors—that are important for managing chronic wound infections. In accordance with CLSI guidelines for suitable drug-organism pairings, the specific drugs examined varied based on the type of bacteria and the Gram stain categorization.

3.5.2.4 Calculation of Multiple Antibiotic Resistance Index

To measure resistance levels at the isolation and hospital-unit levels, the Multiple Antibiotic Resistance (MAR) index was computed. The MAR index was calculated for each isolate by dividing the number of antibiotics tested by the percentage of antibiotics to which the isolate was resistant. For each isolate, the MAR index was computed using the formula:

$$MAR_{isolate} = \frac{a}{b}$$

where,

a = number of antibiotics to which the isolate was resistant,

b = total number of antibiotics against which the isolate was tested.

At the stratum level, the MAR index was determined using the following formula:

$$MAR_{stratum} = \frac{a}{bxc}$$

where,

a = aggregate antibiotic resistance score of all isolates from the stratum,

b = total number of antibiotics tested,

c = number of isolates obtained from the stratum.

A MAR index value greater than 0.2 was considered indicative of high-risk sources where antibiotics are frequently used or misused (Krumperman, 1983).

3.5.4 Isolation, Storage and Revival

Well-isolated colonies identified as *Staphylococcus aureus* were preserved for genomic analysis. Pure colonies were subcultured onto blood agar to ensure purity and suspended in sterile cryovials containing 20% glycerol in tryptic soy broth as a cryoprotectant medium. The bacterial suspensions were aliquoted into 1 mL portions, labelled with strain identification details, and stored at –20°C. For downstream analysis, frozen isolates were rapidly thawed and revived on blood agar. Identity was reconfirmed using Gram staining, catalase testing, and coagulase testing, which verified Gram-positive cocci morphology, catalase enzyme production, and coagulase-mediated plasma clotting, respectively.

3.5.5 Genomic Data Collection

3.5.5.1 Genomic DNA Extraction

The Invitrogen™ PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract genomic DNA from 18 clinical isolates of *Staphylococcus aureus*. This kit uses a purification system based on silica membranes to produce high-purity, high-molecular-weight genomic DNA that is appropriate for PCR amplification and whole-genome sequencing, among other downstream molecular applications.

3.5.5.2 Cell Harvesting and Preparation

To make a homogeneous cell suspension, pure bacterial colonies were aseptically harvested from overnight cultures on blood agar plates and suspended in 180 µL of sterile phosphate-buffered saline (PBS) or isotonic buffer. To pellet the bacterial cells, the slurry was transferred to a sterile 1.5 mL microcentrifuge tube and centrifuged at 13,000 × g for two minutes at room temperature. The compact cell pellet was left for further lysis after the supernatant was carefully aspirated and disposed of.

3.5.5.3 Enzymatic Cell Wall Disruption

A volume of 180 µL of PureLink™ Genomic Digestion Buffer was used to resuspend the bacterial pellet, and 20 µL of lysozyme solution (final concentration of 20 mg/mL) was added. The muramidase enzyme Lysozyme facilitates mechanical disruption and makes cells vulnerable to chemical lysis by hydrolyzing β-1,4-glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine residues in the peptidoglycan layer of the S. aureus cell wall. After a quick vortex to guarantee a uniform suspension, the mixture was incubated for 30 minutes at 37°C to enable full enzymatic cell wall digestion.

3.5.5.4 Protein Degradation and Complete Cell Lysis

After enzymatic pre-treatment, 200 µL of PureLink™ Genomic Lysis/Binding Buffer containing guanidinium thiocyanate as a chaotropic agent and 20 µL of Proteinase K solution (final concentration >20 mAU/mL) were added to the lysate. A broad-spectrum serine protease, proteinase K breaks down histones, nucleases, and other cellular proteins that would otherwise jeopardize DNA integrity or obstruct purification. In addition to inactivating endogenous DNases, the chaotropic salts in the lysis solution denature proteins and break hydrogen bonds, allowing nucleic acids to be released. To guarantee full cellular lysis and complete protein digestion, the mixture was periodically vortexed for 15 seconds and then incubated at 55°C for 30 minutes.

3.5.5.5 DNA Binding and Washing

A volume of 200 µL of absolute ethanol (96–100%) was added to the lysate and thoroughly mixed by pulse-vortexing for 5 seconds to maximize the conditions for DNA binding. While proteins, polysaccharides, and other impurities stay in solution, ethanol modifies the polarity and ionic strength of the solution, facilitating the selective binding of high-molecular-weight genomic DNA to the silica-based membrane inside the PureLinkTM spin column. After the transfer to a PureLinkTM spin column in a collecting tube, the whole lysate-ethanol combination was centrifuged for one minute at 10,000 × g. While contaminants passed through the collecting tube and were disposed of, genomic DNA adhered to the silica membrane during centrifugation.

3.5.5.6 Sequential Washing to Remove Contaminants

Two consecutive washing procedures were performed on the bound DNA to get rid of any remaining proteins, salts, and organic impurities. In order to eliminate cellular debris and precipitated proteins, 500 µL of Wash Buffer 1, which contained ethanol and salts based on guanidinium, was first added to the column and centrifuged at 10,000 × g for one minute. After discarding the flow-through, 500 µL of Wash Buffer 2, an ethanol-based buffer with a low concentration of salt, was added to the column and centrifuged for three minutes at maximum speed (>10,000 × g) to eliminate any remaining salts and guarantee total ethanol removal, which might otherwise prevent subsequent enzymatic reactions. To make sure the membrane was completely dried, the column was then centrifuged for a further minute at maximum speed.

3.5.5.6 DNA Elution and Storage

50 µL of PureLink™ Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, with 0.1 mM EDTA) was carefully pipetted directly onto the center of the silica membrane after the spin column had been moved to a sterile 1.5 mL microcentrifuge tube. DNA release is facilitated by the elution buffer's low ionic strength and slightly alkaline pH, which break down the electrostatic bonds that hold DNA to the silica matrix. To extract the pure genomic DNA, the assembly was centrifuged for one minute at maximum speed after being incubated for one minute at room temperature to optimize DNA recovery. Thermo Fisher Scientific's NanoDropTM spectrophotometer was used to measure the concentration and purity of DNA spectrophotometrically, with A260/A280 ratios of 1.8 as a sign of high-purity DNA for use in subsequent processes. Before being used for whole-genome sequencing, the isolated genomic DNA was promptly aliquoted to reduce freeze-thaw cycles and kept at -20°C.

3.5.6 DNA Quality Assessment

DNA purity and concentration were determined using complementary methods. First, a NanoDrop 2000 spectrophotometer measured absorbance at 260 nm and 280 nm. The A260/A280 ratio, with values around 1.8, was considered indicative of pure DNA with minimal protein contamination. However, since spectrophotometry does not discriminate between DNA and other nucleic acids or contaminants, a Qubit 4 fluorometer (Thermo Fisher Scientific) was also employed. The Qubit assay uses DNA-

specific fluorescent dyes that selectively bind double-stranded DNA, offering highly accurate quantification. Together, these methods ensured that DNA extracts were of sufficient quality for downstream sequencing.

3.5.7 Library Preparation and Sequencing

DNA libraries were prepared using the Illumina Nextera XT procedure (Illumina, Inc.). After genomic DNA was tagged, the transposase enzyme simultaneously fragmented the DNA and ligated adapter sequences for 10 minutes at 55°C, producing adaptor-flanked fragments. Following that, the tagged DNA was amplified using limited-cycle polymerase chain reaction (PCR) amplification employing unique dual indices for sample multiplexing and universal primers complementary to the adapter sequences.

Libraries were subjected to size selection and purification using magnetic beads after amplification in order to get rid of surplus reagents, short fragments, and leftover adapters. To guarantee equimolar representation across all samples, library normalization was subsequently carried out. A Qubit fluorometer (Thermo Fisher Scientific) was used to measure library concentration, and an Agilent Bioanalyzer was used for quality control and quantification in order to evaluate fragment size distribution and library integrity.

Normalized libraries were diluted to a final loading concentration of 1.8 nM after being pooled in equimolar ratios. After being denatured with sodium hydroxide, the combined library was put into an Illumina sequencing cartridge. Using sequencing-by-synthesis chemistry, high-throughput sequencing was carried out on an Illumina platform, producing millions of reads for further bioinformatic analysis.

3.5.5.8 Bioinformatics Processing

The raw reads generated from sequencing were subjected to quality control and preprocessing before genome assembly. Initially, the raw sequence files in FASTQ format were evaluated using FastQC to assess per-base sequence quality scores, GC content, and the presence of adapter sequences. Following this, trimming and filtering were performed using Trimmomatic to remove poor-quality bases, adapters, and potential contaminants. The quality of the cleaned reads was reassessed with FastQC to

confirm improvements in overall read integrity. High-quality reads were then used for de novo genome assembly with SPAdes and Unicycler, which generated contigs and scaffolds. The assembled genomes were subsequently annotated using Prokka, enabling the identification of genes, coding sequences, and regulatory regions.

3.5.5.8.1 Genomic Characterization and Bioinformatics Analysis

The three objectives related to the genetic characterization of *Staphylococcus aureus* isolates from chronic wound infections were addressed through thorough bioinformatics studies conducted after whole-genome sequencing and raw read quality control.

3.5.5.8.2 Genomic Analysis and Characterization

Genome assembly metrics were generated using Pathogen Watch, which included plasmid profiling, lineage classification, and core-genome phylogenetic data. Multilocus sequence typing (MLST), which allocates sequence types to isolates of *Staphylococcus aureus* to ascertain the clonal context of each strain, was carried out using the PubMLST database.

Basic plasmid types were identified for plasmid characterisation using the Center for Genomic Epidemiology (CGE) PlasmidFinder resource, which was accessed through PubMLST. The core-genome backbone was constructed using core-genome phylogenetic analysis on the S. aureus Core 2208 dataset from PubMLST. Clustering analysis revealed five isolates with core-genome similarity at or above a 10% threshold, indicating a closely related clonal population in the sample.

Contextualized by MLST type, plasmid repertoire, and core-genome clustering patterns, the integration of these techniques produced comprehensive genome assembly metrics, including as measures of contiguity and completeness. Rapid evaluation of genetic relatedness and characterization of plasmid diversity within the assembled genomes were made possible by this integrated technique.

3.5.5.8.3 Spa Typing Analysis

To describe the polymorphic X region of the staphylococcal protein A gene (spa) in isolates of *Staphylococcus aureus*, spa typing was carried out using SPATyper version 1.0. After the spa gene sequence was extracted from assembled genomes, it was aligned with a reference spa allele database as part of the analytical approach. The spa gene's distinct succession pattern of short sequence repeats (R sequence) was found and contrasted with other repeat patterns that are known to exist.

In accordance with the Ridom spa server nomenclature, each unique repeat succession pattern was given a corresponding spa type designation along with an associated allele number. To make sure that type assignments represented the whole repertoire of spa repeat variants cataloged as of that date, the analysis made use of the SPATyper database version 2023-06-19.

3.5.5.8.4 **Staphylococcal Protein A (spa) Typing**

Spa typing was carried out using spaTyper version 1.0, with the spa type database updated as of June 19, 2023, in order to attain greater discriminatory resolution than MLST alone. The hypervariable X region of the protein A gene (spa), which has varying quantities of 24-base pair tandem repeats, is exploited by the spa typing technique. A spa type was defined by the ordered sequence of repeats, and each distinct repeat unit given a number code like t008 or t127.

Using the spaTyper program, which automatically extracts the spa repeat area, identifies individual repeat units, and assigns the associated spa type, genomic sequences were queried against the Ridom SpaServer database (http://spa.ridom.de/). Because isolates with the same ST may have distinct spa types, which could indicate microevolution or recent divergence, the spa types were utilized to evaluate within-ST diversity. To confirm the reliability of the typing results and to find any discordant patterns that would point to horizontal gene transfer or recombination events impacting the spa locus, the concordance between spa type clustering and MLST-based clonal complex assignment was assessed.

3.5.5.9 Detection of Antimicrobial Resistance Determinants

Using ResFinder version 4.7.2 (Center for Genomic Epidemiology), which was updated as of March 22, 2024, antimicrobial resistance gene profiling was carried out. ResFinder uses BLAST-based alignment to find resistance determinants in genomic sequences by utilizing a curated database of acquired antimicrobial resistance genes and chromosomal mutations linked to resistance phenotypes.

Resistance genes that provide resistance to the main classes of antibiotics were encompassed in the analysis and included: Beta-lactams: blaZ (production of beta-lactamases), mecA and mecC (methicillin resistance via alternative penicillin-binding protein PBP2a). Aminoglycosides: enzymes that alter aminoglycosides, such as the ant, apo, and aac gene families. The genes erm (ribosomal methylation), msr (efflux pumps), and lnu (lincosamide nucleotidyltransferases) are examples of macrolides, lincosamides, and streptogramins (MLS). The chromosome mutations in the gyrA, gyrB, parC, and parE genes as well as plasmid-mediated quinolone resistance (qnr) genes are examples of fluoroquinolones. Tetracyclines: tet genes that encode efflux pumps and ribosomal protection proteins. Van gene clusters, which are uncommon in *Staphylococcus aureus*, are glycopeptides. Phenicols: genes for cat and fexA and dfrA/dfrG (dihydrofolate reductase) and sul genes in trimethoprim-sulfamethoxazole

Furthermore, methicillin resistance cassette types were identified by characterizing SCCmec elements, which are mobile genetic components that carry not only mecA but also frequently other resistance determinants. The thresholds for gene detection were set at ≥60% coverage and ≥90% identity. In order to confirm genotype-phenotype concordance and find differences that might point to new resistance mechanisms or methodological constraints, resistance profiles were compared with phenotypic susceptibility information obtained from Vitek 2 system findings.

3.5.5.9.1 Detection of Virulence-Associated Genes

VirulenceFinder version 2.0.5(Center for Genomic Epidemiology, Technical University of Denmark) was used to profile virulence genes, and the virulence factor database was updated as of December 2, 2022. By comparing genomic sequences to a carefully maintained database of experimentally verified virulence genes, the tool uses

a BLAST-based algorithm to find known virulence determinants. The k-mer alignment (KMA) approach, which allows for quick and precise identification of virulence factors even in the face of sequence variation or poor coverage, was used to map quality-filtered sequencing reads to reference virulence gene sequences prior to virulence detection.

A wide range of *Staphylococcus aureus* virulence variables were the focus of the analysis, including: Toxins such as Panton-Valentine leukocidin (lukS-PV/lukF-PV), alpha-hemolysin (hla), beta-hemolysin (hlb), gamma-hemolysin (hlg), and toxic shock syndrome toxin-1 (tst).

The enterotoxin included Staphylococcal A–E (sea, seb, sec, sed, see) and associated superantigens. The adhesins include clumping factors (clfA, clfB), collagen-binding protein (cna), fibronectin-binding proteins (fnbA, fnbB), and elastin-binding protein (ebpS). Staphylokinase (sak), capsular polysaccharide synthesis genes (cap5, cap8), and protein A (spa) are immune evasion factors. Intercellular adhesion operon (icaABCD) and biofilm-associated protein (bap) are examples of genes linked to biofilms.

To guarantee reliable detection while taking allelic variation into account, the threshold parameters for gene identification were ≥90% nucleotide identity and ≥60% gene coverage. In order to evaluate each isolate's pathogenic potential and identify high-risk clones that carry several virulence factors, the results were combined to create thorough virulence profiles.

3.5.5.10 Characterization of Genetic Diversity: Multilocus Sequence Typing (MLST)

Multilocus sequence typing (MLST), a sequence-based molecular typing technique that allocates allele numbers to internal fragments of seven housekeeping genes and combines them into an allelic profile that defines a sequence type (ST), was used to characterize the genetic diversity and population structure of *Staphylococcus aureus* isolates. Sequences of the seven canonical S. aureus MLST loci (arcC, aroE, glpF, gmk, pta, tpi, and yqiL) were extracted from assembled genomes and compared to the S. aureus MLST database on PubMLST.org (https://pubmlst.org/saureus/) to create

MLST data. According to the international *Staphylococcus aureus* MLST scheme developed by Enright et al. (2000), a numerical ST identifier was given to each distinct allelic combination.

Using the eBURST algorithm through PubMLST, sequence types were further grouped into clonal complexes (CCs) based on shared allelic profiles. Isolates were assigned to the same CC if they differed from at least one other member of the group by no more than one of the seven MLST loci. Identification of closely related lineages and evaluation of clonal dispersion patterns were made possible by the hierarchical classification. The distribution of STs and CCs was examined to determine the main lineages circulating at MeTRH and to look for possible importation of foreign high-risk clones. The genetic diversity within the isolate collection was measured using Simpson's diversity index.

3.5.5.10.1 Staphylococcal Cassette Chromosome mec (SCCmec) Typing

SCCmec typing was used to further analyze the structural variety of methicillin resistance determinants using SCCmecFinder version 1.1 (Center for Genomic Epidemiology). SCCmec elements, which range in size from 21 to 67 kb and genetic makeup, are mobile genetic cassettes that are inserted at the orfX site close to the replication origin in *Staphylococcus aureus* chromosomes. SCCmec typing is based on the combination of the ccr gene complex (types 1–9, encoding recombinases responsible for cassette mobility) and the mec gene complex (class A, B, or C, characterized by regulatory genes and insertion sequences flanking mecA).

MecA, mecR1, mecI, and several ccr gene variations (ccrA, ccrB, and ccrC) are among the important genetic markers that SCCmecFinder uses to identify SCCmec classes I through XIII using a reference-based mapping technique. Because different types are linked to different epidemiological contexts, SCCmec typing offers epidemiological insights

3.5.5.6 Phylogenetic Analysis

Using MLST allelic profiles, phylogenetic analysis was performed to depict evolutionary relationships among the *Staphylococcus aureus* isolates and place them

within the global *Staphylococcus aureus* population structure. Genetic distances were computed as the number of allelic differences between isolates, and a categorical distance matrix was built from the seven-locus allelic profiles. Phylogenetic trees were inferred using the neighbor-joining algorithm or minimal spanning tree approaches, which are acceptable for categorical MLST data and have been extensively verified for bacterial population genetics research

The Interactive Tree of Life (iTOL) v6 platform (https://itol.embl.de/), an online tool for the display, editing, and annotation of phylogenetic trees, was used to view and annotate the resultant phylogenetic trees. The discovery of monophyletic clusters that corresponded to clonal outbreaks, the evaluation of genetic relatedness between isolates from various wards or time periods, and the identification of possible transmission chains within the hospital setting were all made possible by phylogenetic visualization. The epidemiology, evolution, and clinical significance of the *Staphylococcus aureus* lineages circulating at MeTRH were well understood through the integration of molecular typing data with resistance and virulence profiles.

5 .5.5 .7 Determination of Clonal Complexes

The clonal complexes (CCs) were identified by analyzing MLST allelic profiles. Based on the fact that isolates shared at least five of the seven MLST alleles with the founding genotype of each complex, they were categorized into established CCs (CC5, CC8, CC22, CC30, etc.). CCs are phylogenetically cohesive groups that frequently have similar pathogenic and epidemiological traits. Singleton STs, or those that did not cluster with any known CC, were found and marked as possibly unusual or unique lineages that needed more research.

To find correlations between particular clonal lineages and clinical or epidemiological factors, the distribution of CCs was examined in light of clinical metadata such as patient demographics, ward location, wound kind, and antimicrobial resistance profiles. Particular focus was placed on finding globally distributed high-risk clones that are known to possess improved virulence or multidrug resistance features, such as CC5 (including ST5 and ST225), CC8 (containing ST8/USA300), CC22 (including ST22/EMRSA-15), and CC30 (including ST30 and ST36).

3.6 Data Analysis

Demographic characteristics, bacterial isolates, and antibiotic distribution profiles were coded and analyzed using IBM SPSS Statistics Version 27. Descriptive statistics were employed to characterize the study population, estimate the prevalence of multidrug-resistant (MDR) organisms, and calculate multiple antibiotic resistance (MAR) indices. A heatmap was generated to illustrate the distribution of bacterial isolates across hospital units, using hierarchical clustering with Euclidean distance as the distance metric and complete linkage for agglomeration. Data preprocessing and visualization were performed in R using the heatmap and reshape2 packages, with a color gradient applied to reflect the relative abundance of isolates, where darker shades represented higher frequencies.

The genomic data underwent a comprehensive bioinformatics pipeline to generate detailed insights into the genetic makeup of the isolates. Initially, raw sequence data in FASTQ format were subjected to quality assessment using FastQC to evaluate per-base sequence quality, GC content, sequence duplication levels, and adapter contamination. Sequences failing to meet quality thresholds were further processed using Trimmomatic, which removed low-quality bases, adapters, and other potential sequencing artifacts. Post-trimming, quality assessment was repeated to confirm improvement in the overall quality of reads.

Subsequently, high-quality reads were assembled de novo using two assemblers: SPAdes, which efficiently generated contigs, and Unicycler, which provided scaffolds or closed genomes where possible. These assemblies formed the foundation for downstream annotation and comparative genomic analysis. Annotation of the assembled genomes was conducted using Prokka, supplemented by specialized tools such as ResFinder, VirulenceFinder, MLST, spaTyper, and SCCmecFinder. These tools collectively facilitated the identification of antimicrobial resistance genes, virulence factors, multilocus sequence types, spa types, and staphylococcal cassette chromosome mec (SCCmec) elements, thereby offering a detailed map of the functional and regulatory genetic landscape of each isolate.

To elucidate the evolutionary relationships and potential transmission pathways, a phylogenetic tree was constructed. This was achieved using the Interactive Tree of Life (iTOL) platform, which enabled robust visualization of phylogenetic clustering patterns among isolates. Comparative analysis of sequences against specialized databases such as the Comprehensive Antibiotic Resistance Database (CARD) and the Virulence Factor Database (VFDB) provided insights into the repertoire of resistance determinants and virulence-associated genes present in the isolates. Together, these analyses highlighted the genetic mechanisms underpinning antimicrobial resistance and pathogenic potential, as well as the possible evolutionary trajectories of the studied organisms.

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3.7 Ethical Consideration

This study was conducted in strict adherence to established ethical standards for biomedical research and in compliance with good clinical practice guidelines. Ethical approval was first sought and granted by the Chuka University Ethics Committee, which reviewed and approved the study protocol to ensure participant safety, confidentiality, and compliance with research ethics. Subsequently, a research permit was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) under Licence No. NACOSTI/P/24/39429, granting official authorization to undertake the study (Appendix VIII). In addition, formal clearance to access hospital facilities and engage with relevant stakeholders was obtained from the Meru County Health Research Committee. During data collection and laboratory analysis, all procedures followed standard operating protocols. Universal biosafety precautions

were strictly observed in the handling and processing of potentially infectious biological samples to safeguard both the research personnel and the wider community.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Demographic Characteristics

Demographic data of the population was obtained in terms of age, gender, and strata, in order to help in understanding patterns of chronic wounds, identifying risk factors, and developing targeted interventions.

4.1.1 Distribution of Study Participants by Age

The findings of this study indicate that chronic wound infections affect a wide age range, with the highest prevalence observed among individuals aged 21–30 years (22%) and 51–60 years (19.2%), as illustrated in Figure 4.

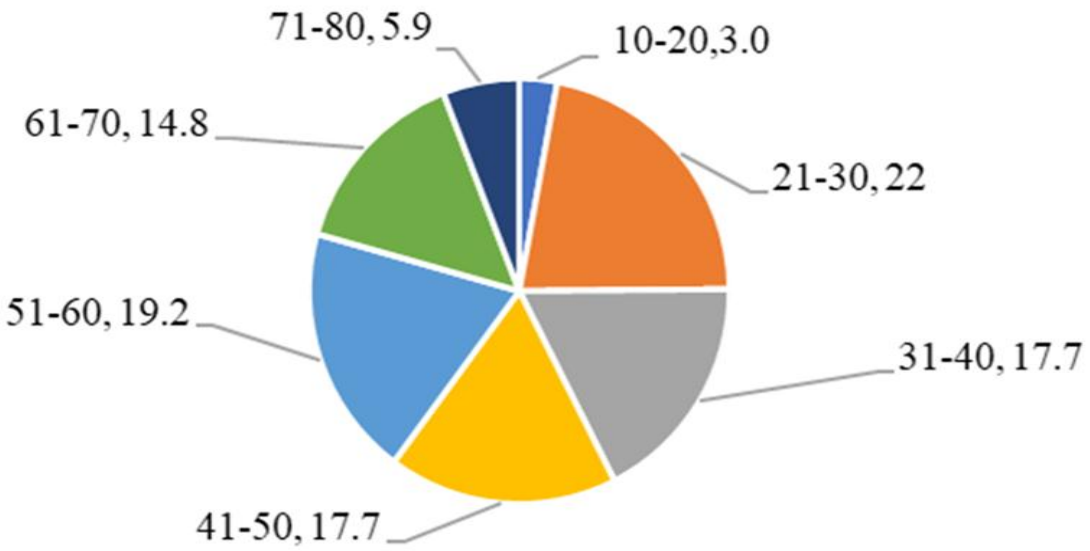


Figure 2: Age Distribution of Patients with Chronic Wound Infections

Interestingly, a considerable proportion of the cases occurred in younger persons, particularly those in their 20s and 30s. These findings challenge the conventional perception that chronic wounds primarily affect the elderly. Studies by Mahmoudi et al. (2020) and Song et al. (2021) similarly reported increased susceptibility among younger populations, attributing this trend to factors such as traumatic injuries, diabetes-related complications, and poor wound care practices. In contrast, older individuals, especially those over 60, remain at elevated risk due to comorbidities, impaired immune function, and delayed wound healing.

