BACTERIAL TYPES, ANTIBIOTIC USE, AND THEIR ANTIMICROBIAL SENSITIVITY PATTERNS IN CHRONIC WOUND IN SELECTED HEALTH FACILITIES, UASIN GISHU COUNTY, KENYA.

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PUBLIC HEALTH DEGREE BY THE MOI UNIVERSITY.

DECLARATION

This dissertation is my original work and has not been presented to any other university/institution, for the award of any degree or certificate.

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DEDICATION

I dedicate these research findings to the Health Care Practitioners and Research Scientists working at the Moi Teaching and Referral Hospital, Eldoret Hospital, Reale Hospital, and St. Luke's Hospital in Uasin Gishu County.

ABSTRACT

Background: Bacterial infections are one of the causes of slow and non-healing wounds. Adding to the increase in incidences of chronic wounds because of other patient factors and the current challenge of antimicrobial resistance experienced worldwide, the management of infected wounds is a challenge. Notwithstanding; the presence of Infection Prevention Control Programs (IPCs) and the practice of prophylactic therapy, infections are still a challenge in these selected Hospitals in Eldoret Town, especially in clinical practice where empirical treatment of infections is routine because of cost challenges that come with targeted therapy.

Objectives: To identify the bacteria isolated from infected chronic wounds, the proportions of the individual strain isolated, the antibiotics used to manage such infected wounds, and their sensitivity patterns against the isolated strains in selected Health facilities, Eldoret town, Uasin Gishu County.

Methods: A cross-sectional study on patients with chronic wounds was adopted, examining microbiology laboratory data on culture and sensitivity tests and patient records of chronic-wound swab culture and sensitivity test results. The sample size for this study was determined using Fischer's formula, taking an average prevalence of 90% of pathogenic bacteria from wound swabs taken from the previous similar studies done in Kenya; at Kenyatta National Hospital (KNH). From this formula, files and records of 138 chronic-wound patients; whereby files that are easy to reach were sampled and recorded first in order of the year the tests were conducted. Sequential sampling of the laboratory data on chronic wound culture and sensitivities was the ideal procedure beginning with the most recent data. The data was collected from Moi Teaching and Referral Hospital (MTRH), Eldoret Hospital, Reale Hospital, and St Luke's Hospital in Eldoret Town Uasin, Gishu County. The sampling order of these hospitals depended on; the number of chronic wound patients they admitted, beginning with the one with the highest numbers. The samples obtained were, therefore, proportionate to the patient numbers. The data was collected retrospectively from April 2022 using a data abstraction form. Collected data was validated, entered, and stored in a Microsoft Excel spreadsheet and analyzed using the statistical software STATA version 16. Frequencies and percentage proportions were calculated to determine the distribution of the demographic variables. To test the independence and the significance of differences between sex, age in years, cause of the wound, and comorbidities against the number of bacteria isolated, we used canonical correlation analysis to explore this relationship. P-values less than 0.05 were considered significant.

Results: Moi Teaching and Referral Hospital provided the most data (75.4%). Most of the wounds were caused by Trauma/Accident, with 65.2% having comorbidities. A total of 19 bacterial strains were isolated. The majority of the bacteria identified were Gram-negative strains. However, Gram-Positive *Staphylococcus aureus* was present in all the mixed isolates. 90.6% indicated the use of antibiotics, both prophylaxis and treatment. Ceftriaxone was the most prescribed antibiotic (16.4%). Less than half of the antibiotics showed susceptibilities above 50% against the bacterial strains tested. High sensitivities were registered by less frequently used antibiotics. Based on Wilks` Lambda test statistics, the cause of the wound, age, and sex did not affect the proportions of bacterial strains isolated.

Conclusion: Polymicrobial bacterial infections were noted in both gram stains. Multiple Drug Resistance (MDR) registered by the most prescribed antibiotics. *Staphylococcus aureus* isolates were definitive of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains as they were resistant to all the Penicillins.

Recommendations: Penicillins are not recommended in managing chronic wound infections. More studies to validate viability of off-patent (generic) antibiotics. Antibiotic combination therapy is recommended based on the established localized antibiogram.