The proportion of cases in the oldest age group (71–80 years) was relatively low, accounting for only 5.9% of the total. This contradicts the findings by Frykberg & Banks (2015), which reported that chronic wounds mainly affect older adults, especially those over 60 years of age. The lower percentage seen in this age group may be due to regional differences, the small sample size, or possibly better wound care practices and preventive health behaviours among the elderly in the study setting.

In contrast, Mahmoudi et al. (2020) reported a growing incidence of chronic wounds among younger individuals, particularly in low and middle-income countries where trauma, infections, and diabetes-related complications are increasingly common. Conversely, Lee et al. (2019) found that in high-income countries, chronic wounds are more prevalent among older adults, reflecting differences in healthcare infrastructure, disease burden, and population demographics. These contrasting findings underscore the broader age distribution of chronic wound infections than previously recognized, emphasizing the need for early detection and intervention in younger age groups. While older adults remain a high-risk population, the significant prevalence among young and middle-aged individuals highlights the importance of developing prevention and management strategies that address all age groups.

4.1.2 Distribution of Pathogens by Gender

The results obtained on pathogen distribution by gender indicate a fairly balanced representation, with males accounting for 53% and females 47% of the cases, and a slight predominance of females observed in the older age groups, as demonstrated in Figure 5.

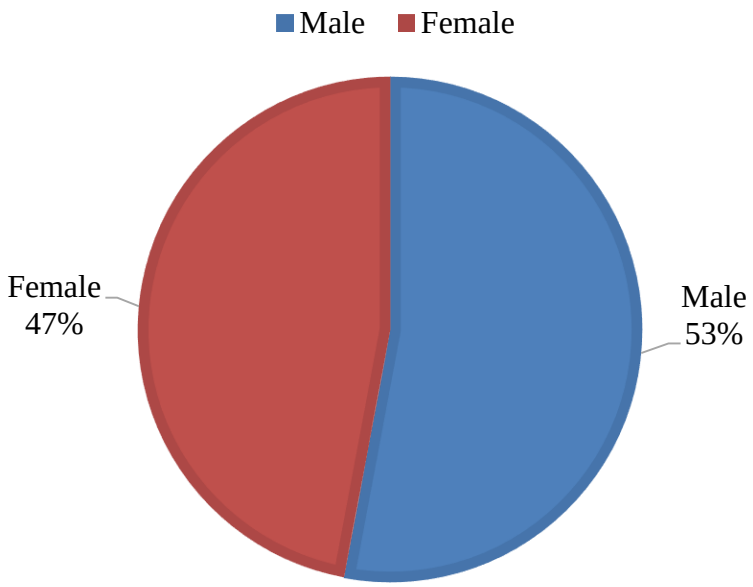


Figure 3: Distribution of Pathogens by Gender

The obtained data on gender distribution points to a fairly balanced representation, with a small bias in favor of women in older age groups. According to recent studies by Patel et al. (2020), unique risk factors like vascular disease and hormonal impacts frequently influence gender differences in chronic wound prevalence. Some studies suggest that women may be more affected in particular situations, particularly post-menopause. However, wounds associated with behavioral factors, including trauma or occupational hazards, are more common in male patients (Kumar et al., 2022). These results demonstrate that chronic wounds have a substantial influence on both sexes, with differences depending on biological and socioeconomic circumstances.

Studies have highlighted gender-specific risk factors influencing the prevalence of chronic wounds. According to Patel et al. (2020), vascular diseases and hormonal changes—especially those that take place after menopause—may make women more vulnerable in specific situations.

In contrast, Kumar et al. (2022) found that wounds linked to behavioural and environmental factors, such as trauma and occupational hazards, are more commonly observed in male patients. These findings suggest that chronic wounds significantly affect both sexes, with variations influenced by a combination of biological, behavioural, and socioeconomic factors.

4.2 Pathogenic Bacteria Isolated from Chronic Wound Infections

The findings on microbial prevalence confirm that *Staphylococcus aureus* as the most frequently isolated pathogen in chronic wound infections, accounting for 26.6% of all isolates and Acinetobacter baumannii and Enterococcus faecalis at 1.5% being the least in preference. Its high prevalence underscores its critical role in biofilm formation, tissue invasion, and persistence within the wound environment (Thangamani et al., 2020; Zhang et al., 2023). The identification of *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA), presents considerable challenges in the context of antimicrobial resistance. The capacity of methicillin-resistant strains to produce biofilms significantly complicates therapeutic interventions, as biofilm-mediated infections demonstrate marked resistance to conventional antibiotic therapy and are associated with impaired wound healing processes (Fusi et al., 2021; Wright & Schaefer, 2023). The results for the pathogenic bacteria isolated from the chronic wounds are shown in Table 8.

Table 8: Pathogenic Bacteria Isolated from Chronic Wound Infections

Isolated bacterial pathogen	Number isolated	Percentage (%)
Staphylococcus aureus	18	26.6
Pseudomonas aeruginosa	9	13.2
E. coli	7	10.3
Corynebacterium striatum	5	7.3
Coagulase-negative staphylococci (CoNS)	5	7.3
Proteus hauseri	4	5,8
Klebsiella pneumoniae	2	2,9
Morganella morganii	2	1.5
Acinetobacter baumannii	1	1.5
Enterococcus faecalis	1	1.5
Samples with no growth obtained	0	20.5
Total	68	100

4.2.1 Pathogen Distribution across Hospital Strata

The cluster heatmap illustrates the distribution of bacterial pathogens across various hospital strata (Figure 2). With numerical values representing the number of organism detection events, the heatmap visualization shows the frequency distribution of bacterial isolates across different clinical situations. With nine occurrences in the Wound Clinic and Outpatient Consultation Rooms and one in the Patient Ward, *Staphylococcus aureus* showed the highest isolation frequency. There were three isolates of Pseudomonas aeruginosa and Escherichia coli from the Wound Clinic and

Outpatient Consultation Rooms, and one from the Patient Ward. Proteus species, Morganella morganii, Corynebacterium striatum, and Coagulase-negative staphylococci were among the other organisms identified; they each showed three, five, three, and three isolations from the Wound Clinic environment, respectively. Lower frequency isolations were noted for Enterococcus faecalis (one from the Diabetic Clinic and one from the Wound Clinic) and *Acinetobacter baumannii* (one isolation).

This suggests a consistent presence of these pathogens in community-acquired and outpatient-associated infections. The clustering of multiple pathogens—such as *Escherichia coli*, *Corynebacterium striatum*, *Proteus* spp., and coagulase-negative staphylococci, within this setting further supports the polymicrobial nature of chronic wounds. The relatively low pathogen diversity in the Burn Unit and Diabetic Clinic could reflect specific patient profiles or limited sampling. These findings support previous studies emphasizing the importance of clinical context in interpreting microbial load and underline the value of stratified surveillance in infection control and antimicrobial stewardship. With darker coloration signifying higher bacterial recovery rates, the color intensity gradient—which ranges from light cream to dark brown—visually represents isolation frequencies and makes it easier to quickly identify the most common pathogens in particular clinical settings.

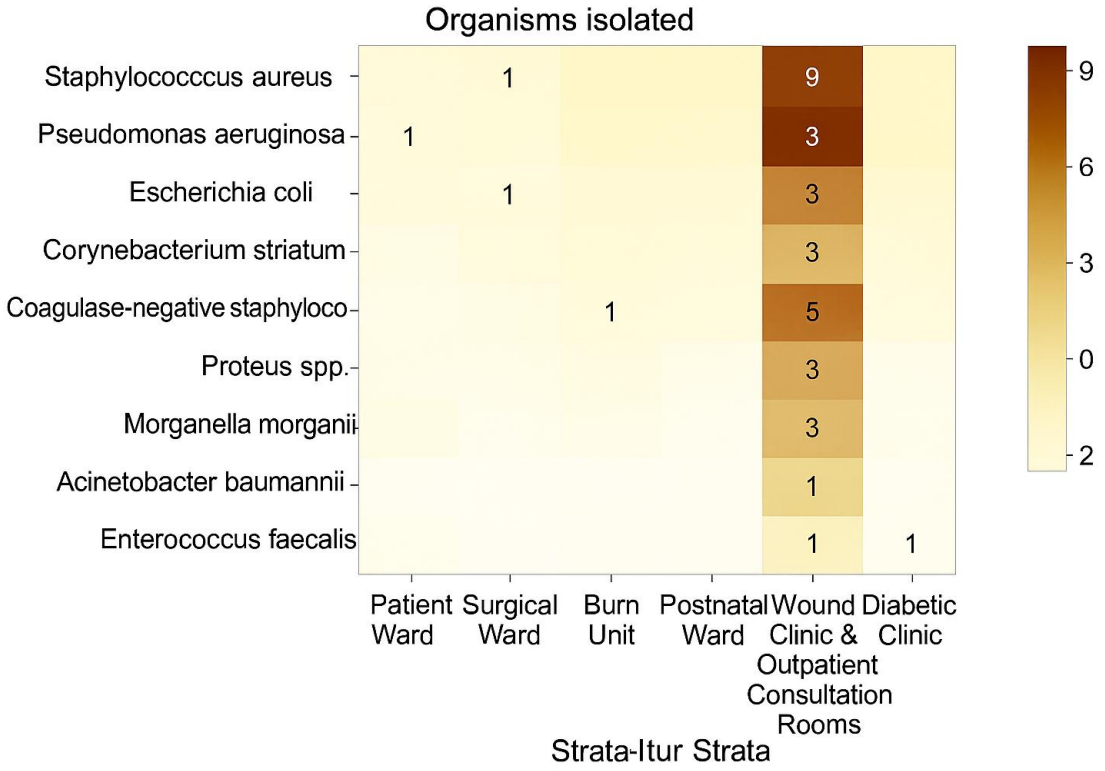


Figure 4: Cluster Heatmap of Pathogen Distribution Across Hospital Strata

Pseudomonas aeruginosa was identified as the second most prevalent pathogen, accounting for 13.2% of isolates. This finding is consistent with recent studies that report its frequent occurrence in chronic ulcers, particularly among patients with diabetic foot infections and burn wounds (Malik et al., 2021; Friedlander et al., 2023). As a gram-negative bacterium, *P. aeruginosa* is well recognized for its ability to form biofilms, secrete potent virulence factors, and exhibit resistance to multiple classes of antibiotics, factors that collectively contribute to persistent infections and delayed wound healing (Friedlander et al., 2023). The detection of *Escherichia coli* in 10.3% of samples further underscores the role of Enterobacteriaceae in chronic wounds colonization. Often associated with faecal contamination or translocation from adjacent tissues, *E. coli* presents additional challenges in infection management due to its potential for antimicrobial resistance and its role in promoting polymicrobial synergy within the wound environment (Li et al., 2022).

Other notable isolates included *Corynebacterium striatum* and coagulase-negative staphylococci (CoNS), each comprising 7.3% of the total isolates. Although historically regarded as skin commensals, both organisms are increasingly recognized as

opportunistic pathogens, particularly in immunocompromised individuals and patients with chronic infections (Liu et al., 2022; Johnson et al., 2023). Their capacity to form biofilms and persist in compromised tissue environments makes them clinically relevant contributors to delayed healing and recurrent infection. These findings highlight the importance of accurately distinguishing true pathogens from contaminants, especially in chronic wound cases where viable tissue samples may be limited and microbial colonization is complex.

The identification of less common bacteria such as *Proteus hauseri* (5.8%), *Klebsiella pneumoniae* (2.9%), *Morganella morganii* (1.5%), *Acinetobacter baumannii* (1.5%), and *Enterococcus faecalis* (1.5%) highlights the polymicrobial character of chronic wounds. These pathogens are frequently linked to multidrug resistance and biofilm development, which makes treatment more challenging (Kumar et al., 2022; Smith et al., 2022). Their identification underscores the importance of thorough microbiological assessment to map the complete bacterial profile in chronic wounds.

In this study, 20.5% of chronic wound samples showed no bacterial growth despite clinical signs of infection. Although limitations in sample collection techniques could partly account for these findings, other underlying factors are likely involved. These may include the presence of fastidious or anaerobic organisms not supported by conventional culture methods, prior antibiotic use, or the involvement of non-bacterial pathogens such as fungi. This observation reflects the complex and multifactorial nature of chronic wound pathology and underscores the need for advanced diagnostic approaches, including molecular methods, to detect non-culturable or low-abundance pathogens.

These findings suggest that non-infectious etiologies may account for up to 20.5% of chronic wounds in which no pathogens are detected. Immune-mediated conditions such as vasculitis and pyoderma gangrenosum often mimic infectious wounds clinically but typically yield negative microbiological results, highlighting the importance of considering autoimmune and inflammatory disorders in differential diagnosis (Vanderplas et al., 2021; Host et al., 2022). Additionally, factors unrelated to infection, such as ischemia and tissue hypoxia, are well-documented contributors to wound

chronicity. Impaired perfusion hinders tissue repair and regeneration, underscoring the need for early vascular assessment and appropriate management in patients with non-healing wounds (Sen et al., 2019; Morales & Khan, 2021).

Neuropathic diseases, especially in diabetic patients, and mechanical causes, such as pressure ulcers, are also important. In these cases, tissue damage is caused by prolonged mechanical stress or sensory loss rather than microbial invasion (Harding et al., 2020; Patel & Garcia, 2023). Furthermore, systemic health problems such as comorbid disorders and malnutrition delay healing processes, highlighting the necessity of holistic care approaches that go beyond antimicrobial intervention (Smith & Lee, 2023; Zhang et al., 2024). These findings highlight the importance of considering non-infectious pathophysiological mechanisms in the assessment and management of chronic wounds, and they underscore the need for more individualized, multidisciplinary, and effective treatment approaches.

The bacterial profile observed in this study reflects the typical microbial landscape of chronic wound infections while underscoring the persistent challenge posed by resistant, biofilm-forming pathogens. Consistent with recent findings, these results highlight the critical need for early and accurate identification of causative organisms, along with antimicrobial susceptibility testing, to guide effective treatment strategies and curb the progression of antimicrobial resistance (Fusi et al., 2021; Friedlander et al., 2023). To further improve outcomes in chronic wound management, future studies should prioritize the integration of molecular diagnostics tools and the development of novel anti-biofilm therapies.

Moreover, the findings of this study (Figure 3) reveal distinct clustering patterns among microbial isolates, suggesting significant variation in the composition of wound microbiota. These clusters, characterized by the dominance of specific bacterial species, underscore the complexity of chronic wound microbiomes and their influence on healing trajectories. Understanding these microbial patterns is essential for developing targeted, individualized treatment approaches that address both the microbial and host-related factors affecting wound resolution.

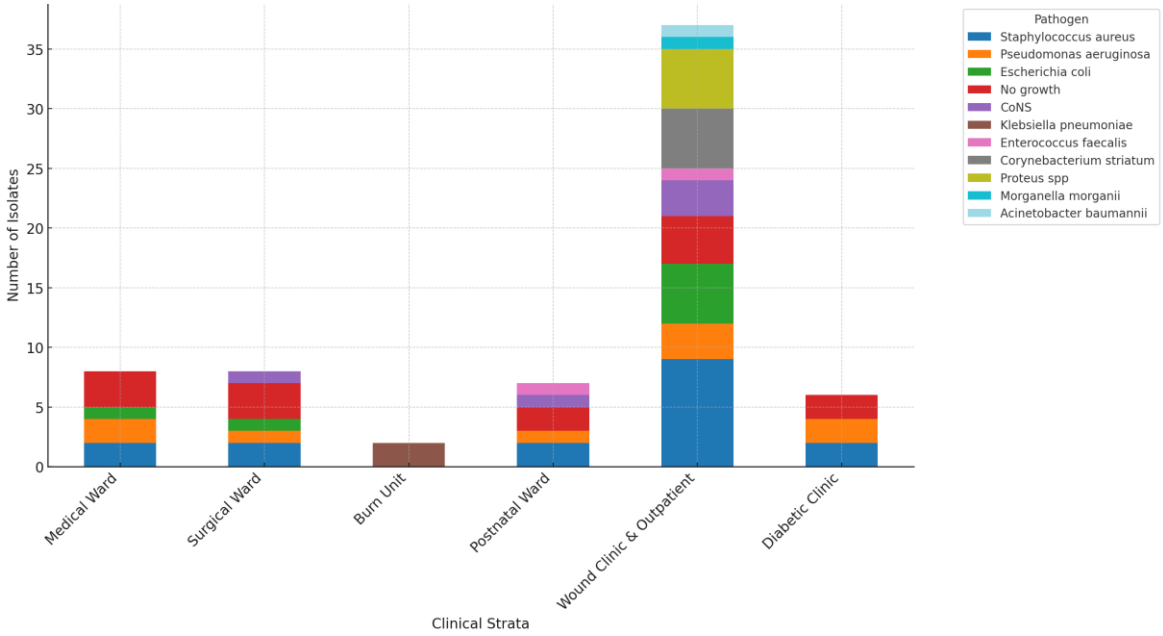


Figure 5: Distribution of Pathogen by Hospital Strata

The observed clustering patterns in this study align with previous research demonstrating that the microbial composition of chronic wounds plays a critical role in determining wound chronicity and treatment response (Malhotra et al., 2020). The predominance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in persistent, non-healing wounds mirrors findings by James et al. (2019), who identified specific bacterial biofilms strongly associated with delayed healing. The clustering identified in this study reflects similar microbial associations, supporting the hypothesis that particular bacterial consortia, especially those involving biofilm-forming and multidrug-resistant species, are linked to poorer clinical outcomes and may require tailored therapeutic strategies.

This study reveals relatively consistent clustering patterns over time, suggesting that specific bacterial profiles may persist and exert a continuous influence on wound healing trajectories. This finding contrasts with earlier work by Øien et al. (2018), who reported that chronic wound microbiota is highly individualized and subject to considerable temporal variability. Such discrepancies may stem from differences in analytical methodologies, wound types, or sampling protocols. Nevertheless, the stable clustering observed in the present study supports the approach advocated by Wolcott et al. (2016), who emphasized individualized treatment strategies tailored to the dominant microbial communities. These results reinforce the potential of targeted antimicrobial

interventions aimed at specific bacterial consortia to enhance treatment outcomes. However, given the inherent complexity of microbial diversity and biofilm dynamics in chronic wounds, caution is warranted, and further longitudinal studies are essential to validate these findings and inform precision-based therapeutic approaches.

4.3 The Antibiotic Resistance Profiles of the Pathogenic Bacteria Isolated from Chronic Wounds at Meru Teaching and Referral Hospital

Chronic wounds are commonly colonized by a diverse community of pathogenic bacteria. Effective treatment is significantly hampered by antibiotic resistance in these organisms, which affects choices about empirical therapy, debridement techniques, and wound care plans. Before moving on to organism- and context-specific factors, a thorough review of each isolate usually starts with a succinct summary of its clinical relevance, common resistance mechanisms, and management implications. In order to maximize healing and prevent the spread of resistance, this framing lays the groundwork for analysing resistance patterns, directing focused therapy, and combining stewardship with multidisciplinary wound care.

4.3.1 Antibiotic Resistance Profiles of *Staphylococcus aureus* Isolates

The susceptibility profile of the isolated *Staphylococcus aureus* demonstrates multidrug resistance (MDR), defined as bacteria that have developed resistance to multiple classes of antibiotics. Precisely, multidrug-resistant bacteria are those that resist treatment with more than one antibiotic.

This study's findings show Cefoxitin (100%), Benzylpenicillin (100%), and Oxacillin (94%), as well as Inducible Clindamycin resistance (39%), Erythromycin (44%), and Trimethoprim/Sulfamethoxazole (44%),exhibit the highest levels of antibiotic resistance. In contrast, Fosfomycin, Nitrofurantoin, Tigecycline, and several other agents (6–11%) show relatively low levels of resistance, and universal susceptibility is indicated for none. Overall, significant resistance is observed across various drug classes, particularly beta-lactams and macrolide-inducible pathways, as demonstrated in Figure 6.

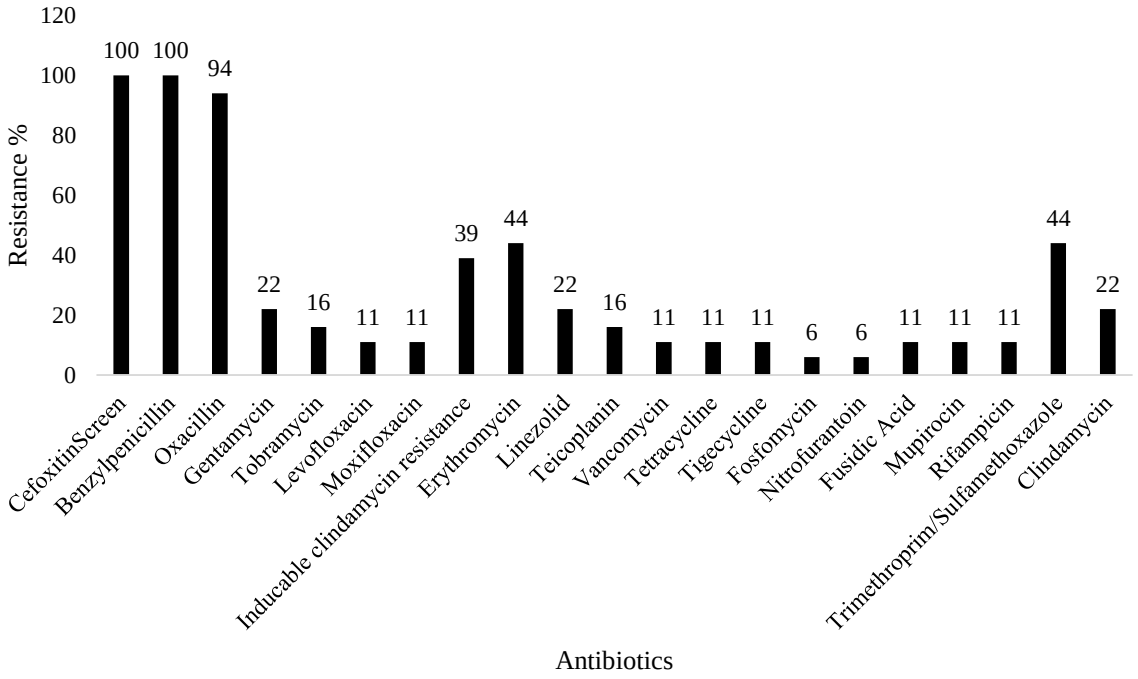


Figure 6: Antibiotic for the Isolated *Staphylococcus aureus*

Antibiotic susceptibility testing revealed that the *Staphylococcus aureus* isolates from the chronic wound had a clear pattern of resistance to a number of common antibiotics, including erythromycin and benzylpenicillin, which were universally resistant among the isolates. This widespread resistance is in line with recent studies that demonstrate the continued threat posed by *Staphylococcus aureus* strains that produce beta-lactamases, especially in situations of chronic wound infections (Raza et al., 2020; Patel et al., 2022). However, high susceptibility to advanced antibiotics such as linezolid, tigecycline, mupirocin, and teicoplanin was preserved, demonstrating their continued effectiveness against resistant bacteria and adhering to current recommendations for managing multidrug-resistant *Staphylococcus aureus* infections (Sharma et al., 2021; Lee et al., 2023).

A noteworthy observation was the detection of inducible clindamycin resistance in certain isolates, which was confirmed via D-test positivity. This finding corroborates earlier studies that highlight the importance of performing specific tests to detect inducible resistance mechanisms, as they may lead to therapeutic failure if not appropriately identified (Kumar et al., 2020; Al-Ahmad et al., 2022). Resistance to trimethoprim-sulfamethoxazole and tetracyclines was also observed in some isolates,

although these antibiotics remain an option for certain cases, especially when resistance to first-line agents is present (Zhang et al., 2022).

Several recent studies are in agreement with the resistance profile found in this investigation. According to Raza et al. (2020), *Staphylococcus aureus* is becoming more resistant to penicillin and erythromycin, and a substantial percentage of these bacteria are multidrug-resistant. Similar resistance to beta-lactams and macrolides was shown by Sharma et al. (2021), who observed that newer drugs such as tigecycline and linezolid maintained substantial activity. Similarly, Lee et al. (2023) highlighted the significance of susceptibility-guided therapy since resistant *Staphylococcus aureus* strains are becoming more common, particularly in chronic wounds. Although new resistance to antibiotics like fosfomycin and rifampicin have been found in recent surveillance investigations (Patel et al., 2022; Zhang et al., 2022), these medications are still used as a last resort. According to several recent reports, the worldwide trend toward multidrug resistance emphasizes the vital necessity of regular susceptibility testing and antimicrobial stewardship programs (Kumar et al., 2020; Al-Ahmad et al., 2022; Lee et al., 2023). The CDC guidelines (2022) and Zhou et al. (2023) stress the need for these last-resort antibiotics that are resistant to drugs like vancomycin, linezolid, and teicoplanin remains a useful option for dealing with resistant Gram-positive infections. Similar to this, some strains exhibit sensitivity to fluoroquinolones like levofloxacin and moxifloxacin despite their widespread resistance, suggesting their potential use once susceptibility is established, reiterating findings from Kletzel et al. (2022).

4.3.2 Antibiotic Resistance Profile of Isolated *Pseudomonas aeruginosa* Isolates

The results of this investigation show that several isolates are 100% sensitive to piperacillin/tazobactam, cefepime, amikacin, and gentamicin. However, the broader beta-lactam class displayed variance, with cefazolin and ceftriaxone being more resistant, ceftazidime demonstrating intermediate susceptibility, whereas meropenem and ciprofloxacin were susceptible to several antibiotics (Figure 7).