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ABBREVIATIONS AND ACRONYMS

AMR- Antimicrobial Resistance

EPS- Exopolysaccharide

MTRH- Moi Teaching and Referral Hospital

SOP- Standard Operating Procedure

WCSK- Wound Care Society of Kenya

MDT- Maggot Debridement Therapy

PCR- Polymerase Chain Reaction

IREC- Institutional Research and Ethics Commission

BHS- Beta Hemolytic Streptococcus

CoNS- Coagulase-negative Staphylococcus

KNH- Kenyatta National Hospital

MIU- Motility Indole Urea

MRSA- Methicillin-Resistant *Staphylococcus Aureus*

MSSA-Methicillin Susceptible *Staphylococcus Aureus*

NLF- Non-Lactose Fermenters

PBP- Penicillin Binding Protein

SPP- Species

SSI- Surgical Site Infection

WHO-World Health Organization

MDR-Multi-Drug Resistant

OTC-Over the Counter

IPC- Infection Prevention and Control Program

DEFINITION OF OPERATIONAL TERMS

BIOFILM- A collective of one or more types of microorganisms that can grow on many different surfaces.

BACTERIAL COLONIZATION- the presence of bacteria on a body surface.

ANTIMICROBIAL STEWARDSHIP-proper use of antimicrobials according to WHO guidelines.

EPITHELIALIZATION- A process where epithelial cells migrate upwards and repair the wounded area.

SEPTIC CHRONIC WOUND- Infected chronic wound that has failed to heal for more than three months.

EXOPOLYSACCHARIDE-Extracellular macromolecules excreted a as tightly bound capsule or slime layer that holds bacteria biofilm together.

ANTIBIOGRAM-Collective data of culture and sensitivity test of specific bacteria against several antibiotics in a hospital facility

NOSOCOMIAL-Hospital acquired infections

IATROGENIC-infections after medical or surgical management

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Chronic non-healing wounds are a silent epidemic, a problem reported to be affecting a growing population in developing countries, with notable effects on the healthcare system and public health (Järbrink *et al.*, 2016). According to the Wound Healing Society, there are three main types of chronic wounds; infectious, surgical, and ulcers (arterial, diabetic, traumatic, venous, and pressure ulcers). An ageing population, bacterial infections, an increasing number of accidents, and an increase in diabetes and obesity contribute to rising chronic wound incidences. Furthermore, a threat to public health because chronic wounds have devastating consequences for patients, healthcare systems, and societies. If not managed well, chronic wounds can lead to prolonged hospital stays, high cost of treatment, trauma, stress, and mental illnesses due to anxiety and depression resulting from social isolation, decreased quality of life, and low self-esteem (MacDonald, 2009).

Bacterial infection is one of the factors affecting the wound-healing process, contributing to the pathogenesis of chronic wounds. Where the healing process is impaired or delayed, bacteria often colonize the wound bed, forming biofilms. These biofilms create a protective environment for bacteria, making them resistant to the body's immune response and conventional treatments. While bacteria in infected chronic wounds may exacerbate the condition, it also highlights the need for targeted interventions to manage and eliminate these infections effectively. Moreover, bacteria can serve as indicators of the underlying issues that contribute to the wound's chronicity. Infection hampers the normal healing process of wounds by forming colonies encased in an Exopolysaccharide (EPS) and stimulating chronic inflammation on the wound site. EPS makes these bacteria less

metabolically active and resistant to treatment (Mihai *et al.,* 2018). More so, the surface of the infected wound houses complex colonies of bacteria that include more than one species (Wolcott *et al.,* 2016). The high cost of managing bacterial wound infection is associated with the cost of antibiotics and patient hospital stays; because of prolonged healing or comorbidities (Gottrup *et al.,* 2013).

Despite a lack of enough evidence concerning the effective treatment of chronic wounds, the current practice is antibiotics usage. These patients receive considerably more antibiotic prescriptions (both systemic and topical) than any other patient with different forms of bacterial infection (Tzaneva *et al.,* 2016). World Bank (2021) declared that approximately 700,000 people die yearly because of AMR. By 2050, these numbers are likely to increase upto10 million deaths annually. Those people living in low and middle-income countries are the most vulnerable. Therefore, measures to combat AMR must be adapted and implemented. The susceptibility of bacteria to antibiotics depends on the following factors; the environment, microbe strain type, the continuing evolution of the microbe, and the pattern of antibiotic use or abuse. It is clinically essential to conduct antimicrobial sensitivity studies continuously; to tackle the growing rate of bacteria mutations and resistance (Omoyibo *et al.,* 2018). Therefore, conducting bacterial isolation, identification, and characterization in chronic wounds is essential. As indicated, it will inform specific therapy for better choices of antibiotics or combinations and aseptically improved handling of chronic wounds in hospital facilities.