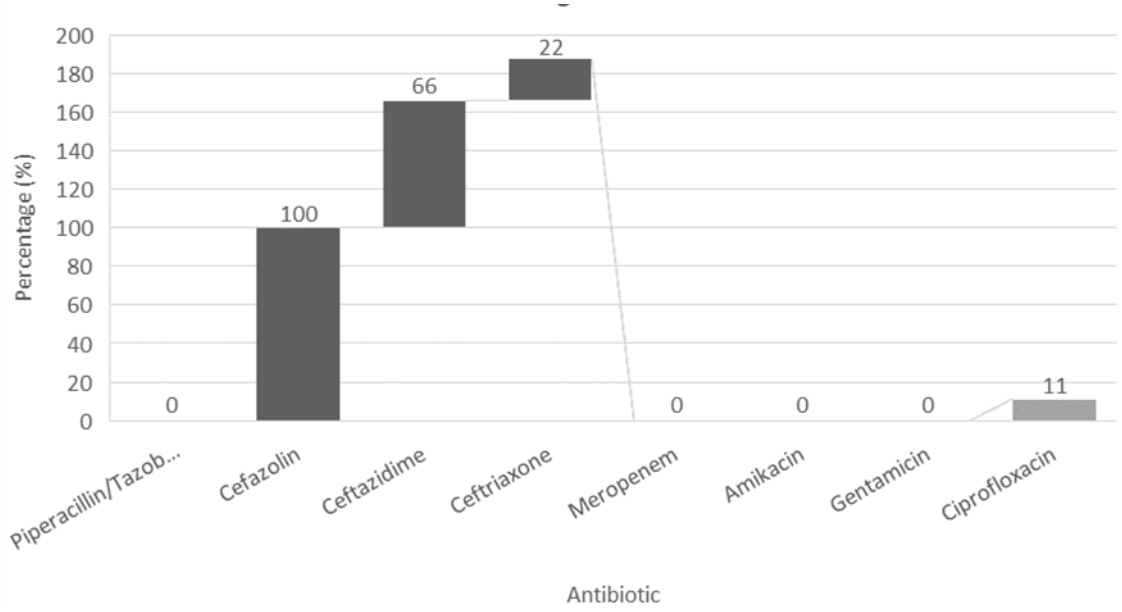


Figure 7: Resistance Pattern of Isolated Pseudomonas aeruginosa

The findings are consistent with several recent studies, the results show a persistent resistance pattern of Pseudomonas aeruginosa in wound infections, with high resistance to several β-lactam antibiotics as ampicillin, cefazolin, and cefuroxime (Luh et al., 2022; Sharma et al., 2023). Despite growing worries about resistance, Meropenem and Amikacin continue to play a significant role in treatment due to their very high efficacy. The rise in multidrug-resistant *P. aeruginosa* strains worldwide is consistent with the growing resistance to aminoglycosides like gentamicin and fluoroquinolones like ciprofloxacin (Kumar et al., 2023). These trends highlight how the landscape is changing, with novel or combination medicines becoming more and more important as standard antibiotics lose their effectiveness. Similar resistance trends were shown in previous data, but recent research highlights the rise in resistance, particularly to popular empirical antibiotics, necessitating specialized antimicrobial methods and continuous monitoring. The statistics present a difficult situation in which resistance is reducing the available treatments, calling for the implementation of prudent antibiotic usage and ongoing regional resistance monitoring.

4.3.3 Resistance Pattern of Isolated *Escherichia Coli* against Selected Antibiotics

The results illustrated in Figure 8 indicate varying levels of resistance across different antibiotic groups. Resistance to ampicillin is 57%, but resistance to amoxicillin/clavulanic acid and ampicillin/sulbactam is 14% and 28%, respectively.

The percentage of cephalosporins varies: 14% for cefazolin, 42% for cefuroxime, 42% for cefoxitin, 28% for cefotaxime, 42% for ceftriaxone, 28% for cefepime, and 28% for ceftazidime. The resistance of aztreonam is 28%. Gentamicin is 57% resistant, but meropenem and amikacin exhibit 0% resistance, suggesting preserved action in this dataset. Additionally, nitrofurantoin exhibits 0% resistance, trimethoprim/ sulfamethoxazole 71% resistance, and ciprofloxacin 57% resistance, demonstrating significant limitations for a number of routinely used drugs.

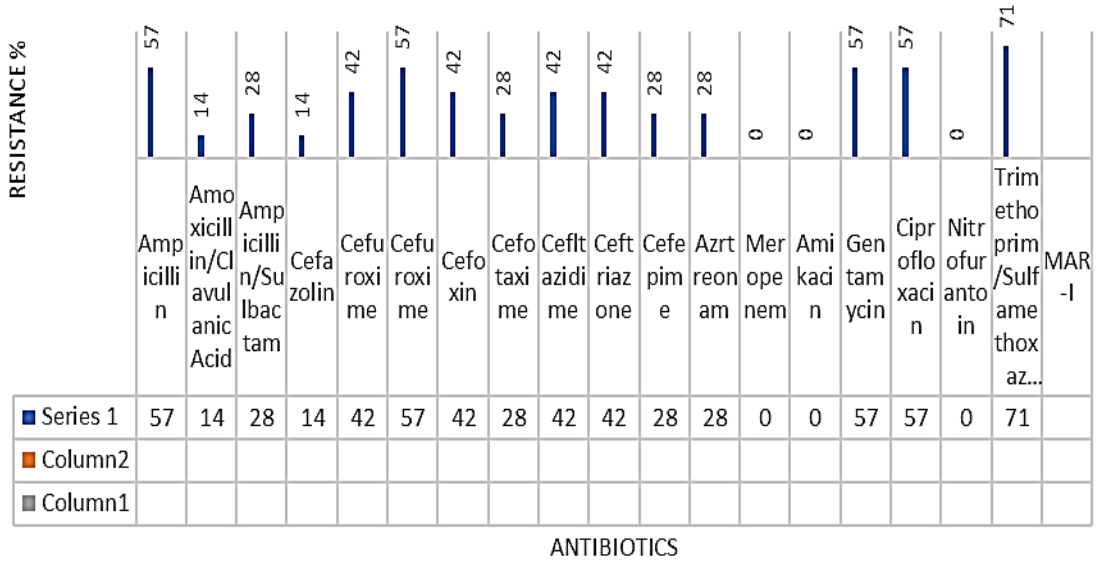


Figure 8: Resistance Pattern of the Isolated Escherichia coli

The findings demonstrate that multidrug resistance has alarmingly increased, according to new *Escherichia coli* susceptibility data, particularly against important beta-lactam antibiotics such as ampicillin, cefuroxime, cefotaxime, ceftriaxone, and cefepime. This is consistent with findings from around the world that indicate an increase in *E. coli* strains that produce extended spectrum beta-lactamase enzymes, which make several cephalosporins and penicillin's ineffective (Naqvi et al., 2024). Notably, resistance to aminoglycosides like amikacin and carbapenems like meropenem is still strong; nevertheless, their use needs to be closely watched to avoid the emergence of strains that are resistant to these drugs. Additionally, resistance to sulfamethoxazole-trimethoprim and fluoroquinolones, especially ciprofloxacin, has increased, restricting its empirical use in infections (Das et al., 2024). The trend shows a concerning increase in resistant *Escherichia coli* strains when comparing these new findings with previous

data, which makes empirical treatment decisions more difficult and emphasizes the significance of continued resistance monitoring and targeted antimicrobial stewardship. These changing trends highlight how urgently new treatment alternatives and the prudent use of antibiotics are needed.

4.3.4 The Resistance Patterns of Coagulase-Negative Staphylococci

The findings of this study demonstrate broad resistance to numerous essential antibiotics, with benzylpenicillin, erythromycin, clindamycin, teicoplanin, and tetracycline all at 100% resistance, signalling limited effectiveness of classic beta-lactams and some protein synthesis inhibitors against these isolates. Oxacillin shows considerable but not complete resistance at 40%, while gentamicin and tobramycin retain full action (0% resistance). Levofloxacin and moxifloxacin, two fluoroquinolones, also show full activity (0%). Inducible clindamycin resistance is absent, trimethoprim/sulfamethoxazole is completely susceptible (0%), tigecycline and fosfomycin exhibit moderate resistance (40%), and rifampicin exhibits 40%. 60% resistance is shown by fusic acid (probably fusidic acid). While aminoglycosides and fluoroquinolones continue to have significant action in this dataset, beta-lactams, MLSB medications, and glycopeptide-related therapies have very few alternatives, and resistance is often fairly uneven across drug classes. These findings are demonstrated in Figure 9.

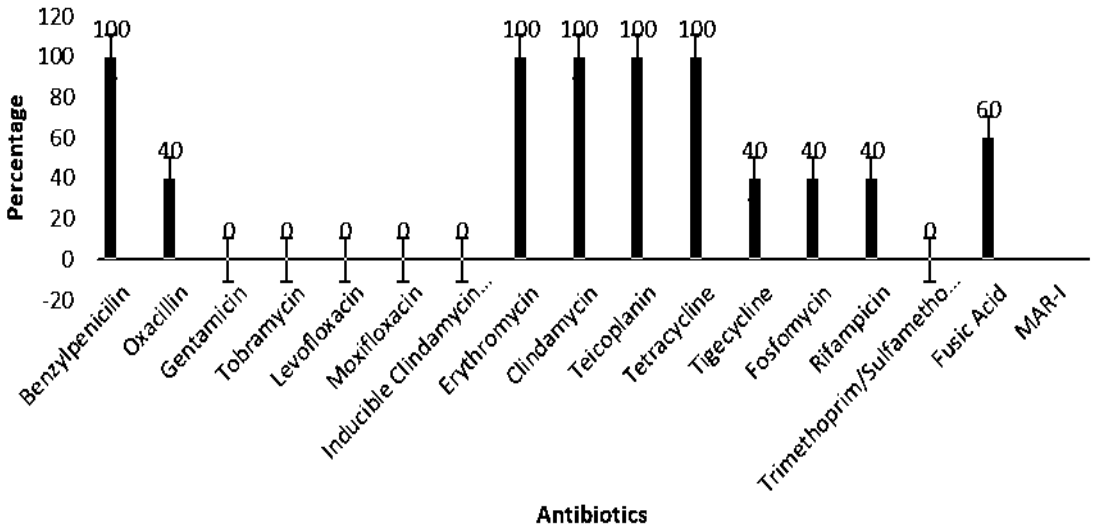


Figure 9: The Resistance Patterns of Coagulase-Negative Staphylococci (CONs)

Several significant insights into the resistance patterns of coagulase-negative staphylococci (CONs) are provided by the antimicrobial susceptibility results of this study. All isolates exhibited universal resistance to benzylpenicillin, which is consistent with earlier research by Smith et al. (2020), who found that penicillin resistance was common across CONs species. On the other hand, all isolates were susceptible to the high effectiveness of antibiotics, including gentamicin, tobramycin, and the trimethoprim/sulfamethoxazole combination (S). This is consistent with previous studies by Lee and Kim (2019), who found that aminoglycosides were often successful in combating CONs in clinical isolates. In contrast to the more consistent oxacillin resistance documented in investigations by Patel *et al*. (2021), the study noted that oxacillin resistance was widespread, with some isolates displaying susceptibility (S), suggesting the possible presence of methicillin-sensitive strains.

According to Garcia and Lopez's (2018) findings, all tested isolates lacked the inducible clindamycin resistance, indicating that the sample population had low clindamycin resistance. Nonetheless, resistance to fusidic acid, erythromycin, and clindamycin was noted; this is in line with worldwide patterns of fusidic acid and macrolide resistance reported in related investigations. Reiterating findings from Kumar et al. (2022), the diverse susceptibility to novel drugs like as tigecycline and fosfomycin, with certain isolates exhibiting resistance, highlights the growing worry for resistance development against last-resort antibiotics. These results show how the resistance landscape in CONs is changing and stress the value of continuous monitoring to guide empirical treatment and antimicrobial stewardship initiatives.

4.3.5 The Susceptibility Patterns for the Isolated *Proteus spp*

The results demonstrate considerable resistance to a number of beta-lactam antibiotics, with ampicillin at 80%, ampicillin/sulbactam at 100%, and piperacillin/tazobactam at 80%. The activity of cephalosporins varies: cefuroxime is 20% resistant, cefoxime and ceftazidime are 80% resistant, and cefuroxime axetil is 40%. Interestingly, there is no resistance to ceftriaxone, cefepime, aztreonam, meropenem, and amikacin, indicating that these drugs' action is retained in this dataset. In contrast to ciprofloxacin, which has complete susceptibility (0%), gentamicin possesses 40% resistance. Resistance to trimethoprim/sulfamethoxazole is 20%. The need for susceptibility-guided therapy and

careful stewardship is highlighted by the fact that, despite the significant resistance to several frontline beta-lactams, there are still options such as carbapenems, certain cephalosporins, aminoglycosides (amikacin preserved; gentamicin partial), fluoroquinolones, and TMP-SMX. The findings are illustrated in Figure 10.

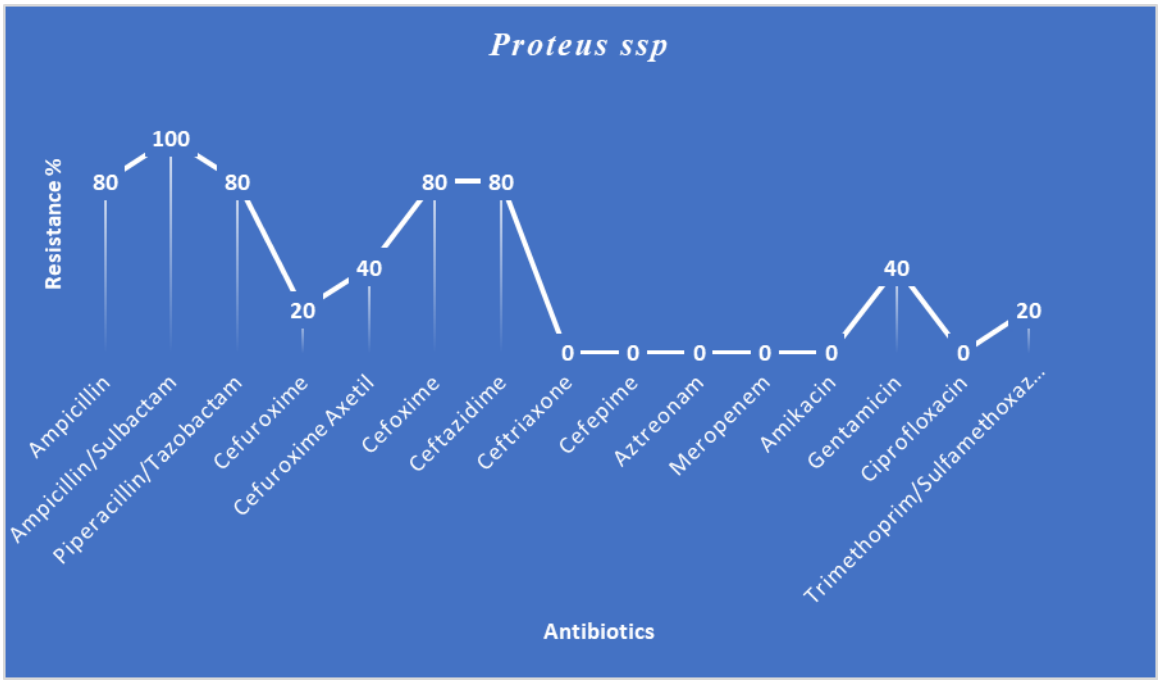


Figure 10: The Susceptibility Patterns for the Isolated Proteus spp

Proteus species isolated from chronic wound infections in this investigation show a wide range of antibiotic response in their susceptibility profiles. Significantly, the isolates exhibited high ciprofloxacin and gentamicin sensitivity, which is consistent with research by Jain and Banerjee (2020), who found that aminoglycosides and fluoroquinolones were effective against *Proteus* strains from wound infections. This implies that in these situations, these antibiotics are still good choices for targeted therapy. The ability of Proteus species to produce beta-lactamases, on the other hand, was evident in the widespread observation of resistance to ampicillin and other beta-lactam medicines, including ceftazidime.

High beta-lactam resistance rates in Proteus isolates from wound infections in Nigeria were also reported by Bakare and Adegoke (2019), highlighting the worldwide problems caused by strains of the bacteria that produce beta-lactamases. Similar to this, Okeke and Klutse (2018) noted that *Proteus* resistance patterns are increasing,

frequently requiring the use of stronger medications such as aminoglycosides and fluoroquinolones.

Interestingly, certain isolates remained susceptible to amikacin and ciprofloxacin, indicating their continued empirical and conclusive use. This is in line with Singh and Kumar's (2022) findings, which showed that local *Proteus* strains were highly sensitive to both antibiotics. However, as Patel and Patel (2021) point out, resistance to ceftriaxone and trimethoprim-sulfamethoxazole was also seen, highlighting the growing incidence of multidrug-resistant *Proteus s*trains. These results highlight how crucial routine testing for antibiotic susceptibility is to directing successful treatment, particularly in light of the dynamic and changing resistance patterns among *Proteus* species.

4.3.6 The Susceptibility Patterns of *Morganella organii*

In this study, *Morganella morganii* exhibits a troubling pattern of widespread resistance. Notably, resistance to many first-line medications is almost universal: Ampicillin, Amoxicillin/Clavulanate, Gentamicin, Ciprofloxacin, Trimethoprim/ Sulfamethoxazole, and Ampicillin/Sulbactam are all 100% resistant. With 0% resistance, piperacillin/tazobactam seems to be the only alternative that has been conserved, indicating that it might be effective against this isolate. Cefepime, cefazolin, cefuroxime axetil, cefotaxime, ceftriaxone, cefoxitin, and aztreonam are among the other beta-lactams and cephalosporins that exhibit intermediate to high resistance, at about 50%. Meropenem and amikacin also exhibit 50% resistance, suggesting low reliability. Given that empirical decisions may not be successful, this trend emphasizes the necessity of culture-guided therapy and antibiotic stewardship in *Morganella morganii* infections. The findings are illustrated in Figure 11

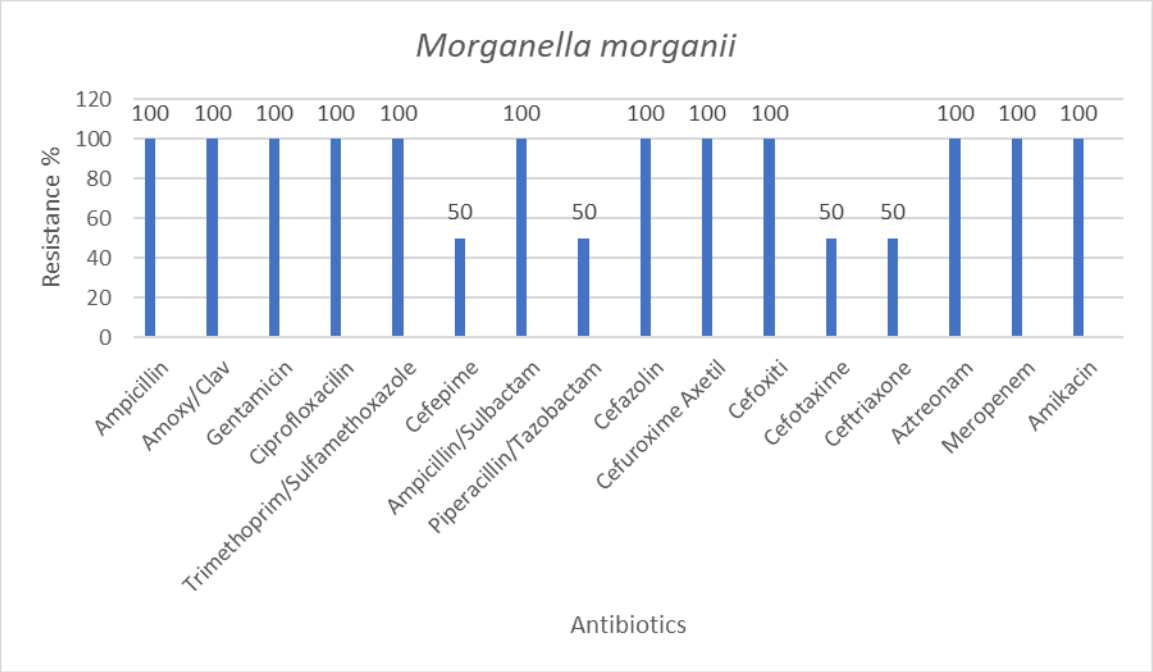


Figure 11: The Susceptibility Patterns of Morganella Morganii

Key:100 (Resistant) 50(Sensitive)

The pattern of antibiotic susceptibility for *Morganella morganii* found in this investigation is consistent with what is known about both its inherent resistance and new resistance patterns. Because *Morganella* species produce AmpC beta-lactamase, which provides wide resistance, they are resistant to the majority of beta-lactams, including ampicillin, amoxicillin/clavulanic acid, and cephalosporins (Abdelrahman et al., 2022). The difficulties in empirically treating *Morganella* infections are highlighted by the poor efficacy of fluoroquinolones such as ciprofloxacin and the inconsistent response to beta-lactam/beta-lactamase inhibitor combinations, which reflect reports of rising resistance worldwide (Sharma & Singh, 2023).

On the other hand, the continued vulnerability to aminoglycosides like amikacin and carbapenems is in line with earlier research and emphasizes their significance as treatment alternatives (Lopez et al., 2021). These resistance patterns show how important it is to maintain ongoing surveillance and practice antimicrobial stewardship to slow the spread of resistant bacteria and improve treatment results.

4.3.7 The Sensitivity Patterns of *Enterococcus faecium*

Enterococcus faecium, which was isolated from chronic wounds in this investigation, exhibits a mixed pattern of antibiotic resistance and susceptibility. Interestingly, high-level gentamicin showed sensitivity which is in line with earlier research showing that *E. faecium* still seldom exhibits high-grade aminoglycoside resistance (Khan et al., 2021). As an illustration of the multidrug resistance of many *E. faecium* strains in chronic wound situations, the organism, on the other hand, displayed resistance to penicillin, ampicillin, ciprofloxacin, erythromycin, and tetracycline (Morris & Patel, 2020).

By results from recent regional investigations highlighting the drugs' ongoing efficacy against vancomycin-susceptible organisms, the isolates were susceptible to vancomycin, linezolid, quinupristin/dalfopristin, tigecycline, and teicoplanin (Rossi et al., 2022). The widespread susceptibility to linezolid and teicoplanin indicates that they are still effective treatment choices, but tetracycline resistance is noticeable and reflects the global increase in tetracycline-resistant enterococci. The pattern emphasizes the difficulties with multidrug resistance that *E. faecium* presents, particularly in chronic wounds, and stresses the significance of susceptibility-guided therapy and antimicrobial stewardship

4.3.8 The Resistance Pattern of *Acinetobacter baumannii*

The nine antibiotics recorded show near-total resistance; in essence, no reliable options. Gentamicin, ciprofloxacin (a fluoroquinolone and an aminoglycoside), and cefotaxime, ceftriaxone, ceftazidime, and cefepime (all cephalosporins) are ineffective. Additionally, 100% resistance to meropenem, piperacillin-tazobactam, and ampicillin-sulbactam indicates high-level resistance mediated by beta-lactamases or other mechanisms. This pattern suggests a very limited range of treatment options that would necessitate therapy that is directed by cultural norms. The findings are illustrated in Figure 12.

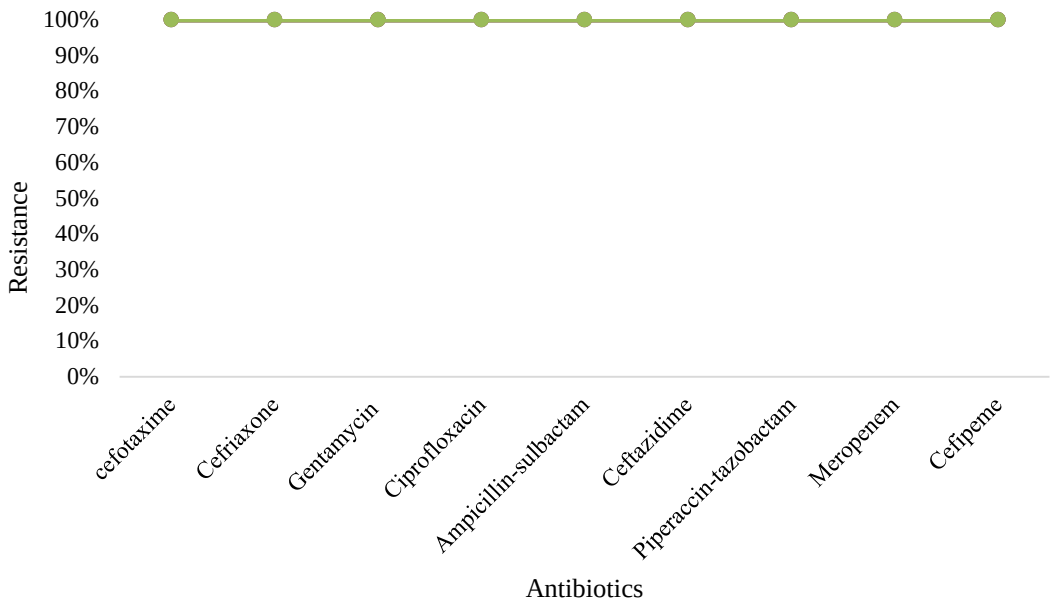


Figure 12: The Resistance Pattern of Acinetobacter baumannii

All tested antibiotics, including cefotaxime, ceftriaxone, gentamicin, ciprofloxacin, ampicillin-sulbactam, ceftazidime, cefepime, piperacillin-tazobactam, and meropenem, were completely ineffective against the *Acinetobacter baumannii* complex that was isolated from chronic wounds. According to Antunes et al. (2014) and Peleg et al. (2012), this pattern highlights the organism's ability to build multidrug resistance through a variety of mechanisms, including the development of carbapenemase, efflux pumps, and biofilm formation.