The practice of empirical therapy rather than specific therapy and the heavy usage of antibiotics in chronic wound healing encourages antimicrobial resistance (AMR). The World Health Organization (Africa) reported a case of a Kenyan health worker who had a

chronic infected leg wound and was using different kinds of antibiotics for several months but eventually developed antimicrobial resistance (WHO, 2019). The WHO recommended way forward is defining effective ways of managing infected wounds and informed choices of antibiotics. Continuous local bacterial sensitivity data with established prevalent bacterial types, is a critical tool that guides antibiotic selection to improve rational specific therapy at outpatient facilities. More so, further studies are required to evaluate the roles of each bacteria strain in the healing of chronic wound infections. From this, we can now establish effective treatment options that also encourage principles of antimicrobial stewardship, which include giving the right antibiotic for the right; duration, timing, and indication.

1.2 Problem Statement

Chronic wounds are becoming more prevalent worldwide because of increasing underlying conditions such as diabetes, obesity, trauma, antibiotic resistance, and ageing populations. Infection is one of the many hampering factors in the healing and management of chronic wounds (Kadam *et al.,* 2019). A systematic literature review on technology and recent advances in managing chronic wounds in Kenya by Ongarora (2022) suggest that 50% of patients admitted to Hospitals have chronic wounds. There has been an improvement and attempts to provide aseptic conditions in the surgical wards through Infection prevention Control programs (IPC). Despite this, the incidence of wound infection is increasing. The prevalence of wound infection in the Kenyatta National Hospital (KNH) (Kenya) surgical pediatric ward was 82 % shown by Elamenya *et al.,* (2015) and 94% in diabetic foot ulcers (Mutonga *et al.,* 2019).

Bacterial infections are difficult to treat because they form many colonies on the wound bed, some encased in an Exopolysaccharide layer. They also develop resistant strains that cause antibiotic ineffectiveness and resistance. Furthermore, these bacterial colonies cause wound malodor, a cause of psychological and psychosocial effects on the patient, relatives, and caregiver. 79% of surgical patients at Moi Teaching and Referral Hospital (MTRH) had SSI (surgical site infections) (Otieno Stephen, 2018). Similar study by Akoru *et al.*, 2016 at MTRH, revealed high infections and similar ethologic agent *staphylococcus aureus.* Most cases of infected chronic septic wound management are done empirically at outpatient facilities. The main reasons for this practice include the lack of enough funds from patients to enable specific treatments, minimal time to perform wound swab culture and sensitivity tests, and lack of equipment.

1.3 Justification

Currently, chronic wounds pose a significant healthcare challenge, affecting individuals of all ages and backgrounds. These wounds often exhibit delayed healing, leading to severe complications if left untreated. The clinical significance of these wounds lies in their association with prolonged healing times, increased morbidity, and elevated mortality rates. Chronic wound patients experience severe pain, prolonged hospitalization, significant emotional and physical distress, reduced mobility, social isolation, low self-esteem, and loss of social and economic stability (Sen 2019). Chronic wounds' physical and emotional tolls can have far-reaching consequences, affecting individuals' overall well-being and daily activities. The prolonged healing time and frequent recurrences of chronic wounds result in repeated healthcare visits and resource-intensive treatments (Sen 2021).

This study, identifies the specific and prevalent bacterial strains in chronic wounds and in turn assist healthcare professionals to gain insights into the wound's microbial profile and tailor treatment strategies accordingly. This approach allows for more targeted antimicrobial therapies, including antibiotics or advanced wound care products, to eradicate the bacteria and promote wound healing. Therefore, understanding the role of bacteria in infected chronic wounds can aid in developing effective treatment plans and improving patient outcomes. Together with the local sensitivity data, the findings of this research will form a basis to inform effective septic wound therapy at the early stages. The sensitivity data obtained will form a temporal sensitivity pattern to inform microbial-targeted choices of antibiotics and improve the rational empiric treatment of the wound infection. Furthermore, it is significant at outpatient facilities where a definitive treatment option is challenging.