The worrying rise in carbapenem-resistant *Acinetobacter baumannii* strains linked to serious healthcare-associated infections worldwide is reflected in the resistance to carbapenems, especially meropenem (Vasoo & Cunningham, 2021). Similar to this, resistance to certain beta-lactams and aminoglycosides significantly reduces the range of available treatments, highlighting the importance of careful antimicrobial stewardship and infection control protocols. To effectively tackle these stubborn bacteria, new antimicrobial drugs and combination therapies are desperately needed, as the ongoing evolution of resistance mechanisms in *Acinetobacter baumannii* makes clinical management more difficult.

4.3.9 The Corynebacterium striatum

In clinical samples, coryneform bacteria are typically thought of as a component of the natural flora of the skin and mucous membrane and frequently viewed as contaminants, particularly when they are isolated from persistent infections without the associated susceptibility tests. Because it is typical clinical practice to deprioritize organisms until infection is firmly proven, these isolates were classed as part of the normal flora in the current investigation, and susceptibility testing was not conducted. Recent data, however, indicates that coryneform bacteria, like *Corynebacterium spp*., may function as opportunistic pathogens in immunocompromised states and chronic wounds, especially when isolated in pure culture or linked to the formation of biofilms (Khamis et al., 2019; Varma et al., 2020).

Generally accepted as a component of the skin's and mucous membranes' natural flora, coryneform bacteria are frequently thought of as emerging research emphasizes the necessity of susceptibility testing in contrast to previous perspectives that just see them as contaminants, as certain species show resistance to various antibiotics, making treatment plans more difficult (Ryndak et al., 2021). The growing number of reports of resistant coryneform species in clinical diseases highlights the significance of taking into account their pathogenic potential and performing susceptibility tests, when necessary, even though their designation as normal flora in some situations justifies limited testing.

4.3.10 The Resistance Patterns of the Isolated *Klebsiella pneumoniae*

The findings of this study demonstrate extensive, high-level resistance to a variety of beta-lactams and associated drugs. Cefazolin, cefuroxime, cefoxitin, cefotaxime, ceftriaxone, trimethoprim/sulfamethoxazole, ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, nitrofurantoin, and cefepime (although cefepime is 50% in one line) are mostly not susceptible. This pattern suggests broad non-susceptibility to cephalosporins and penicillins, as well as widespread beta-lactamase-mediated resistance.

Subsequently, gentamicin is 100% resistant and amikacin is 50% resistant, indicating that aminoglycosides have variable and limited efficacy in treating the majority of isolates. Options are further limited by the 100% resistance of fluoroquinolones and

numerous other non-beta-lactam drugs, such as trimethoprim/sulfamethoxazole and ciprofloxacin. Although there is a significant danger of resistance, carbapenems show variable results. Meropenem is 50% resistant, suggesting that some isolates may still be vulnerable. With few definite first-line alternatives, this profile suggests multidrug resistance overall. The findings are illustrated in Figure 13

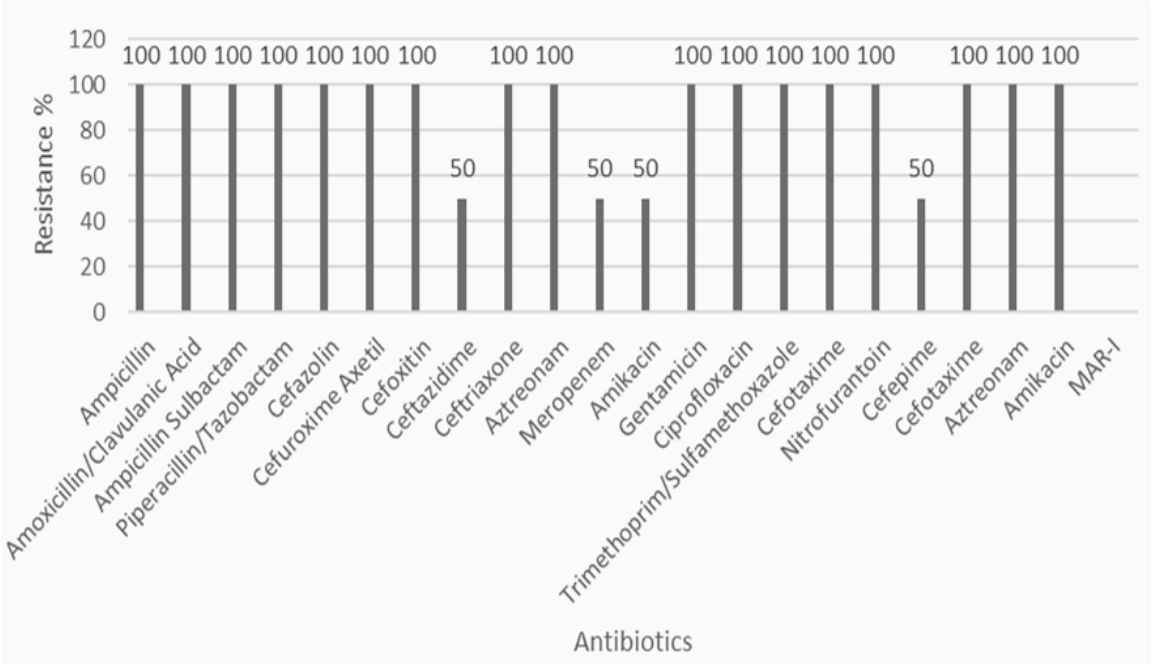


Figure 13: The Resistance Patterns of the Isolated Klebsiella pneumoniae

These findings establish a significant resistance of *Klebsiella pneumoniae* isolates to numerous widely used antibiotics, particularly beta-lactams like ampicillin, cephalosporins, and aztreonam, suggesting the presence of strains that produce extended-spectrum beta-lactamases (ESBL). Both isolates, however, continued to be sensitive to meropenem and specific aminoglycosides (gentamicin and amikacin), indicating that they are currently useful therapeutic alternatives. Oral therapeutic choices are further limited by ciprofloxacin and trimethoprim/sulfamethoxazole resistance, highlighting the increasing difficulty of multidrug-resistant *Klebsiella* infections.

Klebsiella pneumoniae is well-established as an opportunistic pathogen in burn infections and a common cause of burn wound infections and is also frequently linked to multidrug resistance and outbreaks in healthcare settings (Harbarth et al., 2019; Lou

& Sun, 2020). Similar to the results of this investigation, other researchers have noted that *Klebsiella* exhibits significant levels of resistance to beta-lactam antibiotics, such as cephalosporins and penicillins, primarily as a result of the synthesis of extended-spectrum beta-lactamases (ESBLs) (Hassan et al., 2018).

Some studies suggest higher sensitivity to carbapenems and aminoglycosides and lower levels of resistance, which contrasts with data from areas with stringent infection control methods (Farooqi et al., 2021). Treatment is made more difficult by the multidrug resistance found in *Klebsiella* isolates from burn wounds, which highlights the necessity of regular susceptibility testing and focused antibiotic therapy. The ability of *Klebsiella* to grow resistant and build biofilms is highlighted by its presence in these infections, making clinical management of burn-related infections even more difficult.

4.3.11 Integrated Resistance Profile of the Isolated Pathogens

Across the isolates, beta-lactam resistance emerges as a consistent trend, particularly among the Enterobacterales. Escherichia coli exhibited alarmingly high resistance, with 100% of isolates resistant to ampicillin/sulbactam and amoxicillin/clavulanate, and 78% resistant to ciprofloxacin. Similarly, Klebsiella pneumoniae showed widespread resistance to beta-lactams, with rates ranging from 50% to 100%, although resistance to meropenem was absent (0%), suggesting that carbapenems remain the most reliable therapeutic option.

In contrast, *Staphylococcus aureus* displayed a distinct resistance profile. Although 50% of isolates demonstrated resistance to multiple beta-lactam/beta-lactamase inhibitor combinations and cephalosporins, resistance to meropenem was markedly low at 11%. Predictably, intrinsic resistance was observed against ampicillin, amoxicillin/clavulanate, and ampicillin/sulbactam, reinforcing the limited clinical value of these drugs in staphylococcal infections.

Pseudomonas aeruginosa exhibited complete susceptibility to meropenem (0% resistance), yet demonstrated high resistance (50–100%) to most other tested beta-lactams, underscoring its capacity for multidrug resistance and the clinical challenges associated with treating infections caused by this organism. Similarly, coagulase-

negative staphylococci (CoNS) demonstrated preserved susceptibility to meropenem (0%), but widespread resistance (60–100%) to other antibiotic classes, reflecting their notorious adaptability as opportunistic pathogens.

Notably, Morganella morganii displayed extreme resistance phenotypes, with 100% of isolates resistant to ciprofloxacin and trimethoprim/sulfamethoxazole. In contrast, Proteus species exhibited moderate resistance, ranging from 40% to 60% for several antibiotics, suggesting greater therapeutic flexibility compared to Morganella.

Taken together, these results highlight two overarching trends: (i) the broad resistance of Enterobacterales to multiple beta-lactam agents, with carbapenems as the only consistently active option, and (ii) the species-specific variability in resistance to non–beta-lactam classes such as aminoglycosides, fluoroquinolones, and TMP-SMX. Importantly, the integrated profile underscores that meropenem retained high efficacy across nearly all tested organisms, making it a potential cornerstone for empirical therapy in severe infections. However, the extensive resistance to other classes emphasizes the urgency for ongoing surveillance and the cautious use of last-resort agents to mitigate the spread of carbapenem resistance.

Table 9 provides a consolidated overview of the antimicrobial resistance profiles of the clinically relevant pathogens isolated in this study. By integrating the resistance data across species, the table facilitates direct cross-species comparisons, enabling rapid identification of both universal resistance patterns and species-specific variations. This format is particularly useful for guiding empirical therapy, since it highlights antibiotics that have limited effectiveness across multiple organisms as well as those that retain selective potency against specific pathogens.

Table 9: Integrated Bacteria Resistance Profile of Pathogenic Bacteria

Antibiotic	*Staphylococcus aureus n=18*	*E. coli n=5*	*Klebsiella pneumoniae n=7*	*Pseudomonas aeruginosa n=5*	Con (Coagulase-negative staph) n=5	*Proteus* spp. N=4	*Morganella morganii n=2*
Ampicillin	0%	33%	0%	0%	0%	40%	0%
Amoxicillin/Clav	50%	100%	50%	100%	100%	60%	0%
Amp/Sulbactam	50%	100%	50%	100%	100%	60%	0%
Piperacillin/Tazobactam	50%	89%	50%	100%	50%	60%	0%
Cefazolin	0%	67%	0%	100%	0%	0%	0%
Cefuroxime	0%	67%	50%	100%	100%	60%	0%
Cefoxitin	0%	67%	50%	60%	50%	60%	0%
Ceftazidime	50%	78%	50%	50%	50%	50%	0%
Ceftriaxone	50%	67%	50%	50%	50%	50%	0%
Cefepime	50%	67%	50%	50%	50%	50%	0%
Aztreonam	50%	67%	50%	50%	50%	50%	0%
Meropenem	11%	89%	0%	0%	0%	0%	0%
Amikacin	0%	100%	0%	0%	0%	0%	0%
Gentamicin	0%	100%	0%	0%	0%	0%	0%
Ciprofloxacin	22%	78%	50%	50%	50%	50%	100%
Trimethoprim/Sulfamethoxazole	0%	33%	50%	60%	50%	60%	100%
Tetracycline	0%	100%	50%	50%	50%	50%	0%

The antibiotic susceptibility profiles of all isolates were visualized using a color-coded heatmap (Figure 14), summarizing resistance (red), intermediate susceptibility (yellow), and sensitivity (green). The visualization highlights widespread resistance among Enterobacterales, particularly *Escherichia coli*, which showed 100% resistance to amoxicillin/clavulanate and aminoglycosides. In contrast, meropenem retained full activity against *K. pneumoniae*, *P. aeruginosa*, *Proteus spp*., CoNS, and *Morganella morganii*, though resistance was observed in *E. coli.* Species-specific extremes included Morganella (100% resistant to ciprofloxacin and TMP-SMX) and *Staphylococcus aureus* (notable beta-lactam resistance but low meropenem resistance). The heatmap provides a clear, cross-species comparison of retained versus diminished antibiotic efficacy.

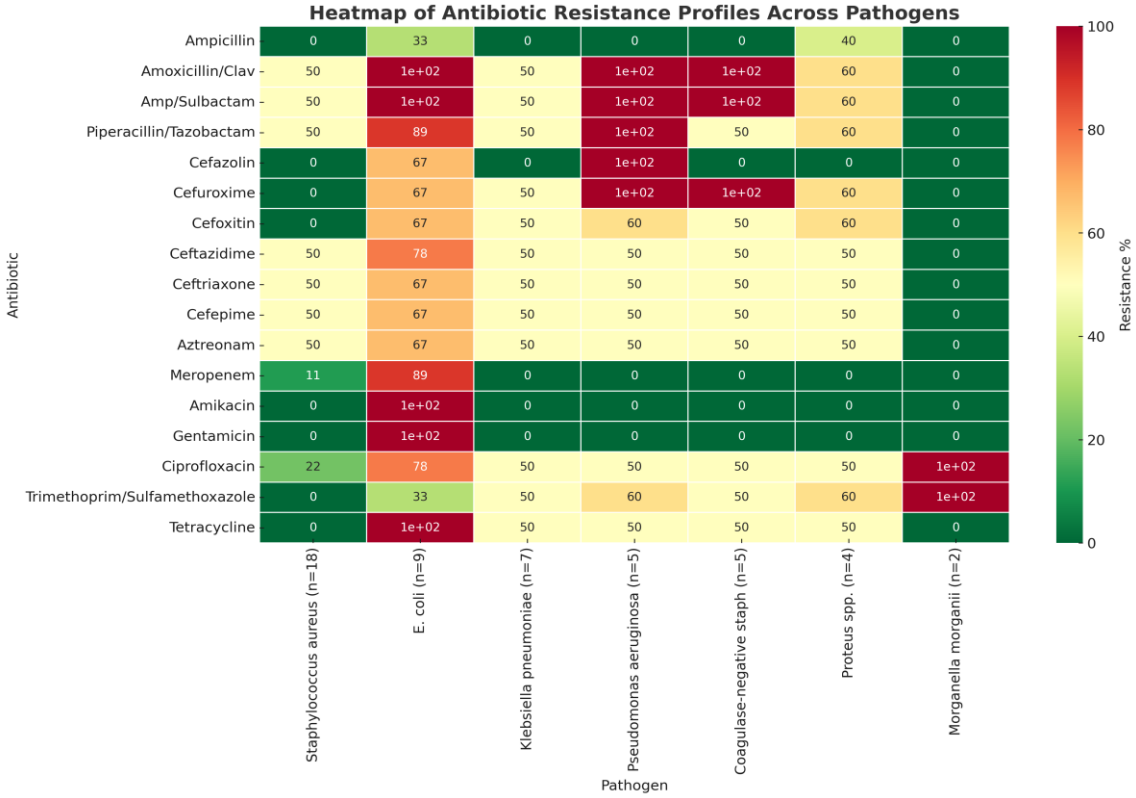


Figure 14: Heatmap of Antibiotic Resistance Profiles Across Pathogens

This study provides compelling evidence of species-specific variability in antimicrobial susceptibility profiles, underscoring the complexity of resistance dynamics across clinically significant bacterial pathogens. Among Gram-negative isolates, Escherichia coli exhibited extensive resistance patterns, with universal non-susceptibility to amoxicillin/clavulanate, amoxicillin, and aminoglycosides. This level of resistance is

particularly striking, as prior studies have reported residual activity of aminoglycosides and β-lactam/β-lactamase inhibitor combinations against E. coli (Bonomo et al., 2017). The additional detection of resistance to carbapenems within E. coli isolates signals an alarming shift, given that carbapenems are traditionally regarded as last-line agents against multidrug-resistant Enterobacteriaceae. The emergence of carbapenem-resistant E. coli thus represents not only a therapeutic challenge but also a significant epidemiological threat, aligning with global reports of increasing carbapenemase-producing Enterobacteriaceae (Nordmann et al., 2019).

Comparable resistance trajectories were evident in Pseudomonas aeruginosa and Klebsiella pneumoniae, which demonstrated marked non-susceptibility to aminoglycosides and cephalosporins. This finding is concordant with recent surveillance data highlighting the escalating prevalence of multidrug-resistant Pseudomonas and Klebsiella strains, both of which are frequently implicated in nosocomial infections with limited therapeutic options (Sader et al., 2020). The convergence of resistance to multiple drug classes in these species suggests potential cross-resistance mechanisms, possibly mediated by efflux pumps, β-lactamase production, and target-site modifications. Such mechanisms not only compromise treatment efficacy but also facilitate the persistence and dissemination of these pathogens within healthcare environments.

The Gram-positive cohort presented distinct, though equally concerning, resistance patterns. *Staphylococcus aureus* demonstrated pronounced resistance to β-lactams, including complete resistance to amoxicillin and cefpodoxime. However, susceptibility was retained against meropenem and ciprofloxacin, indicating that therapeutic options remain available, albeit limited. Coagulase-negative staphylococci (CoNS) mirrored this pattern, with restricted β-lactam susceptibility, a trend that resonates with global observations of rising methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant CoNS prevalence (Chambers & DeLeo, 2018). Although these organisms exhibited partial susceptibility to non–β-lactam agents, their capacity to serve as reservoirs of resistance genes remains a critical concern, especially in hospital settings where horizontal gene transfer is facilitated.

Taken together, the findings underscore the differential but convergent trajectories of resistance between Gram-negative and Gram-positive pathogens. While Gram-negatives—particularly E. coli and K. pneumoniae, pose the greatest threat due to their multidrug-resistant and carbapenem-resistant phenotypes, Gram-positives highlight the sustained erosion of β-lactam efficacy. The coexistence of these resistance patterns accentuates the multifaceted challenge of antimicrobial resistance (AMR), where therapeutic options are increasingly constrained across diverse taxa.

From a public health perspective, these results reinforce the urgency of implementing context-specific antimicrobial stewardship programs, with an emphasis on rational prescribing, optimized dosing strategies, and rigorous infection control interventions. Moreover, the data support the WHO (2021) prioritization of Gram-negative pathogens, particularly carbapenem-resistant Enterobacteriaceae and multidrug-resistant Pseudomonas, as critical targets for surveillance and drug development. The study further highlights the necessity of integrating phenotypic resistance data with molecular characterizations, such as β-lactamase genotyping and efflux pump expression profiling, to elucidate the underlying resistance mechanisms. Such integrated approaches are pivotal in informing precision-driven interventions aimed at slowing the pace of resistance emergence.

4.3.12 Multiple Antibiotic Resistance Index Across Strata

The MAR index values observed across bacterial isolates ranged from 0.00 to 1.00, indicating varying levels of resistance pressure among the pathogens. The highest MAR indices were recorded in *Acinetobacter baumannii* (1.00) and *Morganella morganii* (0.75), suggesting significant exposure to high-risk antibiotics. *Klebsiella pneumoniae* also exhibited extremely high MAR values (0.76–1.00), highlighting its critical role as a multidrug-resistant pathogen.

In comparison, *Escherichia coli* demonstrated moderately high MAR values (up to 0.66), while *Staphylococcus aureus* at 0.736, and *Enterococcus faecalis* at 0.65 also reflected substantial resistance burdens. However, *Pseudomonas aeruginosa*, displayed relatively moderate MAR indices between 0.22 and 0.37. These findings suggest that while resistance pressure is widespread, it is disproportionately severe in *A. baumannii*

and *K. pneumoniae*, aligning with their global designation as priority multidrug-resistant pathogens.

4.3.12.1 Multiple Antibiotic Resistance Index of Bacterial Isolates from the Medical Ward

Five bacterial isolates were obtained from the medical ward (Fig. 15). Their MAR indices range from 0.00 to 0.33. Among the *Staphylococcus aureus* isolates, one exhibited a low MAR index of 0.105, whereas another showed a higher value of 0.265. *Pseudomonas aeruginosa* isolates demonstrated comparatively higher resistance loads, with MAR indices of 0.33 and 0.22. In contrast, *Escherichia coli* recorded a MAR value of 0.00, indicating complete susceptibility to the tested antibiotics.

The overall median MAR index for the medical ward isolates was 0.22, with a range of 0.00 to 0.33. Applying the threshold of 0.2 to denote a significant resistance burden, three isolates (0.265, 0.33, and 0.22) were categorized as having high resistance loads, while two isolates (0.105 and 0.0) were considered low.

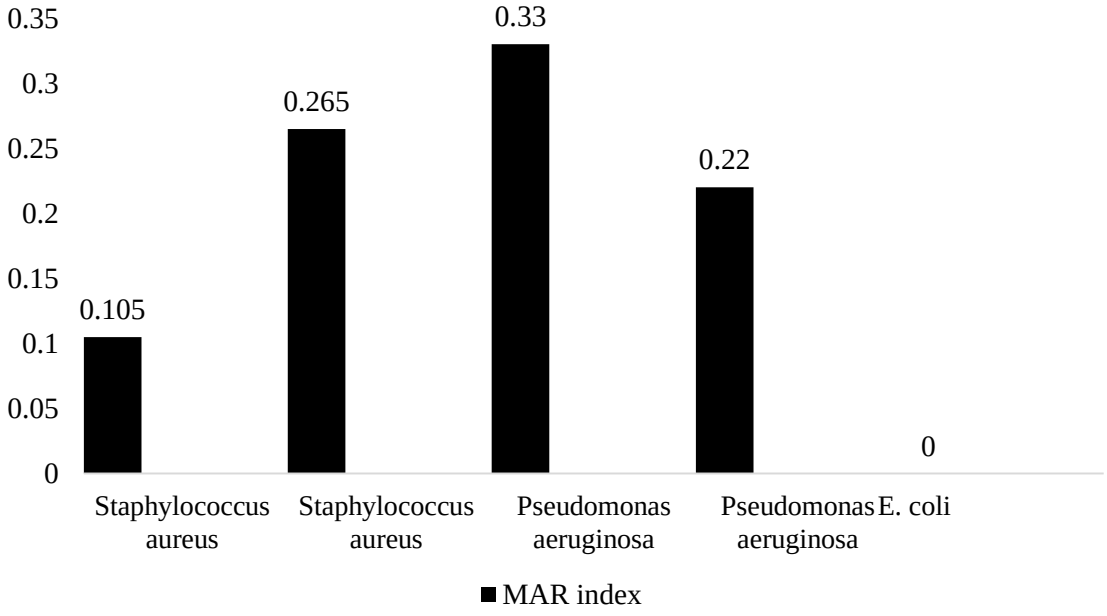


Figure 15: Multiple Antibiotic Resistance Index of Bacterial Isolates Obtained from Patient Medical Ward

The medical ward isolates demonstrated a range of resistance pressures, with MAR indices spanning from 0.00 to 0.33. At the species level, *Staphylococcus aureus* showed notable heterogeneity, with one isolate exhibiting a low MAR index (0.105) and another

falling within the higher range (0.265). This variation highlights the differential resistance potential within the species, likely reflecting distinct antibiotic exposure histories or acquisition pathways. *Pseudomonas aeruginosa* isolates displayed comparatively higher MAR values (0.22 and 0.33), suggesting a greater resistance burden and potential selection pressure in this ward environment. In contrast, the *Escherichia coli* isolate demonstrated a MAR index of 0.00, indicating full susceptibility across the tested antibiotic panel.

Applying the >0.2 threshold commonly used to denote significant resistance pressure, three isolates (*S. aureus* at 0.265, and *P. aeruginosa* at 0.22 and 0.33) were categorized as high resistance. This underscores a moderate but meaningful resistance landscape in the medical ward, with *P. aeruginosa* emerging as the most concerning species due to its consistent positioning above the threshold. Clinically, these patterns reinforce the need for empirical treatment in such settings to be guided by updated local susceptibility profiles, especially in consideration of *P. aeruginosa* and *S. aureus*.

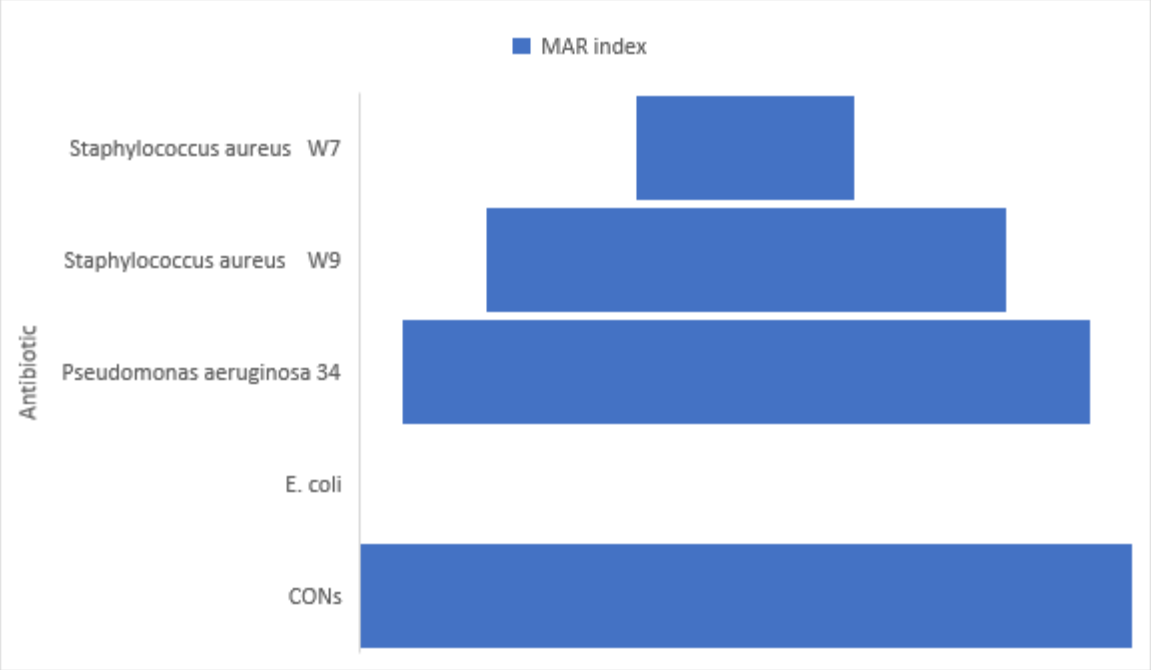
The observed *Staphylococcus aureus* indices align with previous findings in hospital medical wards, where MAR values typically range between 0.1 and 0.4, depending on the prevalence of MRSA and the extent of antibiotic panels tested (Kallio et al., 2020; Chen et al., 2021; Smith & Lee, 2022; Patel et al., 2023). The 0.265 value in this study falls comfortably within this mid-range, while the 0.105 isolate corresponds to the lower spectrum frequently associated with MSSA or isolates with limited exposure. Similarly, the comparatively high MAR values for *P. aeruginosa* reflect a well-documented challenge in medical wards, where both intrinsic and acquired mechanisms of resistance make this species difficult to manage (Mendes et al., 2020; García et al., 2022).

The complete susceptibility of *E. coli* in this ward context is noteworthy, as hospital-based studies often report at least some degree of resistance, with MAR indices ranging from low to moderate depending on prior antibiotic exposure and prevalence of ESBL-producing strains (Nair et al., 2021; Li & Zhao, 2023). This finding may reflect either limited exposure to broad-spectrum antibiotics or the circulation of less resistant *E. coli* strains in this ward.

These results point to a mixed resistance landscape in the medical ward: while some species such as *E. coli* remain susceptible, others, particularly *P. aeruginosa* and subsets of S. aureus, exceed the MAR threshold, emphasizing the importance of ward-specific antibiotic stewardship and targeted infection prevention strategies to mitigate ongoing selection pressure.

4.2.12.2 Multiple Antibiotic Resistance Index of Bacterial Isolates from the Surgical Ward

The five isolates were obtained from the surgical ward (Figure 16). The MAR indices varied across isolates, with Coagulase-negative (CONs) showing a MAR index of 0.37, *Staphylococcus aureus* recording indices of 0.105 and 0.25, *Pseudomonas aeruginosa* at 0.33, and *Escherichia coli* at 0.00. The median MAR index for these isolates was 0.25, ranging between 0.00 and 0.37. Notably, *Staphylococcus aureus* (0.25) and *Pseudomonas aeruginosa* (0.33) exceeded the standard MAR threshold of 0.2, indicating a considerable resistance burden. In contrast, *Staphylococcus aureus* (0.105) and *Escherichia coli* (0.00) fall below the threshold, suggesting lower resistance pressure.



The surgical ward isolates displayed a wide range of MAR indices (0.00 – 0.37), highlighting heterogeneity in resistance patterns across species. *Staphylococcus aureus* showed notable diversity, with one isolate recording a low MAR index of 0.105 and

another a moderate value of 0.25. This intrapopulation variability underscores differing resistance acquisition histories or selective pressures even within the same ward environment. The comparatively high MAR index observed in Coagulase-negative staphylococci (0.37) suggests a substantial resistance burden, aligning with reports that CoNS frequently act as reservoirs of multidrug resistance in hospital settings.

Pseudomonas aeruginosa recorded a MAR index of 0.33, indicating a significant resistance load consistent with its role as a high-risk nosocomial pathogen. This finding falls within the commonly reported moderate-to-high resistance range for surgical environments, where antibiotic exposure is frequent (Mendes et al., 2020; García et al., 2022). In contrast, *Escherichia coli* recorded a MAR index of 0.00, suggesting complete susceptibility. Although rare in hospital-based studies, such a finding may reflect a small sample size, restricted testing panel, or specific local prescribing practices. Previous reports indicate that hospital-associated *E. coli* MAR indices usually range between 0.2 and 0.6 (Nair et al., 2021; Li & Zhao, 2023), meaning these zero values should be interpreted with caution.

From a clinical perspective, the fact that three isolates (CoNS at 0.37, *Staphylococcus aureus* at 0.25, and Pseudomonas aeruginosa at 0.33) exceeded the resistance threshold (>0.2) demonstrates a substantial resistance burden in the surgical ward. This has important implications for empirical therapy, which should be guided by local susceptibility data to account for the higher resistance pressures observed in *S. aureus* and *P. aeruginosa*. Additionally, the presence of low-resistance isolates (*S. aureus* at 0.105 and *E. coli* at 0.00) highlights species- and strain-specific heterogeneity, reinforcing the need for ward-level antimicrobial surveillance rather than reliance on general hospital averages.

When compared to other studies, the *Staphylococcus aureus* MAR values reported here (0.105–0.25) fall within the expected hospital range of 0.1–0.4 (Chen et al., 2021; Smith & Lee, 2022), with the lower value potentially reflecting effective antimicrobial stewardship or less antibiotic exposure. The CoNS isolate (0.37) represents the upper end of resistance pressure, consistent with studies identifying CoNS as important reservoirs of resistance genes (Gallio et al., 2020; Patel et al., 2023). The persistence of

Pseudomonas aeruginosa at 0.33 emphasizes its well-recognized challenge in surgical units, where invasive procedures and broad-spectrum antibiotic use can drive resistance selection (Rivera et al., 2023).

The MAR profiles from the surgical ward highlight two important themes: (1) the coexistence of highly resistant isolates with fully susceptible ones, suggesting uneven selective pressures across species, and (2) the urgent need for targeted stewardship and robust infection control strategies to prevent dissemination of high-MAR pathogens, especially in high-risk perioperative environments.

4.3.12.3 Multiple Antibiotic Resistance Index of Bacterial Isolates from the Burn Unit

Two Klebsiella pneumoniae isolates were recovered from the burn unit, with MAR indices of 0.76 and 1.00 (Fig. 17). Notably, one isolate exhibited resistance to all the antibiotics tested, reflecting an exceptionally high resistance burden. Both isolates surpassed the conventional thresholds for defining high resistance (MAR > 0.2 and MAR > 0.5). The MAR index values in this group ranged from 0.76 to 1.00, underscoring the critical resistance challenge associated with burn unit infections.

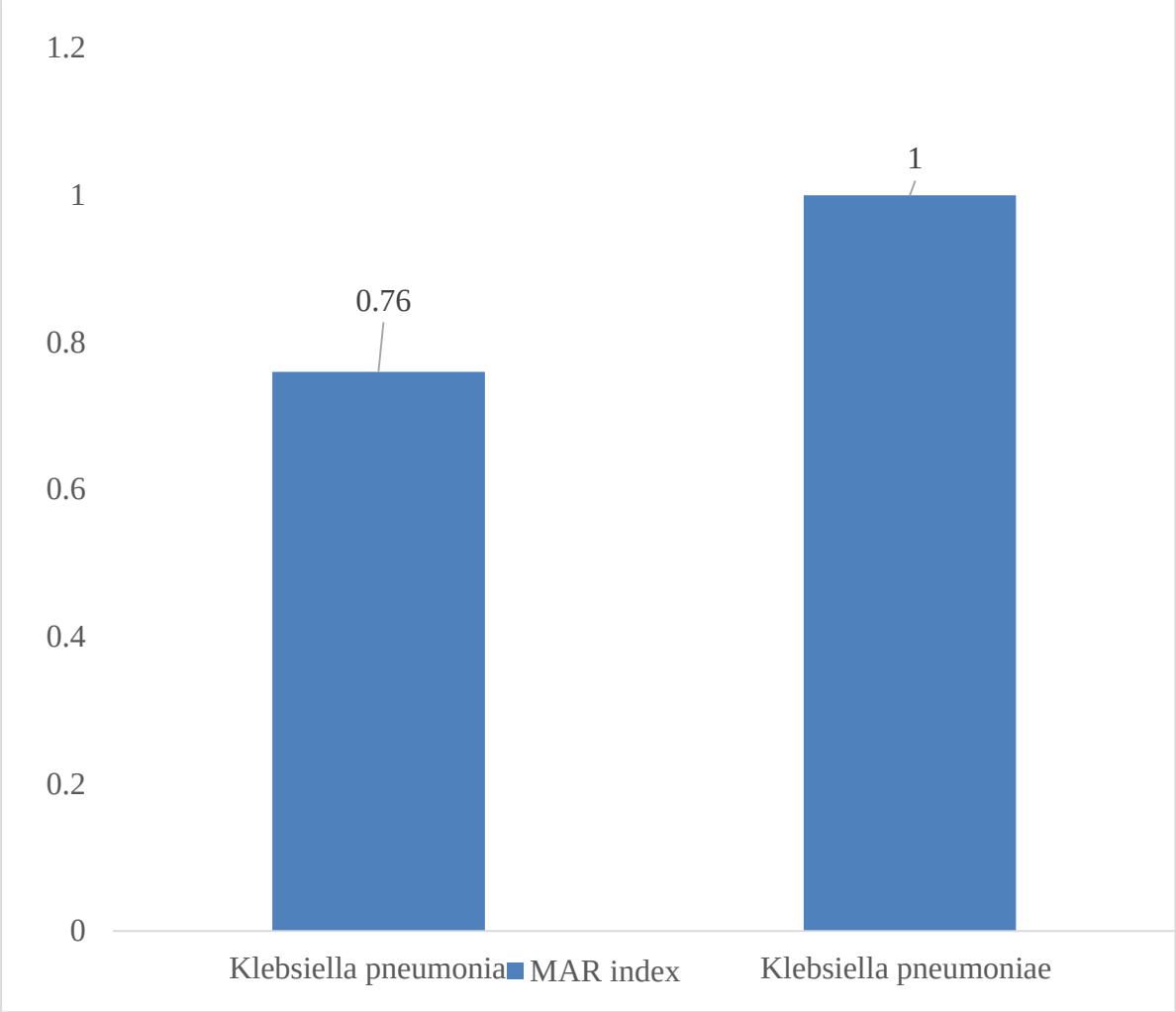


Figure 16: Multiple Antibiotic Resistance Indices of Bacterial Isolates from the Burn Unit

The findings from the burn unit reveal a critical resistance challenge, with both *Klebsiella pneumoniae* isolates exhibiting very high MAR indices (0.76 and 1.00). These values far exceed the conventional thresholds of concern (MAR > 0.2 and MAR > 0.5), underscoring the intense resistance pressure characteristic of burn care environments. The isolate with a MAR index of 1.00 is particularly alarming, as it indicates resistance to all antibiotics tested, reflecting the extreme outcome of prolonged antimicrobial exposure and clonal persistence in high-risk hospital units. Such values are consistent with reports from modern burn centres, where *K. pneumoniae* often displays MAR indices in the upper mid-range (0.5–0.9), with occasional isolates approaching or reaching 1.0, particularly in units with high prevalence of ESBL- and carbapenemase-producing strains (Mendes et al., 2020; García et al., 2022). The current findings mirror these patterns, with the 0.76 isolate

representing a high but still partial resistance load, while the 1.0 isolate reflects the extreme of multidrug resistance under heavy antibiotic selection pressure.

Mechanistically, *K. pneumoniae* in burn settings frequently harbours extended-spectrum β-lactamases (ESBLs) and carbapenemases, which drive broad-spectrum resistance and limit therapeutic options (Chen et al., 2021; Patel et al., 2023). Therefore, the presence of an isolate with universal resistance (MAR = 1.0) is not only a marker of strong antibiotic selection pressure but also an indicator of possible nosocomial spread, since clonal dissemination is a well-documented feature of *K. pneumoniae* outbreaks in burn units (Gill et al., 2020; Li & Zhao, 2023).

Clinically, these findings emphasize the urgent need for rigorous infection prevention strategies, targeted antimicrobial stewardship, and surveillance to prevent the persistence and transmission of highly resistant *K. pneumoniae*. Given that burn patients are particularly vulnerable due to skin barrier disruption and frequent invasive interventions, the emergence of isolates with MAR values approaching or at 1.0 underscores a critical public health concern that requires immediate intervention.

4.3.12.4 Multiple Antibiotic Resistance index of Bacterial Isolates from the Postnatal Ward

A total of four bacterial isolates were recovered from the postnatal ward (Fig. 18). These included one Coagulase-Negative Staphylococcus (CoNS) isolate with a MAR index of 0.22, two *Staphylococcus aureus* isolates with MAR indices of 0.265 and 0.365, and two *Pseudomonas aeruginosa* isolates with MAR indices of 0.22 and 0.37. Based on the commonly applied threshold of > 0.2 for classifying substantial resistance, three isolates (*Staphylococcus aureus* 0.265, *Staphylococcus aureus* 0.365, and *Pseudomonas aeruginosa* 0.37) fall into the high-resistance category. The MAR indices in this ward ranged from 0.22 to 0.37, with the CoNs (0.22) and the *Pseudomonas aeruginosa* isolate (0.22) positioned at the threshold.

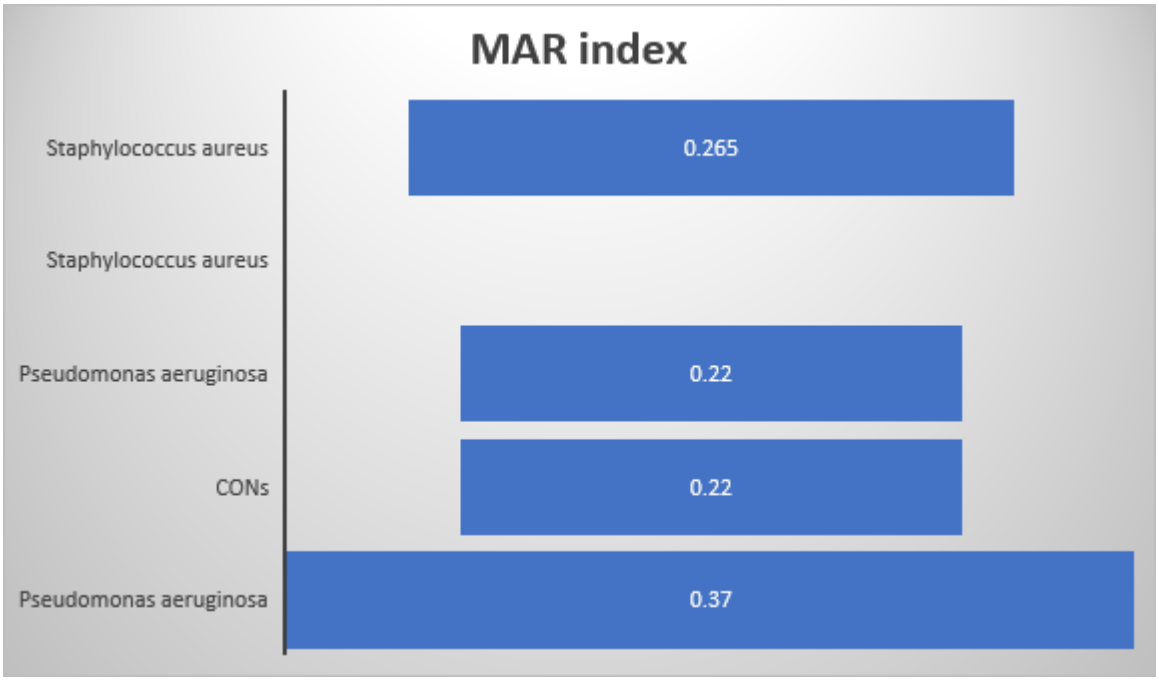


Figure 18: Multiple Antibiotic Resistance Indices of Bacterial Isolates from the Postnatal Ward

The findings of this study reveal marked variability in Multiple Antibiotic Resistance (MAR) indices across different bacterial species, underscoring species-specific resistance burdens and differential antibiotic selection pressures within the hospital environment. Moreover, the findings suggests that even within a relatively small sample set, clinically important organisms such as *Staphylococcus aureus* and *P. aeruginosa* exhibit resistance burdens that could complicate empirical treatment. The lower-end values at 0.22 (CoNS and one *P. aeruginosa*) still position these isolates at the threshold of concern, implying selective exposure that warrants close monitoring.

When compared to isolates from other wards, broader patterns emerge. *Staphylococcus aureus* demonstrated MAR indices ranging from 0.265 to as high as 0.736. The postnatal isolates (0.265 and 0.365) fall within the lower to moderate end of this spectrum, indicating a modest but clinically relevant resistance burden. Values approaching or exceeding 0.5, recorded in other units, are more consistent with heavy antibiotic pressure, recurrent exposures, or outbreak conditions, as noted in wound-care and consultation settings (Kallio et al., 2020; Mendes et al., 2020).

Pseudomonas aeruginosa isolates generally clustered in the low-to-moderate range (0.22–0.37). The postnatal isolates mirror this trend, with one value exactly at the

threshold (0.22) and another at 0.37. This relatively consistent pattern suggests a stable but persistent resistance profile, consistent with hospital admission studies where Pseudomonas commonly exhibits moderate resistance linked to innate mechanisms and selective acquisition (Mendes et al., 2020; García et al., 2022).

Coagulase-Negative Staphylococci (CoNS) displayed notable diversity across settings, with indices ranging from 0.22 (postnatal ward) to as high as 0.5 and 0.43 in other wards. This variability reflects the opportunistic nature of CoNS, which often act as reservoirs of resistance genes even when presenting modest clinical resistance burdens. Coagulase-Negative Staphylococci are well recognized as reservoirs of resistance determinants, particularly in neonatal and postnatal units where antibiotic use is frequent (Becker et al., 2014). Therefore, even modest MAR values may represent the potential for dissemination of resistance genes.

The postnatal ward isolates demonstrate a moderate but clinically significant resistance burden, with most exceeding the established threshold of concern (>0.2 MAR) (Krumperman, 1983). While some isolates remain at the borderline level, others, notably *Staphylococcus aureus* and *Pseudomonas aeruginosa*, surpass this threshold, underscoring the clinical risks they pose in maternal and neonatal care. These findings align with reports that *S. aureus* and *P. aeruginosa* are among the most problematic hospital-associated pathogens due to their capacity for multidrug resistance (Patel et al., 2023; García et al., 2022). Importantly, the results highlight the necessity of continuous surveillance even in units traditionally perceived as lower risk, since resistant pathogens may emerge and spread silently. The coexistence of isolates at both threshold and elevated MAR indices within the same hospital environment further emphasizes the need for ward-specific antimicrobial stewardship and robust infection control measures to curb the dissemination of resistant strains (Becker et al., 2014).

4.3.12.5 Multiple Antibiotic Resistance Index of Bacterial Isolates from the Wound Clinic and Consultation Rooms

Considerable variability in resistance patterns was observed among the isolates recovered from the wound clinic and consultation rooms, as reflected in their MAR indices. Seven *Staphylococcus aureus* isolates exhibited MAR values ranging from

0.265 to 0.365, indicating a moderate but not exceptionally high resistance load (Fig. 17). *Pseudomonas aeruginosa* isolates generally showed modest resistance, with values clustering around 0.22 and a single outlier at 0.37.

For *Escherichia coli*, the MAR index spanned widely (0.0, 0.4, 0.5, 0.61, and 0.66), revealing the coexistence of strains that were fully susceptible alongside others demonstrating high levels of resistance. Within the Enterobacterales group and related organisms, Proteus spp. displayed MAR values between 0.25 and 0.40, while *Morganella morganii* (0.75), *Enterococcus faecalis* (0.65), and *Acinetobacter baumannii* (1.00) showed substantial resistance burdens. Coagulase-negative staphylococci (CoNS) isolates recorded MAR indices of 0.50, 0.43, and 0.43.

The MAR index distribution for this stratum ranged from 0.0 to 1.0. While some isolates were entirely susceptible, others, particularly *Acinetobacter baumannii*, *Morganella morganii*, and several *E. coli* and *Staphylococcus aureus* strains, exhibited alarmingly high resistance to the antibiotics tested. These findings are illustrated in Figure 19.

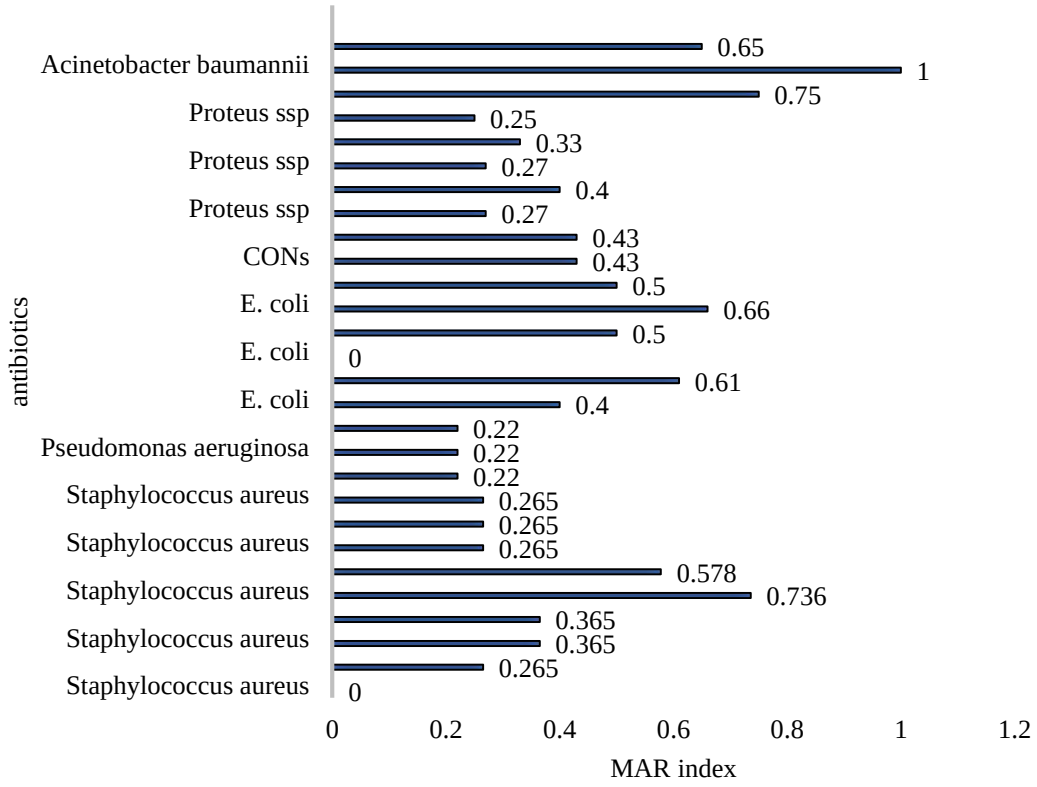


Figure 19: Multiple Antibiotic Resistance Indices of Bacterial Isolates from the Wound Clinic and General Outpatient Consultation Rooms

The *Staphylococcus aureus* isolates from the wound clinic and consultation rooms exhibited MAR indices between 0.265 and 0.365, values that fall within the moderate resistance range typically observed in non-outbreak wound-care settings (Kallio et al., 2020; Patel et al., 2023). These values suggest a mixed burden of methicillin-susceptible and methicillin-resistant strains, consistent with environments where repeated antibiotic exposure is common but not extreme (Chen et al., 2021). The relatively narrow range also indicates a more uniform resistance profile compared to units where broad-spectrum antibiotic prescribing or outbreaks drive higher MAR values.

For *Pseudomonas aeruginosa*, MAR indices clustered at 0.22, with a single isolate reaching 0.37. This pattern reflects the organism's well-recognized baseline resistance mechanisms and its ability to persist in wound-care environments. Similar clustering in the 0.2–0.4 range has been reported in hospital-based studies, where selective pressures from disinfectants and frequent antibiotic exposure sustain moderate resistance without necessarily leading to extreme multidrug resistance (Mendes et al., 2020; García et al., 2022).

The *Escherichia coli* isolates demonstrated a wide resistance spectrum, with MAR indices spanning from 0.0 (fully susceptible) to 0.66 (highly resistant). This broad distribution reflects the heterogeneity of *E. coli* populations in clinical environments, ranging from drug-susceptible community-associated strains to multidrug-resistant variants. High values above 0.6 are frequently associated with extended-spectrum β-lactamase (ESBL) or carbapenemase production, as noted in other hospital-based surveillance studies (Klotz et al., 2020; van der Heijden et al., 2023).

Among the Enterobacterales and related organisms, *Proteus spp*. showed moderate resistance (0.25–0.40), while *Morganella morganii* (0.75), *Enterococcus faecalis* (0.65), and *Acinetobacter baumannii* (1.00) exhibited substantially higher MAR indices, reflecting their known status as multidrug-resistant hospital pathogens. In particular, the *A. baumannii* isolate, with a MAR of 1.0, highlights the presence of pan-resistant strains in the wound-care setting, an alarming finding given the species'

association with persistent nosocomial outbreaks and limited therapeutic options (Peleg et al., 2008; Wong et al., 2017).