The informed selection of antibiotics targeting the prevalent bacteria strains increases the chances of achieving a better therapeutic outcome. This study will positively affect health service delivery by strengthening the infection prevention and control programs (IPC) and policy in our health facilities; to minimize Hospital-acquired infections (HAIs), the possibility of AMR, and infection re-occurrence. Furthermore, it will have a positive effect on both patients and the health care system as a whole: in terms of reduced time of treatment, cost of treatment, reduced patient hospitalization, minimized economic and social burden, antibiotic stewardship, and improved chronic wound management in health facilities. The major public health concern is to prevent the; psychological, social, and economic effects of chronic non-healing wounds. Therefore, the goal is to minimise

antimicrobial resistance (AMR), maintain antimicrobial stewardship, and improve patient hospital costs and safety.

1.4 Research Questions

This research answered the following questions:

1. What types of bacteria were isolated and their prevalence in chronic wounds from the selected health facilities in Eldoret Town, Uasin Gishu County?
2. Which antimicrobials were used to manage chronic wounds and their bacterial sensitivity patterns from the selected health facilities in Eldoret Town, Uasin Gishu County?

1.5 Objectives

1.5.1 Broad objective

-To identify the bacteria types isolated from chronic wound swabs, antibiotics used and bacterial sensitivity patterns from the selected health facilities in Eldoret Town, Uasin-Gishu County.

1.5.2 Specific objectives

i. To establish the bacterial species prevalence in chronic wound swab isolates from the selected health facilities in Eldoret Town, Uasin-Gishu County.

ii. To determine the most prevalent bacterial pathogen in chronic wound swab isolates from the selected health facilities in Eldoret Town, Uasin-Gishu County.

iii. To evaluate the antibiotics used to manage chronic wounds in the selected health facilities in Eldoret Town, Uasin-Gishu County.

iv. To analyze the resistance and susceptibility patterns of the antibiotics used in the selected health facilities in Eldoret Town Uasin-Gishu County.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Chronic wounds are a growing medical problem such that their impact on the health and quality of life of patients and their families is of concern. It represents a significant healthcare challenge worldwide, posing a considerable burden on patients, healthcare systems, and society as a whole. These wounds, which fail to progress through the normal stages of wound healing within an expected timeframe, can lead to prolonged suffering, impaired quality of life, and increased healthcare costs.

According to Sen (2019), an analysis of human wounds and their burden in the United States showed that 8.2 million people had wounds with or without infections in 2018. Their estimated cost of treatment ranged from $28.1 billion to $96.8 billion annually. Furthermore, he notes that the high costs of health care; were associated with an ageing population, isolation of difficult-to-treat biofilms, and the rising threat of diabetes and obesity. Due to these factors, chronic wounds are a substantial clinical, social, and economic challenge worldwide. The COVID-19 pandemic has directly affected the management of wounds and aspects of the wound care process, such as patient clinic visits. It has prompted alternative approaches to wound care, such as Telemedicine, creating videos to help with wound dressing, encouraging self-care methods, and homemade remedies. Since chronic wounds are associated with comorbidities such as diabetes and obesity, the mortality rates of chronic wound patients have risen as far as COVID-19 infection is concerned (Sen, 2021). Therefore, it calls for a more structured approach to clinical management, investment, education, and related research in wound care.

2.2 Chronic wounds

Failure of wounds to heal through the normal healing process and remain open for more than three months; are considered chronic wounds (Iqbal *et al.,* 2017). According to Järbrink *et al.* (2016), approximately 2% of the population in developing countries will battle chronic wounds during their lifetime. According to the United States National Institutes of Health, chronic wounds affect 6.5 million patients in the United States alone, which will likely increase (Sen *et al.,* 2009). There are many causes of chronic wounds; some of these examples include infections, diabetes, and obesity and are considered the highest risk factors. There is an ongoing debate about whether to consider chronic wounds as a disease on their own or not. However, they are complicated by many comorbidities, creating a challenge in differentiating and tracking them clinically. Consequently, it has also made funding and research on chronic wounds globally and regionally very low (Sen, 2019).

Chronic wounds can arise from a variety of underlying conditions and factors, including vascular insufficiency, neuropathy, pressure, trauma, infection, and systemic diseases such as diabetes mellitus. Understanding the underlying etiology and pathophysiology of chronic wounds is essential for guiding appropriate management strategies and optimizing patient outcomes. These are some of the etiological factors that lead to development of chronic wounds (Gardner *et al.,* 2013).