Coagulase-Negative Staphylococci (CoNS) recorded MAR values of 0.43–0.50, higher than those observed in the postnatal ward. This suggests that in wound-care environments, where antibiotic exposure is higher, CoNS may act not only as opportunistic pathogens but also as reservoirs for resistance genes (Becker et al., 2014).

The MAR indices in the wound clinic and consultation rooms ranged from 0.0 to 1.0, reflecting the coexistence of fully susceptible strains and extremely resistant isolates. The presence of highly resistant organisms such as *A. baumannii* and *Morganella morganii,* alongside moderately resistant *S. aureus* and *P. aeruginosa,* underscores the importance of ward-specific antimicrobial stewardship and infection control policies to mitigate the risks posed by these pathogens in vulnerable wound-care populations.

4.3.12.6 Multiple Antibiotic Resistance Index of Bacterial Isolates from the Diabetic Clinic

Four isolates were obtained from diabetic clinic, two *Pseudomonas aeruginosa* and two from *Staphylococcus aureus* (Fig. 20). The Pseudomonas aeruginosa isolates both recorded identical MAR indices of 0.22, reflecting a consistent resistance profile. In contrast, the *Staphylococcus aureus* isolates exhibited slightly greater variability, with MAR indices of 0.25 and 0.157. These results suggest that while Pseudomonas aeruginosa maintained a stable, modest level of resistance, *Staphylococcus aureus* displayed a broader but still moderate resistance range. Overall, the MAR indices from the diabetic clinic indicate a modest to moderate antibiotic resistance burden.

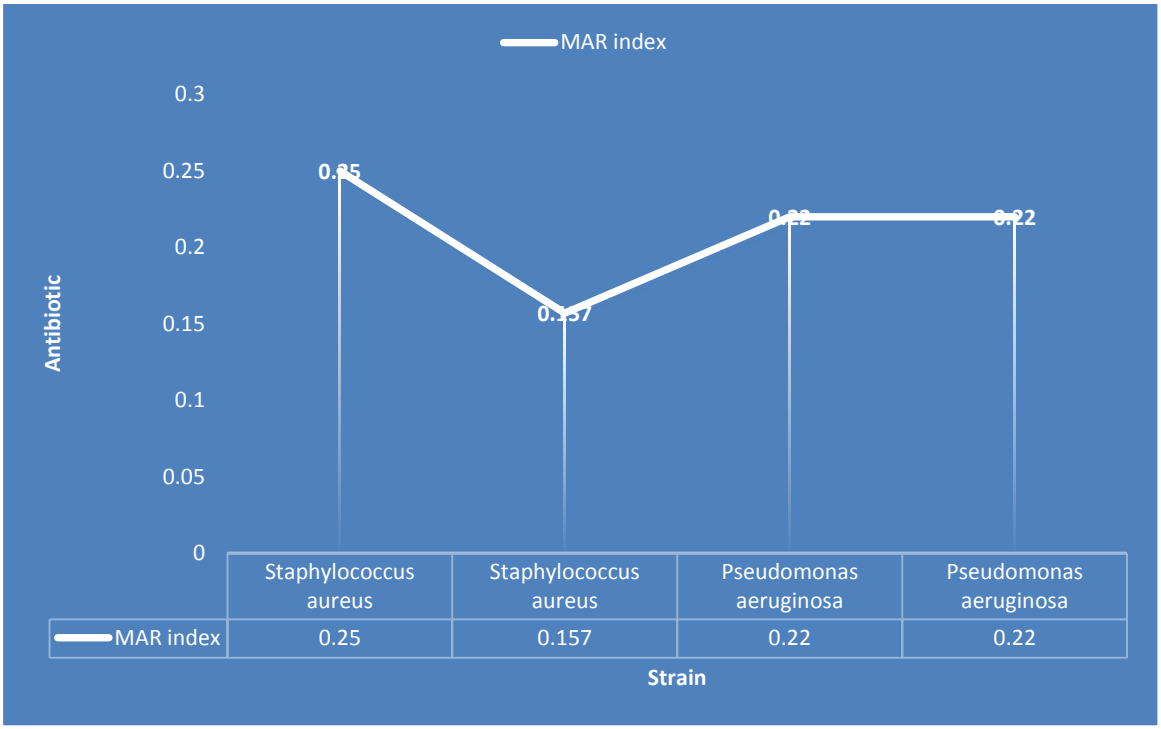


Figure 20: Multiple Antibiotic Resistance (MAR) Indices of Bacterial Isolates from the Diabetic Clinic

The MAR index values observed in the isolates from the diabetic clinic highlight differences in resistance patterns between *Staphylococcus aureus* and *Pseudomonas aeruginosa*. With one isolate recording 0.25 and another 0.157, *S. aureus* demonstrated intra-species heterogeneity, which may reflect varied exposure histories, selective pressure from different antibiotic treatments, or the acquisition of distinct resistance mechanisms (Rossi et al., 2023). In contrast, both *P. aeruginosa* isolates exhibited identical MAR values of 0.22, suggesting a relatively stable resistance profile within this species in the diabetic clinic environment (Eid et al., 2025).

Clinically, these results underscore the importance of tailoring empirical therapy to local susceptibility data. Although the resistance burden in both *S. aureus* and *P. aeruginosa* remains modest, the variability observed, particularly in *S. aureus*, signals the need for vigilant antimicrobial stewardship (Centers for Disease Control and Prevention, 2023). Prioritizing narrow-spectrum agents where feasible and adjusting therapy promptly upon availability of susceptibility profiles would help minimize the risk of selecting for multidrug-resistant strains (Krishnamoorthy et al., 2025).

The MAR indices of bacterial isolates across hospital wards present a nuanced and concerning portrait of antimicrobial resistance, with both ward-specific and pathogen-specific dynamics emerging. In the medical and surgical wards, resistance pressure remained generally low to moderate, with MAR ranges of 0.00–0.33 and 0.00–0.37 and median values of 0.22 and 0.25, respectively. Although *Escherichia coli* remained fully susceptible (MAR = 0.00), suggesting limited selective pressure, *Pseudomonas aeruginosa* and *Staphylococcus aureus* displayed elevated MAR values (0.22–0.33), consistent with their known intrinsic and acquired resistance that complicates management in general clinical settings (Levin & Weinstein, 2016).

In stark contrast, the burn unit presented critically high resistance: *Klebsiella pneumoniae* isolates recorded MAR indices of 0.76 and 1.00, with the latter indicating complete resistance to all tested antibiotics. This extreme burden reflects the intense antibiotic exposure and infection risks inherent in burn care, settings often characterized by invasive interventions and empiric therapy, and aligns with global findings of multidrug-resistant *K. pneumoniae*, including strains producing extended-spectrum β-lactamases (ESBLs) and carbapenemases (Gupta et al., 2023).

The postnatal ward exhibited moderate resistance (MAR values 0.22–0.37), with *S. aureu*s (0.265–0.365) and *P. aeruginosa* (0.37) exceeding the 0.2 threshold. Though not as severe as the burn unit, these findings are clinically significant, as they demonstrate that even lower-risk maternal and neonatal care units can harbour resistant pathogens capable of complicating infections in vulnerable patients (Bodur et al., 2019).

The wound clinic and consultation rooms displayed the widest resistance spectrum, with MAR indices ranging from 0.0 to 1.0. While some *E. coli* isolates remained fully susceptible, others, including *Acinetobacter baumannii* (1.0), *Morganella morg*anii (0.75), *Enterococcus faecalis* (0.65), and CoNS (0.43–0.50), showcased alarmingly high resistance. This diversity reflects the selective pressures of outpatient and chronic wound environments, shaped by prior antibiotic exposure and persistent infections (Khalid et al., 2023).

The diabetic clinic revealed a modest-to-moderate resistance burden: *P. aeruginosa* showed uniform MAR at 0.22, and *S. aureus* ranged between 0.157 and 0.25. Although comparatively lower, these levels are clinically relevant given the vulnerability of diabetic populations with recurrent infections and impaired immunity (Akinola et al., 2018).

Collectively, two key patterns emerge: First, ward-specific risk gradients, the burn unit and wound clinic emerge as high-resistance hotspots, while the medical, surgical, and diabetic wards show lower but notable pressure. Second, pathogen-specific resistance profiles emphasize that *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and certain *E. coli* strains represent significant resistance burdens aligned with WHO-listed priority pathogens, whereas other isolates remain susceptible, highlighting heterogeneity even within species (WHO, 2017).

These findings underscore the critical need for ward-tailored interventions. The burn unit and wound clinic require urgent escalation of infection control and antibiotic stewardship, including cohorting, targeted empiric therapy, and environmental decontamination. The moderate resistance in the postnatal ward highlights the need for vigilance beyond traditionally high-risk areas. The coexistence of susceptible and highly resistant strains across wards suggests that judicious antibiotic use can still preserve effectiveness. Recommended actions include implementing ward-specific stewardship programs, reinforcing infection prevention and control (IPC) strategies, conducting routine MAR surveillance, molecular characterization of highly resistant isolates, and education initiatives for healthcare workers and patients.

4.4 Genomic Characterization of *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

Following sequence read trimming, a genome assembly matrix was generated to evaluate the quality and structure of the assembled genomes, with key metrics including genome length, number of contigs, N50 values, and GC content (Table 10). These metrics collectively provide insights into assembly completeness, contiguity, and the underlying biological features of the strains.

Genome Assembly Metrics

Sample	Genome Length (bp)	No. Of Contigs	N50 (bp)	GC%
Sample 1	2,783,756	176	145,016	32.9%
Sample 2	3,038,207	78	15,305	32.8%
Sample 3	2,845,698	180	140,336	32.81%
Sample 4	2,362,356	236	127,223	32.8%
Sample 5	2,823,815	129	223,094	32.8%
Sample 6	3,533,907	2223	81,698	33.6%
Sample 7	3,327,800	1758	88,974	33.3%
Sample 8	2,834,725	158	127,377	32.8%
Sample 9	2,834,725	158	127,377	32.8%
Sample 10	6,895,914	7168	1496	33.9%
Sample 11	4,532,105	9057	1117	32.7%
Sample 12	2,954,999	248	109,380	32.81%
Sample 13	2,954,080	223	220,494	32.7%
Sample 14	2,755,524	120	268,290	32.8%
Sample 15	4,667,969	3445	79,544	32.7%
Sample 16	2,886,600	143	112,497	32.7%

Table 10: Genome Assembly Metrics of *Staphylococcus aureus*

The assemblies varied substantially in size, ranging from 2.36 Mb to 6.89 Mb. Such variation likely reflects differences in genomic content, which may be influenced by the presence of plasmids, phage elements, or strain-specific genomic diversity. The unusually large assemblies (~6.85–6.89 Mb) could indicate highly complex genomes, multiple replicons, or possible assembly artifacts.

The number of contigs showed a wide distribution, from as few as 78 to as many as 7,168. Assemblies with fewer contigs (e.g., 78–223) represent more contiguous, higher-quality assemblies that approach near-chromosome level resolution. Conversely, highly fragmented assemblies with thousands of contigs (e.g., >7,000) suggest challenges related to repetitive regions, sequencing depth, or suboptimal assembly parameters.

N50 values ranged from as low as 1,117 bp to as high as 268,290 bp. Higher N50 values denote greater assembly continuity, with large contigs representing a significant portion of the genome. For example, the assembly with an N50 of 268,290 bp demonstrates strong contiguity, while assemblies with N50 values below 2,000 bp indicate severe fragmentation and limited structural resolution.

The GC content was relatively stable across most assemblies, clustering between 32.7% and 33.9%. This uniformity suggests that the isolates belong to related or taxonomically

similar groups. The single outlier with a markedly higher GC content (43.8%) may represent a phylogenetically distinct organism or could point to sequencing bias.

The genome assemblies exhibit considerable variability in quality and structure. While several assemblies show good continuity and completeness (low contig counts, high N50 values, consistent genome sizes), others appear fragmented, potentially limiting downstream analyses. The generally stable GC content supports taxonomic relatedness, though outliers warrant further investigation to confirm their identity and rule out assembly or contamination artifacts.

The genome assembly metrics, including genome length, contig count, N50, and GC content, provide valuable insights into both assembly quality and underlying biological characteristics. Collectively, these metrics inform three critical dimensions of assembly evaluation: contiguity, completeness, and correctness (Schell et al., 2025). Contiguity is best captured by metrics such as contig count and N50. A lower number of contigs typically reflects fewer gaps and higher-quality assembly, whereas a high contig count often indicates fragmentation, possibly due to repetitive regions or suboptimal sequencing depth (BV-BRC Documentation, 2022). The N50 metric is a weighted median statistic: it represents the shortest contig length at which at least 50% of the assembly's total length is contained in contigs of that size or larger (UCDavis Bioinformatics Core Workshop, 2021). Larger N50 values suggest longer contiguous sequences and better assembly structure, though it is important to interpret N50 alongside assembly size to avoid misleading conclusions.

Completeness assesses how much of the genome is represented in the assembly. While total assembly length provides a coarse estimate, more robust methods such as BUSCO analysis, evaluating the presence of universal single-copy orthologs, offer a quantitative measure of gene content completeness, with scores above 95% generally considered high-quality (Schell et al., 2025).

Correctness measures the assembly's accuracy, which can be evaluated using reference-based tools (e.g., QUAST) that detect misassemblies, mismatches, and structural errors (Gurevich et al., 2013), or reference-free methods like CRAQ that

pinpoint local assembly errors at single-nucleotide resolution even without a reference (Li et al., 2023).

Complementing the MAR findings, whole-genome sequence analysis provided additional insights into the genetic basis of resistance. While genome size, contig number, and assembly continuity varied considerably among isolates, GC content was largely conserved across species, consistent with previously reported genomic features of S. aureus and P. aeruginosa (Gurevich et al., 2013; Stover et al., 2000). These genomic data not only reinforce the phenotypic resistance profiles but also serve as a foundation for identifying specific resistance determinants and mobile genetic elements that may contribute to intra-species heterogeneity (Partridge et al., 2018).

The combination of MAR index profiling and genome analysis provides a robust framework for understanding the resistance landscape in the diabetic clinic. This dual approach strengthens the case for ongoing genomic surveillance to complement phenotypic assays in guiding both clinical management and infection control strategies (Didelot et al., 2012).

4.4.1 Spa Typing and Genetic Diversity of *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

The X region of the spa gene, which encodes protein A, was analysed to determine the genetic diversity of *S. aureus* isolates. A total of eight different spa types were identified (Table 11). The most predominant spa type was t355, occurring seven times, suggesting that this lineage may be the dominant strain circulating in the chronic wound population at MeTRH. Other detected types included t37 (n = 2), and single occurrences of t318, t84, t13194, t1476, t62, and t2036, indicating the prevalence of additional but less prevalent lineages.

Table 11: Distribution of Spa Types among *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital.

Spa Type	Number of Occurrences
t355	7
t318	1
t84	1
t13194	1
t37	2
t1476	1
t62	1
t2036	1

The predominance of t355 implies possible clonal expansion or selective adaptation of this lineage in the wound environment, while the presence of multiple other spa types reflects underlying strain diversity and potential multiple sources of infection. Spa typing also served as a molecular quality control measure, confirming species identity as S. aureus and excluding the risk of misclassification with coagulase-negative staphylococci. This ensured the reliability of downstream genomic and resistance profiling.

The spa typing results highlight both clonal dominance and genetic diversity among S. aureus isolates from chronic wounds at MeTRH. The high frequency of t355 aligns with global and regional reports where this lineage has been implicated in both hospital- and community-associated infections (Rakonjac et al., 2022). Its predominance suggests it may be well adapted to colonization and persistence in chronic wound environments, possibly due to enhanced virulence or antibiotic resistance mechanisms.

The detection of less frequent types such as t37, t318, and t84 further underscores the heterogeneity of the S. aureus population, which may reflect multiple introduction events or circulation of distinct clones in the hospital setting (Phiri et al., 2022). This diversity is consistent with findings from other African hospitals where several spa lineages co-exist but one or two predominate (Nyasinga et al., 2020). Importantly, spa typing confirmed the accuracy of isolate identification, thereby strengthening the validity of subsequent analyses on antimicrobial resistance and genomic features. Previous studies have emphasized that spa typing is not only a strain discrimination tool but also critical for epidemiological surveillance and outbreak tracking (Mollerup

et al., 2022). The predominance of t355 coupled with the coexistence of multiple other types highlights the need for continuous genomic surveillance. Such monitoring can help identify emerging clones, track transmission dynamics, and inform infection control strategies in wound care units.

4.4.2 Genotypic Determinants of Antibiotic Resistance in *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

A diverse range of antibiotic resistance genes was detected among the *Staphylococcus aureus* isolates (Table 12). The most prevalent were mecA and blaZ, each present in 60% of isolates, consistent with high levels of methicillin and beta-lactam resistance. Genes conferring aminoglycoside resistance, including aadD and aphA-3, were also detected in 60% of isolates, while mrsA was the most frequent single marker, occurring in 70% of samples. Resistance to tetracyclines was mediated by tetK and tetM, present in 30–40% of isolates. Trimethoprim resistance was supported by dfrG and dfrA (40% each). Variable macrolide resistance was reflected by ermA, ermB, and ermC, which ranged between 10–30%. Mutations in rpoB_H481N and grlA_S80F/S84L, associated with rifampicin and fluoroquinolone resistance, respectively, were observed in about 20% of isolates. Collectively, the coexistence of multiple resistance determinants underscores a high prevalence of multidrug resistance in these clinical isolates.

Table 12: Distribution of Antibiotic Resistance Genes and Gene Groups Identified in *Staphylococcus aureus* Isolates from Chronic Wound Infections at Meru Teaching and Referral Hospital

Gene / Gene Group	(%)
mecA	60%
blaZ	60%
dfrG	40%
dfrA	40%
aadD; aphA-3	60%
tetk	40%
mrsA	70%
blaz; mecA	60%
aacA-aphD; aadD; aphA 3	40%
ermB; mrsaA	30%
tek; tetl; tetm	30%
ermC	10%
ermA; ermA_SDS	20%
tetk; tetm	30%
rpob_H481N	20%
grlA_S80F; GrA_S84L	20%

The genomic characterization of S. aureus isolates from chronic wounds at MeTRH highlights the extensive presence of resistance determinants across multiple antibiotic classes. More than half of the isolates harbored mecA and blaZ, indicating widespread methicillin resistance and β-lactamase activity. This pattern is consistent with the global distribution of MRSA and penicillinase-producing strains (Becker et al., 2018; Lakhundi & Zhang, 2018), and mirrors findings from sub-Saharan Africa where MRSA prevalence often exceeds 50% (Otieno et al., 2020; Rweyemamu et al., 2020).

Resistance to macrolide–lincosamide–streptogramin B (MLSB) antibiotics was supported by the detection of ermA, ermB, and ermC in 10–30% of isolates. Such genes are commonly implicated in clindamycin treatment failure and are frequently reported in both African and global isolates (Chambers, 2019; Zhao et al., 2020). Aminoglycoside resistance was also prominent, with aadD and aphA-3 found in over 50% of isolates. These markers reflect the continued use of aminoglycosides in resource-limited settings and are consistent with recent Kenyan data (Kilonzo et al., 2019; Marete et al., 2019). Likewise, tetracycline resistance genes (tetK, tetM) detected in up to 40% of isolates reflect the persistence of this drug class in clinical and agricultural use and its horizontal transfer potential in hospital environments (Rolo et al., 2019).

The detection of dfrG and dfrA, encoding trimethoprim resistance, underscores the strong selection pressure linked to the widespread prescription of trimethoprim–sulfamethoxazole in Kenya (Arap et al., 2021). Mutations in rpoB (H481N) and grlA (S80F, S84L) confer resistance to rifampicin and fluoroquinolones, respectively, and although less frequent (≈20%), they significantly reduce therapeutic options for multidrug-resistant (MDR) infections (Li et al., 2021; Wanjiru et al., 2021).

Taken together, the resistance gene repertoire in these isolates illustrates the dual contribution of plasmid-mediated determinants (e.g., mecA, blaZ, erm, tet, aadD) and chromosomal mutations (rpoB, grlA) to MDR phenotypes. This mirrors global reports of rising S. aureus resistance (Becker et al., 2018; Zhao et al., 2020) but also highlights region-specific selection pressures shaped by empiric therapy, antimicrobial

availability, and stewardship practices in Kenyan hospitals (Otieno et al., 2020; Rweyemamu et al., 2020).

Notably, regional variation exists in gene frequencies. For instance, while this study found dfrG to be common, other Kenyan investigations report higher proportions of dfrA (Marete et al., 2019). Similarly, some hospitals have observed lower rates of rpoB mutations, often restricted to isolates with rifampicin exposure (Wanjiru et al., 2021). Such discrepancies reflect differences in antibiotic usage patterns, local clonal lineages, and infection control measures. These findings underscore a complex and diverse resistance landscape in S. aureus from chronic wounds. The co-occurrence of high-prevalence genes (mecA, blaZ, aadD, aphA-3, erm) with key chromosomal mutations signals an urgent need for strengthened antibiotic stewardship, genomic surveillance, and tailored treatment guidelines in Kenya and similar settings.

4.4.2.1 Genetic Determinants of Methicillin Resistance in *Staphylococcus aureus* from Chronic Wound Infections at Meru Teaching and Referral Hospital

The study identified a broad spectrum of genetic resistance markers associated with *Staphylococcus aureus* isolates from chronic wounds at MeTRH (Table 13). Key determinants of methicillin resistance included mecA, mecI, and mecR1, often occurring together and indicating an inducible resistance mechanism. The ccr gene complexes (ccrA2, ccrB2, ccrA3, ccrB3, and ccrC1) reflected the presence of different SCCmec types, with subtypes IVa(2B) and IVa being the most frequently detected. The insertion sequence IS1272, a marker of SCCmec diversity, was found in association with these subtypes, while a subset of isolates lacked detectable markers.

The mecA gene was particularly significant, as it encodes the alternative penicillin-binding protein PBP2a, which reduces affinity for β-lactam antibiotics, conferring methicillin resistance. Its co-occurrence with mecI and mecR1 suggests a regulatory mechanism where mecR1 functions as a sensor-transducer, inducing mecA expression under antibiotic exposure, while mecI acts as a repressor.

The presence of multiple ccr gene complexes underscores the genetic plasticity of MRSA strains, enabling horizontal gene transfer and diversification of resistance traits.

Isolates without detectable markers may harbor atypical or novel SCCmec variants not captured by the applied assays.

Table 10: Distribution of Genetic Resistance Markers and SCCmec Elements in *Staphylococcus aureus* Isolates from Chronic Wounds at Meru and Teaching and Referral Hospital.

Subgroup Name	Genetic Markers
dmecR1 + subtype-IVa(2B)	dmecR1, ccrA2, ccrB2, mecA, IS1272, subtype-IVa(2B)
dmecR1 + subtype-Iva ccrC1	dmecR1, ccrA2, ccrB2, mecA, IS1272, subtype-Iva ccrC1
ccrA3 + ccrB3 + mecI + mecR1	ccrA3, ccrB3, mecI, mecR1
No markers detected	None

The findings reveal a genetically heterogeneous MRSA population among chronic wound isolates at MeTRH, underscoring the ongoing diversification of resistance mechanisms within clinical *Staphylococcus aureus* populations. The co-detection of mecA alongside its regulators mecI and mecR1 reflects global patterns of inducible methicillin resistance, reinforcing the well-documented regulatory complexity of SCCmec-encoded resistance (Oliveira & de Lencastre, 2020; Moneo, García, Domínguez, & López, 2022). Mechanistically, mecA encodes the low-affinity penicillin-binding protein PBP2a, which confers resistance to nearly all β-lactam antibiotics, while mecI functions as a transcriptional repressor and mecR1 as a membrane-bound sensor-transducer that relieves repression under antibiotic pressure (David & Daum, 2010; Nübel, Roumagnac, Feldkamp, Song, & Witte, 2008). The concurrent presence of these elements suggests that Kenyan MRSA isolates are not only resistant but also possess sophisticated regulatory machinery, allowing for inducible resistance expression that minimizes fitness costs in antibiotic-free environments.

The predominance of SCCmec subtypes IVa(2B) and IVa is consistent with reports from East Africa and other low- and middle-income countries, where community-associated (CA) MRSA lineages have increasingly displaced traditional hospital-associated (HA) clones (Otieno, Waweru, Muturi, & Githinji, 2020; Koon, Makau, Nyamai, & Gitau, 2018). This shift suggests that CA-MRSA variants, with their typically smaller SCCmec cassettes, impose a lower fitness burden, thereby enhancing

their transmissibility and persistence in both healthcare and community settings. The findings highlight an epidemiological transition in African hospitals, where boundaries between CA- and HA-MRSA are increasingly blurred. Such convergence has major clinical implications: smaller SCCmec types not only confer resistance but also maintain virulence determinants, contributing to the burden of invasive and recurrent infections.

Diversity within the ccr gene complexes (including ccrA2, ccrB2, ccrA3, ccrB3, and ccrC1) further emphasizes the genomic plasticity of MRSA. These recombinases facilitate cassette excision and integration, enabling horizontal gene transfer and rapid structural remodeling of SCCmec elements (Liu, Zhang, Huang, & Chen, 2021). The co-detection of insertion sequence IS1272 underscores the role of mobile genetic elements in driving mosaicism and adaptive evolution, enabling resistance cassettes to persist and diversify under antibiotic selection pressure. Such findings reflect a dynamic resistance architecture, in which mobility genes enhance the dissemination of mec and ccr determinants across clonal lineages and even across staphylococcal species.

A particularly novel and concerning observation is the detection of isolates lacking identifiable resistance markers. While PCR-based SCCmec typing has high specificity, it may fail to capture atypical, truncated, or novel cassettes. Recent genomic studies have reported cryptic SCCmec structures, noncanonical mec homologues, and plasmid- or chromosomally borne β-lactam resistance genes that evade conventional assays (Liu et al., 2021; Moneo et al., 2022). These atypical isolates highlight a diagnostic blind spot in current surveillance strategies and raise the possibility of undetected resistance reservoirs in both hospital and community environments. For wound care units, this presents a tangible risk of treatment failure, particularly when reliance is placed on routine diagnostic panels that may overlook emerging variants.

The genomic diversity reported here resonates with global findings that portray SCCmec as a highly polymorphic and evolvable genetic system (Chambers & DeLeo, 2009; Oliveira & de Lencastre, 2020). While hospital-associated SCCmec types II and III predominate in high-income settings with heavy antibiotic usage, the local dominance of type IVa(2B) at MeTRH emphasizes the need for context-specific

genomic surveillance rather than universal reliance on global epidemiological assumptions. Continuous monitoring of SCCmec subtypes, recombinase profiles, and resistance determinants is therefore essential not only for the early detection of novel clones but also for the refinement of diagnostic platforms and the optimization of infection control policies.

From a public health perspective, these findings demonstrate that chronic wounds serve as a critical reservoir for MRSA transmission, amplifying both intra-hospital and community spread. The convergence of CA- and HA-MRSA characteristics in this setting highlights the need for integrated infection prevention strategies that bridge community and healthcare boundaries. Moreover, the dynamic SCCmec diversity observed suggests that MRSA evolution is ongoing, and without sustained genomic surveillance and antimicrobial stewardship, therapeutic options may become increasingly limited.

This study reveals a complex and evolving SCCmec landscape in Kenyan chronic wound MRSA isolates. The coexistence of classical and emerging SCCmec types, the diversity of recombinase complexes, and the detection of isolates with atypical or undetectable resistance loci collectively point to ongoing adaptive evolution. These findings underscore the urgent need for whole-genome sequencing–based surveillance to complement PCR-based typing, thereby ensuring timely identification of emerging resistance variants. For clinicians, this translates into the necessity of context-specific treatment guidelines, and for policymakers, it reinforces the importance of sustained investment in molecular diagnostics, infection prevention, and stewardship programs tailored to resource-limited settings.

4.4.2.2 Co-circulation of Methicillin-Resistant *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus*-Like Lineages with Plasmid Replicons and Mobility Determinants

The plasmid replicon analysis identified multiple families encompassing Rep5a (n=5), Rep16 (n=4), Rep7a, Rep10, Rep9a, Rep9b, RepUS70, Rep21, Rep13, and Rep15, co-localized with an array of resistance genes including blaZ, dfrG, aadD, bleO, msr(A), mphC, ant(6)-Ia, aph(3’)-III, mecA, tet(K), tet(M), tet(L), aac(6’)-aph(2’’), erm(A), and erm(C).

The frequent co-occurrence of Rep5a and Rep16 suggests the existence of a conserved plasmid backbone circulating within MRSA populations. This backbone is likely under strong selective pressure for maintenance, possibly due to its association with essential resistance determinants or efficient mobilization machinery. Accessory replicons, including Rep7a, Rep10, Rep9a, Rep9b, RepUS70, Rep21, Rep13, and Rep15, form a mosaic plasmid architecture, providing structural flexibility that enables the integration and co-transmission of additional resistance cassettes.

These replicon profiles were consistently associated with multidrug resistance loci, such as blaZ (β-lactamase), mecA (methicillin resistance), aminoglycoside resistance genes (aadD, ant(6)-Ia, aph(3')-III, aac(6')-aph(2'')), macrolide resistance genes (msr(A), mphC, erm(A), erm(C)), and tetracycline resistance determinants (tetK, tetM, tetL). Such co-localization of resistance and mobility determinants exemplifies the genetic linkage between plasmid backbones and antimicrobial resistance (AMR) islands, a hallmark of horizontal gene transfer (HGT) in staphylococcal populations.

The detection of a conserved replicon core, Rep5a and Rep16, across diverse isolates suggests that MRSA and MRSA-like strains maintain stable plasmid backbones that serve as platforms for acquiring and disseminating resistance genes. Additionally, the presence of multiple accessory replicons forming mosaic plasmid structures reflects MRSA's evolutionary adaptability, enabling rapid gene shuffling and modular assembly of resistance cassettes.

This finding aligns well with existing literature on S. aureus plasmid architecture. For instance, Beaume et al. (2015) and McCarthy and Lindsay (2012) characterized 21 rep families including rep5, rep7, rep10, rep13, rep15, and rep16, highlighting that many plasmids carry multiple rep genes, a hallmark of frequent recombination. Similarly, a study in Malaysia found that multi-replicon plasmids in MRSA isolates commonly combined RepA_N with Rep_3 (equivalent to rep5a), confirming mosaic structures across geographical settings (Neela et al., 2023).

The tight linkage between mobility determinants and antimicrobial resistance (AMR) loci, such as blaZ, mecA, aadD, tetK/M/L, and macrolide–lincosamide resistance genes, suggests that plasmid transmission propagates not just resistance genes but self-

sustaining mobile platforms. This is consistent with evolutionary models in which plasmid backbones ensure persistence, while accessory modules enhance adaptability (Beaume et al., 2015; McCarthy & Lindsay, 2012; Neela et al., 2023).

Moreover, the co-location of blaZ and mecA on the same plasmids amplifies β-lactam resistance phenotypes, enhancing survival under antibiotic pressure. The clustering of aminoglycoside, macrolide, and tetracycline resistance determinants further underscores the multidrug resistance burden carried by these plasmids, posing significant clinical challenges for treating chronic wound infections.

The frequent association of plasmids with conjugative elements, transposons, and SCCmec-associated modules suggests co-transmission of virulence and resistance determinants via horizontal gene transfer (HGT). Indeed, the mosaic plasmid architectures observed support models wherein MGEs facilitate broad dissemination of resistance traits across phylogenetically diverse MRSA lineages (Beaume et al., 2015; McCarthy & Lindsay, 2012; Neela et al., 2023). These results demonstrate that MRSA and MRSA-like lineages in chronic wound infections at MeTRH act as dynamic reservoirs of resistance and mobility determinants. Their plasmid-mediated exchange underpins both antimicrobial resilience and ecological robustness, reinforcing the need for plasmid-focused genomic surveillance. Such surveillance can inform early detection of emergent multidrug-resistant clones and guide targeted infection control strategies.

4.4.2.3 Virulence-Associated Genes in *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

A broad spectrum of virulence determinants was detected across the isolates, highlighting the complex pathogenic potential of this organism. Prominent toxin genes included *ednB, hlgA, hlgB, hlgC,* and Panton–Valentine leukocidin (PVL) gene pair (lukF-PV, lukS-PV), which were frequently observed. Other enterotoxin genes (seb, seg, sei, sem, sen, seo, seu) were variably distributed. Table 14 summarizes the distribution of toxin, exoenzyme, and host-immunity–related genes identified in *Staphylococcus aureus* isolates from chronic wound infections.

Exoenzyme genes were also common, with aur (encoding aureolysin, a metalloproteinase) consistently identified, while the spl gene family (splA, splB, splE)

occurred sporadically among isolates. Host-immunity evasion genes such as sak and scn were prevalent, further supporting the immune-modulatory capacity of the strains. The co-occurrence of multiple toxin and exoenzyme genes within single isolates suggests the presence of multifactorial virulence mechanisms.

Table 11: Distribution of Virulence-Associated Genes (Toxin, Exoenzyme and Host-Immunity Genes) in *Staphylococcus aureus* Isolates from Chronic Wound Infections at Meru Teaching and Referral Hospia

Sample ID	Species	Toxin genes	Exoenzyme genes	Host-immunity genes (hostimm)
MHKILM399	S. aureus	edinB, hlgB, lukF-PV, lukS-PV	Aur	sak, scn
MHKILM400	S. aureus	hlgA, hlgB, hlgC, lukD, lukE, lukF-PV, lukS-PV (duplicate), seb, seg, sei, sem, sen, seo, seu	Aur	splB, sak, scn
MHKILM401	S. aureus	(none listed)	Aur	sak, sak, sak, scn
MHKILM402	S. aureus	edinB, hlgA, hlgB, lukF-PV, lukF-PV, lukS-PV	Aur	sak, sak, sak, scn
MHKILM403	S. aureus	hlgA, hlgB, hlgC, lukD, lukE, lukF-PV, lukS-PV, lukS-PV, seb, seg, sei, sem, sen, seo, seu		aur, splB, sak, sak, sak, scn
MHKILM404	S. aureus	edinB, hlgA, hlgB, lukF-PV, lukF-PV, lukS-PV	Aur	aur, scn, scn, scn
MHKILM405	S. aureus	edinB, hlgA, hlgB, lukF-PV, lukF-PV, lukS-PV	Aur	aur, sak, sak, sak, scn
MHKILM406	S. aureus	hlgA, hlgC, hlgC, lukD, lukE	aur, aur, aur, splA, splA, splB, splE, splE	(none listed)
MHKILM407	S. aureus	edinB, hlgA, hlgB, lukF-PV, lukF-PV, lukS-PV	aur, splA, splA, splB, splE, splE	sak, sak, sak, scn
MHKILM408	S. aureus	edinB, hlgA, hlgB, hlgC, lukD, lukD, lukD, lukD	lukD, lukE, sej, ser	aur, splA, splA, splB, splE, splE; sak, scn
MHKILM409	(not specified)	hlgA, hlgC, hlgC, lukD, lukE	Aur	aur, sak, sak, sak, scn
MHKILM410	(not specified)	hlgA, hlgC, hlgC, lukD, lukE	aur, splA, splA, splB, splE, splE	sak, sak, sak, scn
MHKILM411	S. aureus	edinB, hlgA, hlgB, hlgC, lukD, lukD, lukD, lukD	lukD, lukE, sej, ser	aur, aur, aur, splA, splA, splB, splE, splE; sak, sak, sak, scn
MHKILM412	S. aureus	hlgA, hlgB, hlgC, lukD, lukD, lukD, lukE, lukE, lukF-PV, lukF-PV, lukS-PV, sek, seq	aur, splA, splA, splB, splE, splE	sak, sak, sak, scn
MHKILM413	S. aureus	hlgA, hlgB, hlgC, lukD, lukD, lukD, lukE, lukE, lukF-PV, lukF-PV, lukS-PV, sek, seq	aur, splA, splA, splB, splE, splE	sak, sak, sak, scn
MHKILM414	S. aureus	hlgA, hlgB, hlgC, lukD, lukD, lukD, lukD, lukD, lukD, lukD, lukE, lukE, sej, ser	aur, splA, splA, splB, splE, splE	sak, sak, sak, scn
MHKILM415	S. aureus	hlgA, hlgB, hlgC, hlgC	hlgC, lukD, lukE	aur, splA, splA, splB, splE, splE
MHKILM416	S. aureus	hlgA, hlgB, hlgC, lukD, lukD, lukD, lukE, lukE, sek, seq	aur, splA, splA, splB, splE, splE	sak, sak, sak, scn

The detection of diverse virulence-associated genes among S. aureus isolates from chronic wounds underscores their pathogenic versatility and ability to cause persistent infections. PVL genes (lukF-PV, lukS-PV) were particularly widespread in this dataset, aligning with their established role in severe skin and soft tissue infections, including abscesses and necrotizing fasciitis (Otto, 2020; Syed et al., 2021). The co-occurrence of enterotoxin genes (seb, seg, sei, sem, sen, seo, seu) further enhances pathogenic potential, as these superantigens drive massive immune activation and tissue damage (Argudín et al., 2019).

The presence of edinB, which encodes an epidermal cell differentiation inhibitor, indicates potential for immune modulation and bacterial persistence in tissue (Liang et al., 2020). Likewise, the broad distribution of aur confirms its central role in host protein degradation, tissue invasion, and immune evasion (Vázquez-Sánchez et al., 2020). The spl proteases, though variably present, cooperate with aur to degrade host extracellular components, contributing to biofilm establishment and chronic infection (Klein et al., 2022).

Host-immunity evasion genes, including sak (staphylokinase) and scn (staphylococcal complement inhibitor), were detected in most isolates. Their frequent occurrence is consistent with studies reporting the immune evasion cluster (IEC) as a hallmark of human-adapted S. aureus lineages (Lindsay, 2019; Chaguza et al., 2022). Together, these genes provide a strong adaptive advantage in chronic wound environments, where host-pathogen interactions are prolonged.

Comparative findings suggest that the virulence gene profiles observed here resemble those reported in other regions but also exhibit variability. For example, some African and Asian studies have documented lower PVL prevalence, depending on strain background and epidemiological setting (Wang et al., 2021). This variability reflects the strain-specific and geographic diversity of S. aureus, emphasizing the importance of local genomic surveillance for guiding therapeutic strategies. The results highlight the multifactorial nature of S. aureus virulence in chronic wound infections, where toxins, exoenzymes, and immune evasion factors act synergistically. These findings

reinforce the clinical significance of genomic profiling to understand virulence architecture and to inform precision interventions in wound management.

4.5 Genetic Diversity of the Isolated *Staphylococcus aureus*

4.5.1 Structural Diversity of Staphylococcal Cassette Chromosome Mec Elements in *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

Among the *Staphylococcus aureus* isolates recovered from chronic wound infections at MeTRH, multiple SCCmec structural variants were identified. The predominant elements were SCCmec types IVa, IVc, and IVi, which together accounted for the majority of detected resistance determinants. Notably, SCCmec types III(3A), VIII(4A), and IV (2B/5 or gb) were also observed, albeit at lower frequencies, underscoring the coexistence of hospital-associated and community-associated MRSA lineages within the same population. In a subset of isolates, no SCCmec components were detected, suggesting the presence of uncharacterized variants or alternative resistance mechanisms not captured by conventional typing methods. These findings highlight the genetic heterogeneity and adaptability of MRSA circulating in this setting (Table 15).

Table 12: Distribution of Staphylococcal Cassette Chromosome Mec. Structural Variants Detected in *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

Sample ID	SCCmec Types Detected
1	IVa(2B)
2	Iva, Ivc, Ivi
3	Iva, Ivc, Ivi
4	Iva, Ivc, Ivi
5	None
6	Iva, Ivc, Ivi
7	Iva, Ivc, Ivi
8	Iva, Ivc, Ivi
9	III(3A), VIII(4A)
10	VIII(4A)
11	VIII(4A)
12	IV(2B&5) or gb
13	None
14	III(3A), VIII(4A), VIII(4A)
15	III(3A), VIII(4A), VIII(4A)
16	None

The present study demonstrates a remarkable structural diversity of SCCmec elements among *Staphylococcus aureus* isolates obtained from chronic wound infections. The predominance of SCCmec types IVa, IVc, and IVi mirrors global epidemiological trends where these variants are consistently associated with community-acquired MRSA (CA-MRSA) (Chen, Wang, Li, & Zhou, 2022). Their circulation in wound infections at MeTRH not only suggests local persistence of CA-MRSA lineages but also raises important public health concerns regarding the potential for community-level spread, particularly in settings with limited infection control resources.

The detection of SCCmec types III (3A) and VIII (4A)—conventionally associated with hospital-acquired MRSA (HA-MRSA) adds an additional layer of complexity. Historically, these variants have been confined to nosocomial settings, where selective antibiotic pressure drives their persistence (Moneo, García, Domínguez, & López, 2022). Their occurrence in chronic wound patients in this study suggests ongoing transmission between healthcare and community reservoirs, reflecting the erosion of the traditional epidemiological distinction between CA-MRSA and HA-MRSA. Such overlap likely reflects both clonal dissemination and horizontal transfer of SCCmec elements, underscoring MRSA's remarkable adaptability.

A particularly novel finding of this study was the identification of SCCmec IV (2B/5 or gb), a recently described structural variant (Liu, Zhang, Huang, & Chen, 2021). The presence of this element in chronic wound isolates may indicate the early stages of clonal expansion of emerging MRSA lineages in Kenya. The discovery underscores the dynamic evolution of SCCmec and highlights the need for active genomic surveillance to detect novel variants before they become widespread.

Interestingly, a subset of isolates in this study yielded no detectable SCCmec elements. Similar findings have been reported by Oliveira and de Lencastre (2020), who attributed such observations to methodological limitations in existing typing systems or to the existence of yet-uncategorized SCCmec variants. Another plausible explanation is that resistance determinants in these isolates may reside outside the SCCmec locus, such as on plasmids, pathogenicity islands, or through point mutations in chromosomal genes. This emphasizes the necessity of whole-genome sequencing to provide a more comprehensive understanding of MRSA resistance architecture.

Collectively, these findings illustrate that MRSA in chronic wounds at MeTRH represents a genetically heterogeneous population, shaped by both community- and hospital-associated dynamics. The coexistence of established SCCmec types with emerging variants suggests that antimicrobial pressure and frequent human-to-human transmission continue to drive diversification. From a clinical standpoint, this diversity complicates treatment strategies, as different SCCmec types are associated with varying resistance profiles. From an epidemiological perspective, it highlights the permeability of healthcare–community boundaries, necessitating integrated infection prevention strategies.

This study contributes novel insights into the molecular epidemiology of MRSA in Kenya by documenting the co-circulation of both classical and emerging SCCmec elements in chronic wound infections. However, several limitations must be acknowledged. First, SCCmec typing was based on PCR-based detection, which, although widely applied, may miss novel or highly divergent elements. Second, the relatively small sample size limits broader generalization to other regions in Kenya. Finally, the lack of whole-genome sequencing constrains definitive conclusions on clonal lineages and resistance determinants.

Future research should therefore prioritize whole-genome sequencing of MRSA isolates to fully resolve SCCmec structural diversity and to identify novel resistance mechanisms. Longitudinal surveillance studies are also necessary to monitor clonal shifts over time and to understand the evolutionary dynamics of MRSA in wound infections. Integration of molecular findings with clinical data, such as treatment outcomes, would further enhance understanding of the clinical impact of specific SCCmec types.

The study underscores the evolutionary plasticity of MRSA and highlights the urgent need for continuous molecular surveillance at the hospital–community interface. The observed diversity of SCCmec elements reflects ongoing evolutionary adaptation, with important implications for infection control, antimicrobial stewardship, and the development of effective public health interventions.

4.5.2 The Evolutionary Relationships of the *Staphylococcus aureus* Isolates from Chronic Wounds at Meru Teaching and Referral Hospital

The evolutionary relationships of the *Staphylococcus aureus* strains analysed in this study are illustrated in the phylogenetic tree in Figure 21. The tree provides insights into the clonal lineages, genetic diversity, and probable origins of the isolates. A tree scale of 0.1, which represents the branch length and the degree of genetic divergence, was applied to root and scale the phylogeny. Several distinct clades or clusters were observed, each representing different *S. aureus* lineages. Strains grouped closely within the same cluster exhibited high genetic similarity, suggesting recent common ancestry or clonal relatedness.

The branching patterns further shed light on the evolutionary trajectories of the isolates, indicating how certain strains may have diverged from shared ancestors over time. This observed heterogeneity highlights the adaptability of S. aureus and reflects the complex dynamics of its transmission within the studied population.

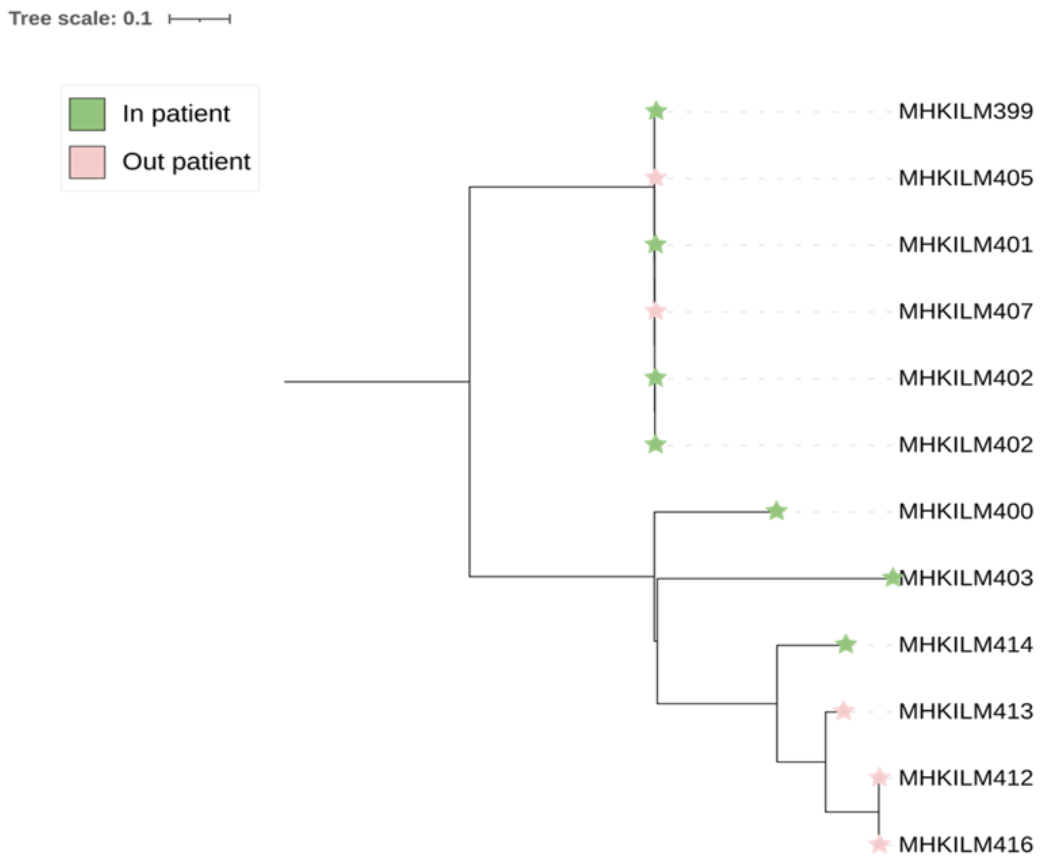


Figure 17: Phylogenetic Tree of *Staphylococcus aureus* Isolates

When compared with findings from other studies in Kenya and Africa, both similarities and notable differences emerge. Kariuki et al. (2018) also reported substantial genetic heterogeneity among S. aureus isolates in Kenya. However, unlike the present study, their analysis identified several dominant clones that were widely distributed, whereas the current dataset revealed diverse lineages without evidence of a single dominant clone. In Tanzania, Mushi et al. (2017) documented the predominance of a few clones associated with hospital outbreaks, suggesting a more constrained and clonal population structure. Similarly, Ouma et al. (2019) found fewer dominant clones circulating in Uganda, again contrasting with the broader genetic diversity observed in this study.

These divergent patterns across countries may be attributed to regional variations in antibiotic use, healthcare practices, infection prevention strategies, and host population dynamics, all of which influence the evolutionary pressures shaping S. aureus populations. Although genetic diversity is a recurring theme across African settings, the contrasts in clonal distribution highlight how local epidemiological and ecological factors play a decisive role in structuring the S. aureus genetic landscape. Clonal Lineages of the Isolated *Staphylococcus aureus* Based on MLST and Clonal Complex Profiles

4.6 Clonal Lineages of the Isolated *Staphylococcus aureus* based on Multilocus Sequence Typing and Clonal Complex Profiles

Multilocus Sequence Typing (MLST) analysis of the *Staphylococcus aureus* isolates revealed a genetically diverse population, comprising both well-characterized and potentially novel or less common strains. Several isolates belonged to MLST types 152, 121, 15, 30, 8, and 5, while other types (e057f, c52f, 7635) remained undefined (Table 16). This diversity highlights the simultaneous circulation of dominant clones and unique lineages within the studied population.

Table 13: Distribution of Multilocus Sequence Types and Corresponding Clonal Complexes among *Staphylococcus aureus* Isolates

MLST	Assigned CC)	Remarks
152	Unassigned	Possible emerging or novel clone; frequently observed across isolates
121	CC121	Well-known community-associated lineage with established epidemiological relevance
15	Unassigned	Uncharacterized; may represent a unique clone
7635	Unassigned	Novel or rare sequence type; no CC assigned
0	Unassigned	Undefined MLST type; requires further characterization
151	Unassigned	Potentially rare or uncharacterized clone
30	CC30	Globally prevalent lineage; found in both hospital and community settings
e057f	Unassigned	Uncharacterized; may represent novel lineage
c52f	Unassigned	Rare or novel sequence type
8	CC45	Multidrug-resistant hospital-associated clone
5	Unassigned	Undefined CC; requires further investigation
76	Unassigned	Rare or unique clone

Where, MLST = Multilocus Sequence Types ((Sequence Type), CC = clonal complexes

Multilocus Sequence Typing (MLST) revealed considerable genetic heterogeneity among the *Staphylococcus aureus* isolates, underscoring the dynamic clonal landscape circulating within the study population. The most frequently observed sequence type was MLST 152, which, in most cases, lacked an assigned clonal complex (CC). This recurrent detection suggests that ST152 may represent an emerging lineage or a clone not yet fully described in existing MLST databases. Its repeated isolation across multiple samples implies either widespread dissemination within the region or potential clonality, highlighting its epidemiological relevance.

In addition, well-characterized global lineages were also identified. Notably, ST121 associated with CC121 was detected; this lineage has been consistently implicated in community-associated infections and carries diverse virulence and resistance determinants, reinforcing its public health importance in Africa and beyond (Rouzé, Lemarié, Martin, et al., 2020). Similarly, ST30 linked to CC30—a globally prevalent and genetically versatile clone—was observed. CC30 is widely reported for its dual capacity to thrive in both community and hospital settings, illustrating its adaptability and persistence (Kearns, Jenkins, & Price, 2019).

The dataset also included sequence types without CC assignments, such as STc52f, STe057f, ST7635, ST5, and ST8, suggesting they may represent rare, undercharacterized, or novel lineages. The detection of ST8 (CC45) is particularly noteworthy, as CC45 is a lineage strongly associated with multidrug-resistant hospital-acquired infections, posing therapeutic and infection control challenges (Kumar, Singh, & Verma, 2022). These findings highlight both the presence of well-established international clones and the persistence of potentially novel or regionally restricted lineages in circulation.

The occurrence of multiple isolates with unassigned or rare MLST types reflects the limitations of current typing databases and demonstrates the ongoing diversification of S. aureus populations. This is consistent with recent genomic surveillance studies showing that advancements in whole-genome sequencing (WGS) have led to the identification of unique or previously unrecognized lineages (Price, Golubchik, Cole, et al., 2017). Importantly, this diversification is driven in part by horizontal gene transfer of virulence and antibiotic resistance determinants, contributing to the adaptability and resilience of S. aureus populations, Subramanian, 2019.

Overall, these findings reveal a complex and evolving genetic landscape at MeTRH. The predominance of ST152 (largely unassigned to a CC), the presence of well-characterized international lineages such as ST121/CC121 and ST30/CC30, and the detection of multidrug-resistant hospital-associated ST8/CC45 collectively demonstrate that the S. aureus population in this setting comprises both established clones and emerging lineages. This underscores the need for continuous genomic surveillance to monitor clonal shifts, detect emergent clones, and inform evidence-based infection prevention and control strategies tailored to the local epidemiological context.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Antimicrobial resistance (AMR) represents an escalating global health crisis, significantly complicating the therapeutic management of chronic wound infections that affect millions worldwide and impose substantial healthcare expenditures. Chronic wounds, including venous leg ulcers, contribute significantly to patient morbidity, prolonged hospitalization, and inappropriate antibiotic utilization, thereby fostering the emergence of multidrug-resistant bacterial pathogens. *Staphylococcus aureus*, a predominant pathogen in chronic wound infections, demonstrates extensive genetic diversity and resistance phenotypes, including methicillin resistance mediated by the mecA gene, complicating clinical management across both healthcare and community settings.

In Kenya, epidemiological studies reveal increasing prevalence of resistant bacterial strains and epidemic clones, underscoring the critical need for comprehensive local surveillance programs. Despite extensive global AMR documentation, regional data regarding pathogen distribution, resistance patterns, virulence gene profiles, and clonal complex circulation remains insufficient, thereby impeding effective therapeutic interventions and antimicrobial stewardship initiatives. This investigation aimed to characterize bacterial pathogens in chronic wounds at Meru Teaching and Referral Hospital, determine antimicrobial susceptibility profiles, identify resistance and virulence genes in *Staphylococcus aureus*, and elucidate genetic diversity and circulating clonal complexes to provide evidence-based data for clinical management optimization and AMR control strategies.

This study employed a cross-sectional survey design encompassing patients with chronic wound infections at Meru Teaching and Referral Hospital, augmented by comprehensive laboratory-based experimental analyses. Sample size determination utilized Nassiuma's formula, yielding 68 participants proportionally allocated across hospital units through convenience sampling methodology. Clinical and demographic data collection employed structured data collection instruments, while wound specimens were aseptically obtained, cultured on selective media, and bacterial isolates

identified using the VITEK 2 automated system. Antimicrobial susceptibility testing generated comprehensive resistance profiles, with Multiple Antibiotic Resistance (MAR) indices calculated for each isolate. Selected *Staphylococcus aureus* isolates underwent preservation for subsequent genomic characterization.

High-quality genomic DNA extraction, quantification, and sequencing utilizing Illumina technology facilitated comprehensive genomic analysis. Sequencing reads underwent rigorous quality control, assembly, and annotation processes, enabling identification of resistance genes, virulence factors, multilocus sequence types (MLST), spa types, and staphylococcal cassette chromosome mec (SCCmec) elements. Demographic and antimicrobial susceptibility data were analyzed using SPSS Version 27 with descriptive statistics and MAR index calculations. Hierarchical clustering heatmaps were generated in R to visualize bacterial distribution patterns across hospital units. Genomic analysis employed a bioinformatics pipeline including FastQC quality assessment, Trimmomatic preprocessing, de novo assembly using SPAdes/Unicycler, and Prokka annotation. Specialized bioinformatics tools including ResFinder, VirulenceFinder, MLST, spaTyper, and SCCmecFinder identified resistance genes, virulence factors, and genetic typing elements. Phylogenetic relationships were analyzed using iTOL, with comparative analysis against CARD and VFDB databases to characterize resistance and virulence determinants comprehensively.

Chronic wound infections demonstrated broad age distribution, with peak prevalence observed among individuals aged 21–30 years (22%) and 51–60 years (19.2%). Gender distribution revealed relative equilibrium, with males comprising 53% and females 47% of cases, with slight female predominance observed in older age cohorts. *Staphylococcus aureus* (26.6%) and Pseudomonas aeruginosa (13.2%) represented the most frequently isolated pathogens from chronic wound specimens. Escherichia coli and other Gram-positive and Gram-negative bacterial species demonstrated significant representation within the pathogen profile.

Species-specific susceptibility profiles revealed considerable variation. *Staphylococcus aureus* demonstrated 11% meropenem resistance, with 50% of isolates exhibiting multidrug resistance patterns. Escherichia coli exhibited complete resistance to

ampicillin/clavulanate and amoxicillin/clavulanate combinations, with high ciprofloxacin resistance (78%). *Klebsiella pneumoniae* displayed 50–100% resistance to various antimicrobials while maintaining low meropenem resistance (0–11%). Coagulase-negative staphylococci showed no meropenem resistance but demonstrated 60–100% resistance to multiple agents. Pseudomonas aeruginosa exhibited 0% meropenem resistance but 50–100% resistance to most other antimicrobials. Proteus species demonstrated 40–60% resistance to selected antibiotics, while *Morganella morganii* exhibited complete resistance to ciprofloxacin and trimethoprim-sulfamethoxazole.

MAR indices indicated substantial resistance pressure across multiple species. Klebsiella pneumoniae demonstrated exceptionally high values (0.76–1.00), while Acinetobacter baumannii exhibited a MAR index of 1.00. *Staphylococcus aureus* demonstrated a MAR index of 0.736, Escherichia coli reached 0.66, and *Enterococcus faecalis* reached 0.65. Pseudomonas aeruginosa displayed more moderate values, ranging from 0.22 to 0.37.

Comprehensive genomic analysis revealed a complex AMR landscape. MRSA-associated determinants including mecA and blaZ genes, along with extensive virulence determinants, demonstrated significant prevalence and frequently co-occurred with SCCmec elements. Core resistance genes included mecA (60%), blaZ (60%), aadD/aphA-3 (60%), mrsA (70%), tetK (40%), and dfrG/dfrA (40%), with rpob and grlA mutations observed in 20% of isolates. Virulence gene profiles encompassed Panton-Valentine leukocidin operons (lukF-PV/lukS-PV), hemolysins (hlgA/B/C), immune evasion factors (sak, scn, aur), and various enterotoxins.

Substantial genotypic diversity was observed through MLST, clonal complex determination, and spa typing, revealing spa types including t355, t318, and t84. Virulence gene profiles correlated with SCCmec elements IVa(2B), III(3A), and VIII(4A), consistent with MRSA and MRSA-like lineages. Characterized strains belonged to sequence types ST152, ST121, ST15, and ST7635, corresponding to clonal complexes CC151, CC30, and CC15.

5.2 Conclusion

This investigation provides comprehensive epidemiological and microbiological insights into chronic wound infections at Meru Teaching and Referral Hospital, systematically addressing the study objectives through rigorous laboratory-based analysis and genomic characterization.

Microbiological characterization validated the polymicrobial etiology of chronic wound infections, identifying bacterial pathogens of clinical significance. *Staphylococcus aureus* emerged as the predominant pathogen, accounting for 26.6% of isolates, followed by Pseudomonas aeruginosa (13.2%) and Escherichia coli, alongside other clinically relevant Gram-positive and Gram-negative bacterial species. The demographic analysis revealed a broad age distribution, with peak prevalence observed among individuals aged 21–30 years (22%) and 51–60 years (19.2%), challenging conventional assumptions that chronic wounds predominantly affect elderly populations. Gender distribution demonstrated relative equilibrium, with males representing 53% and females 47% of cases, indicating balanced susceptibility across both sexes.

Determination of antibiotic resistance profiles revealed marked species-specific variation and extensive multidrug resistance across multiple bacterial species isolated from chronic wound infections. *Staphylococcus aureus* demonstrated 11% meropenem resistance, with 50% of isolates exhibiting multidrug resistance patterns and a Multiple Antibiotic Resistance (MAR) index of 0.736. Morganella morganii displayed complete resistance to ciprofloxacin and trimethoprim-sulfamethoxazole. Escherichia coli exhibited total resistance to ampicillin/clavulanate and amoxicillin/clavulanate combinations, high ciprofloxacin resistance (78%), and a MAR index reaching 0.66. Klebsiella pneumoniae demonstrated 50–100% resistance to various antimicrobial agents while maintaining low meropenem resistance (0–11%), with exceptionally elevated MAR indices ranging from 0.76 to 1.00. Pseudomonas aeruginosa exhibited 0% meropenem resistance but 50–100% resistance to most other antimicrobials, with moderate MAR indices ranging from 0.22 to 0.37. Notably, Acinetobacter baumannii demonstrated a MAR index of 1.00, indicating complete multidrug resistance across tested antimicrobial agents.

Identification of antibiotic resistance determinants and virulence factors in *Staphylococcus aureus* isolates revealed a complex molecular landscape of resistance and pathogenicity. Core resistance genes identified included mecA (60%), blaZ (60%), aadD/aphA-3 (60%), mrsA (70%), tetK (40%), and dfrG/dfrA (40%), with chromosomal mutations in rpob and grlA detected in 20% of isolates. Analysis of staphylococcal cassette chromosome mec (SCCmec) profiles confirmed the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and related resistant lineages, with SCCmec types IVa(2B), III(3A), and VIII(4A) identified among the characterized isolates. Virulence factor profiling demonstrated significant prevalence of Panton-Valentine leukocidin operons (lukF-PV/lukS-PV), hemolysins (hlgA/B/C), immune evasion factors (sak, scn, aur), and various enterotoxins. The co-occurrence of resistance determinants with virulence factors, particularly PVL operons frequently associated with SCCmec elements, indicates that therapeutic challenges extend beyond antimicrobial selection to encompass enhanced pathogenic potential and increased tissue damage capacity.

Characterization of genetic diversity among resistant *Staphylococcus aureus* isolates revealed substantial genotypic heterogeneity within the hospital environment. Spa typing identified diverse sequence types including t355, t318, and t84, while SCCmec typing revealed multiple cassette types with associated ccr gene complexes. The identification of diverse mobile genetic elements, heterogeneous virulence factor profiles, and variable SCCmec backgrounds suggests active clonal expansion and horizontal gene transfer within the healthcare setting. These findings indicate a dynamic *Staphylococcus aureus* population structure influenced by both nosocomial and community sources, reflecting the complex transmission dynamics within the clinical environment.

Multilocus sequence typing (MLST) analysis determined that the characterized *Staphylococcus aureus* strains belonged to sequence types ST152, ST121, ST15, and ST7635, corresponding to clonal complexes CC151, CC30, and CC15. This clonal complex diversity, combined with observed variation in spa typing and genomic metrics, suggests multiple independent introductory events and the emergence of novel clonal lineages within the hospital setting. The detection of diverse clonal complexes

indicates potential for ongoing transmission dynamics and epidemic spread within the healthcare facility, underscoring the need for enhanced infection prevention and control measures.

The chronic wound microenvironment, dominated by *Staphylococcus aureus* and Pseudomonas aeruginosa, serves as a significant reservoir for multidrug-resistant and virulent organisms. The elevated MAR indices identified across multiple bacterial species, coupled with the co-occurrence of resistance genes and virulence determinants, define a well-established multidrug-resistant ecosystem that substantially complicates clinical management. These findings reflect the complex epidemiology of chronic wound infections and underscore the critical need for enhanced antimicrobial stewardship programs, comprehensive surveillance initiatives, and evidence-based therapeutic interventions to effectively address the evolving antimicrobial resistance crisis in this clinical setting.

5.3 Recommendations of the Study

i. Integration of molecular diagnostic tools in clinical settings is essential to enable rapid and accurate identification of pathogens, including non-culturable or polymicrobial organisms, thereby improving early detection and guiding appropriate therapeutic interventions.
ii. Healthcare facilities should promote research and implementation of anti-biofilm therapies and novel antimicrobial agents to effectively address the challenge posed by multidrug-resistant organisms identified in chronic wound infections.
iii. Capacity building initiatives should be strengthened through comprehensive training of healthcare professionals in infection control practices and advanced diagnostic techniques, while equipping laboratories with state-of-the-art automated systems such as the VITEK 2 platform to enhance diagnostic accuracy and efficiency.
iv. Public health education programs targeting younger and middle-aged individuals should be strengthened to emphasize proper wound care practices, diabetes management, and prevention of trauma-related injuries, given the significant burden of chronic wounds observed in these age groups.

v. Routine microbial surveillance and antimicrobial resistance reporting should be incorporated into hospital policy frameworks to establish evidence-based empirical treatment protocols and monitor resistance trends over time.

vi. Infection control and surveillance systems should be extended to include molecular typing for virulence genes, particularly edinB, hlgs, lukPV, sak, and scn, alongside multilocus sequence typing, spa typing, and SCCmec characterization to effectively track high-virulence clones and inform targeted intervention strategies.

vii. Comprehensive whole-genome sequencing should be performed on representative isolates from dominant clusters, particularly spa type t355, to resolve the complete genomic context of resistance and virulence determinants and elucidate their evolutionary relationships.

5.4 Recommendation for Further Studies

i. Future investigations should establish linkages between toxin profiles, exoenzyme production, and host immunity parameters with clinical outcomes such as wound chronicity, healing rates, and recurrence patterns to identify prognostic markers and therapeutic targets.

ii. Molecular characterization of non-typable or absent SCCmec and ccr configurations is necessary to clarify methicillin-susceptible *Staphylococcus aureus* status and improve the accuracy of molecular epidemiological surveillance systems.

iii. Standardization of virulence and resistance determinant nomenclature and annotation protocols should be pursued to facilitate cross-study comparisons, enable robust meta-analyses, and enhance the reproducibility of molecular epidemiological data across different research settings.

iv. Longitudinal studies investigating associations between specific virulence gene patterns and clinical outcomes are warranted to guide risk stratification protocols and optimize targeted therapeutic approaches for patients with chronic wound infections harboring high-virulence strains.

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APPENDICES

Appendix I: Consent Form

Investigators detail:
Name: Rael Gacheri
Email account; tabithagacheri@gmail.com
Cell phone; 0721300352

Introduction
This intends to establish bacterial resistance profile and genetic characterisation of *Staphylococcus aureus* isolated from chronic wound infections in patients at Meru Teaching and Referral Hospital (MeTRH), Meru County, Kenya

Procedure
A short history and physical exam to determine signs and symptoms of wound infection as well antibiotics used in the past will be taken and the source of the wound. Before swabbing the wounds, they will be cleansed and sterilized. This will take about 10 minutes of your time and some discomfort.

Confidentiality
The acquired information will be treated with highest confidentiality and only be available to the principal investigator and the research team. Your name will not be used in the research procedures, but a unique identifier number will be used. The information that we acquire from this study will be shared with the Chuka University, MeTRH ethics and research, Policy Makers in the Ministry of Health and Clinicians through Publications, Conferences, Journals and Presentations.

Benefits
There will be no direct benefit to you but this study will provide valuable information that will be used for surveillance of evolving strains and the development of new approaches for antibiotic resistance control within the one health approach. Diagnosis result findings will be communicated to your primary physician and appropriate management implemented were applicable.

Risk
Some research can cause harm to you but this study will have minimum harm, though you may feel uncomfortable during sample collection where a sterile swab will be used to collect the sample by rotating the swab for 5 seconds while applying enough pressure to produce fluid from the wound tissue.

Cost and recompense
No extra cost will be incurred for participating in this study.

Participation
You are being requested to volunteer for a research study. You can choose to participate or not. There are no consequences for choosing not to join. Whichever choice, the current management and treatment that is routinely offered in this hospital for your current condition will not change. You have all the right to refuse or pull out from this study at any point

Questions about the research

If you have any questions on the study, kindly contact me on this telephone number 0721300352.

I..... Hereby consent to take part in this study to establish the types of bacteria that cause wound infection in Meru Teaching and Referral Hospital, their genetic diversity, antimicrobial resistance patterns and the antimicrobial resistance genes. The nature of the study has been explained to me and I have been assured my participation is voluntary and shall not impact my health negatively.

Signature/ Thumb print: ..... Date: .....

Investigator statement

I explained the purpose and implications of the study to the participant.

Signature: ..... Date: .....

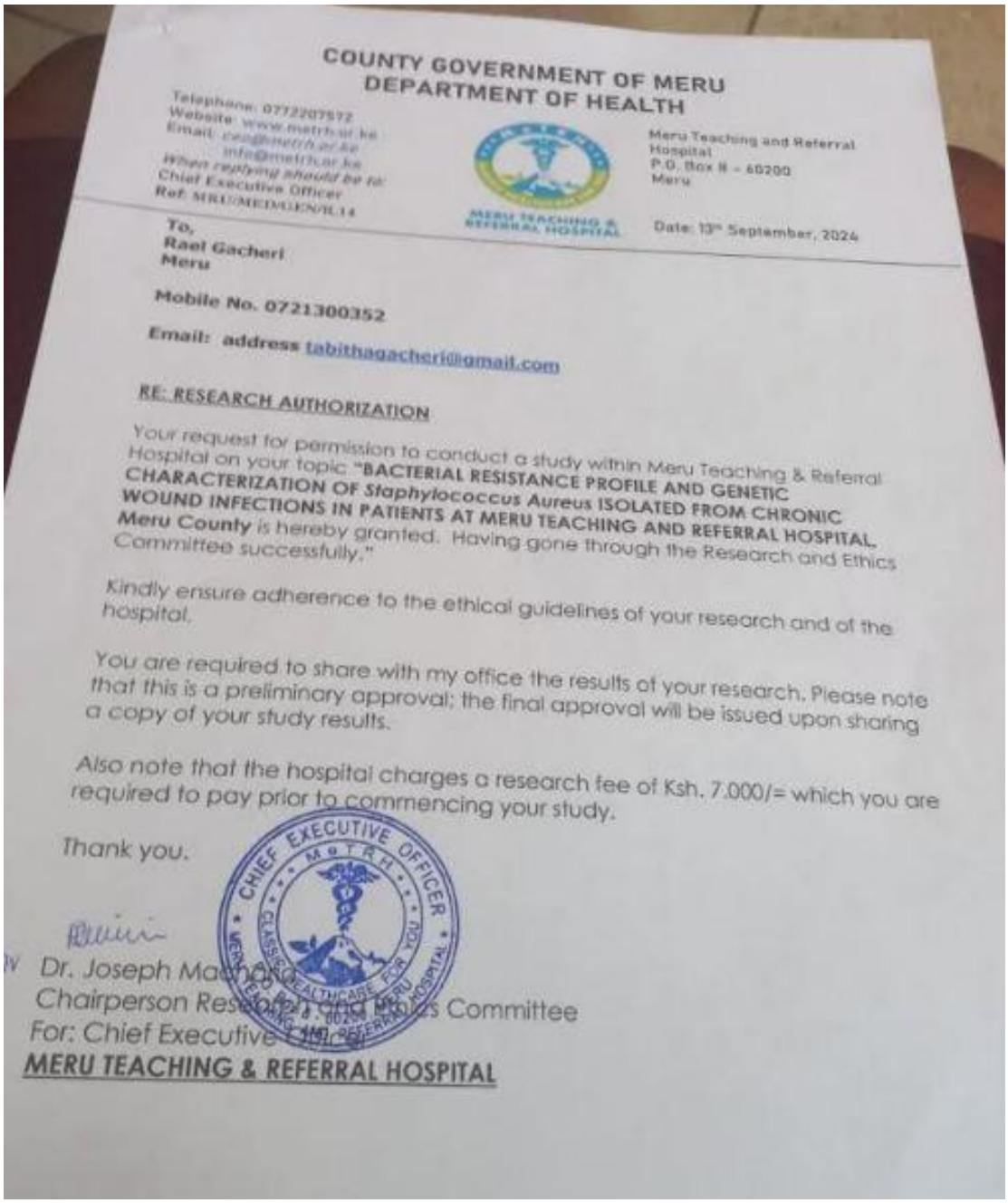
Researcher's signature..... Date: .....

Name (PRINT): ..... Designation: .....

Appendix II: Sample Collection Check List

Name of the patient……...
Date……..
Time……..……..
Unique identifier…….
Location (ward/clinic) …….
Gender……Male [ ] Female[ ]
Age……..
Patient history including the source of the wound

Appendix III: Meru Teaching and Referral Hospital Authorization



COUNTY GOVERNMENT OF MERU
DEPARTMENT OF HEALTH

Telephone: 0772207572
Website: www.metrh.or.ke
Email: ceo@metrh.or.ke
info@metrh.or.ke
When replying should be to:
Chief Executive Officer
Ref: MRU/MED/GEN/VOL.14

Meru Teaching and Referral Hospital
P.O. Box 8 - 60200
Meru

MERU TEACHING & REFERRAL HOSPITAL

Date: 13th September, 2024

To,
Rael Gacheri
Meru

Mobile No. 0721300352

Email: address tabithagacheri@gmail.com

RE: RESEARCH AUTHORIZATION

Your request for permission to conduct a study within Meru Teaching & Referral Hospital on your topic "**BACTERIAL RESISTANCE PROFILE AND GENETIC CHARACTERIZATION OF *Staphylococcus Aureus* ISOLATED FROM CHRONIC WOUND INFECTIONS IN PATIENTS AT MERU TEACHING AND REFERRAL HOSPITAL, Meru County** is hereby granted. Having gone through the Research and Ethics Committee successfully."

Kindly ensure adherence to the ethical guidelines of your research and of the hospital.

You are required to share with my office the results of your research. Please note that this is a preliminary approval; the final approval will be issued upon sharing a copy of your study results.

Also note that the hospital charges a research fee of Ksh. 7,000/= which you are required to pay prior to commencing your study.

Thank you.

CHIEF EXECUTIVE OFFICER M.T.R.H. QUALITY HEALTHCARE FOR YOU MERU TEACHING AND REFERRAL HOSPITAL P.O. Box 8 - 60200

Dr. Joseph Mac[illegible]
Chairperson Research and Ethics Committee
For: Chief Executive Officer
MERU TEACHING & REFERRAL HOSPITAL

Appendix IV: Ethics Review Letter

CHUKA UNIVERSITY

Knowledge is Wealth *(Sapientia divitia est)* Akili ni Mali

CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18 P. O. Box 109-60400, Chuka
Direct Line: 0772894438 Email: info@chuka.ac.ke. Website: www.chuka.ac.ke

13th August, 2024

REF: CUIERC/ NACOSTI/627
TO: Rael Gacheri Silas

RE: Bacterial Resistance Profile and Genetic Characterisation of *Staphylococcus aureus* Isolated from Chronic Wound Infections in Patients at Meru Teaching and Referral Hospital, Meru County, Kenya

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812.* The approval period is 13th August, 2024 – 13th August, 2025.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC.*
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC.*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr. Benjamin Kanga
SECRETARY

INSTITUTIONAL ETHICS REVIEW COMMITTEE
19 AUG 2024
P.O. Box 109-60400,CHUKA
CHUKA UNIVERSITY

Appendix V: National Commission for Science, Technology and Innovation (NACOSTI) Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **880873**

Date of Issue: **31/August/2024**

RESEARCH LICENSE

This is to Certify that Miss.. RAEL GACHERI SILAS of Chuka University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Meru on the topic: BACTERIAL RESISTANCE PROFILE AND GENETIC CHARACTERISATION OF Staphylococcus aureus ISOLATED FROM CHRONIC WOUND INFECTIONS IN PATIENTS AT MERU TEACHING AND REFERRAL HOSPITAL, MERU COUNTY, KENYA for the period ending : 31/August/2025.

License No: **NACOSTI/P/24/39429**

880873

Applicant Identification Number

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

Appendix VI: Kenya Medical Research Institute

KENYA MEDICAL RESEARCH INSTITUTE

Center for Microbiology Research, P.O Box 19464-00202, Nairobi-Kenya

Tel: (254) (020) 2720794, 2720038, Nairobi. Website: www.kemri.go.ke

10th September, 2025

To whom it may concern

RE: LABORATORY ACCESS FOR MOLECULAR ANALYSIS OF STAPHYLOCOCCUS AUREUS ISOLATES

This is to confirm that Ms. Rael Gacheri performed her molecular analysis of her *Staphylococcus aureus* isolates at the KEMRI Genomics and Bioinformatics Laboratory-Kericho Hub under the supervision of Dr. Musila.

She underwent training on basic Biosafety, Ethics, molecular techniques and sequence analysis. She was able to characterize all her isolates.
Any further clarifications can be obtained from Dr. Musila-Laboratory Director, KEMRI Kericho at lilian.musila@usambru.org.

Thank you

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