Vascular Insufficiency:

Peripheral arterial disease (PAD) and venous insufficiency are common causes of chronic wounds, resulting from impaired blood flow to the affected tissues. In PAD, atherosclerosis leads to narrowing or occlusion of the arteries, reducing oxygen and nutrient delivery to

the tissues and impeding wound healing. Venous insufficiency, characterized by venous hypertension and impaired venous return, can result in chronic venous ulcers, typically located on the lower extremities.

Neuropathy:

Neuropathic wounds often occur in patients with diabetes mellitus or other neuropathic conditions, resulting from sensory loss, motor dysfunction, and autonomic neuropathy. Loss of protective sensation predisposes patients to repetitive trauma and pressure injuries, leading to the development of chronic ulcers, particularly on the feet and lower limbs.

Pressure Injuries:

Pressure injuries, also known as pressure ulcers or bedsores, occur due to sustained pressure or friction on the skin and underlying tissues, resulting in tissue ischemia, necrosis, and ulceration. Immobility, prolonged bed rest, friction, and shear forces contribute to the development of pressure injuries, which commonly affect bony prominences and areas subjected to pressure, such as heels, sacrum, and elbows.

Infection:

Infection plays a significant role in delaying wound healing and complicating the management of chronic wounds. Bacterial colonization and biofilm formation impair host defenses, prolong inflammation, and delay the proliferative and remodeling phases of wound healing. Chronic wounds are often polymicrobial.

Understanding the underlying pathophysiology, risk factors, clinical manifestations, and evidence-based management strategies for chronic wounds is essential for healthcare professionals involved in wound care, a clear and accurate clinical assessment and diagnosis are essential for guiding appropriate wound management strategies and

optimizing patient outcomes. A comprehensive evaluation of the wound, patient history, comorbidities, and contributing factors is crucial for developing an individualized treatment plan. Wound assessment involves a systematic evaluation of the wound characteristics, including size, depth, location, tissue type, exudate amount, and presence of necrosis or infection. Various wound assessment tools and scoring systems, such as the Bates-Jensen Wound Assessment Tool and the Pressure Ulcer Scale for Healing, may be used to standardize wound evaluation and track changes over time.

Obtaining a detailed patient history is essential for identifying underlying risk factors, comorbidities, medications, and previous treatments that may impact wound healing. Common risk factors for chronic wounds include diabetes mellitus, peripheral vascular disease, venous insufficiency, neuropathy, immobility, malnutrition, smoking, and immunosuppression. Diagnostic investigations, such as Doppler ultrasound, ankle-brachial index (ABI) measurement, vascular imaging, and wound swab culture, may be performed to assess vascular status, identify underlying pathology, and guide treatment decisions. Imaging modalities, including magnetic resonance imaging (MRI) and computed tomography (CT) angiography, may be utilized to evaluate the extent of tissue damage, assess for underlying osteomyelitis, and guide surgical planning (Sen 2019).

According to Jones *et al.*, 2007, the management of chronic wounds requires a multidisciplinary approach, involving collaboration among healthcare professionals, wound care specialists, and allied healthcare providers. Treatment strategies aim to address underlying etiological factors, promote wound healing, prevent complications, and improve patient quality of life. Examples of evidence based wound management include;

1) **Wound Bed Preparation:**

Wound bed preparation is a fundamental principle in chronic wound management, involving the removal of necrotic tissue, control of infection, optimization of tissue perfusion, and management of wound exudate. Debridement techniques, including sharp debridement, enzymatic debridement, autolytic debridement, and surgical debridement, may be employed to remove necrotic tissue and promote wound healing.

2) **Moist Wound Healing:**

Maintaining a moist wound environment is essential for promoting cell migration, proliferation, and extracellular matrix deposition, key processes in wound healing. Moist wound dressings, such as hydrogels, hydrocolloids, foams, films, and alginate dressings, help maintain an optimal moisture balance, absorb excess exudate, and protect the wound bed from external contaminants.

3) **Compression Therapy:**

Compression therapy plays a key role in the management of venous leg ulcers and other forms of venous insufficiency, aiming to reduce edema, improve venous return, and promote ulcer healing. Compression bandages, stockings, and pneumatic compression devices may be utilized to apply controlled pressure to the affected limb and reduce venous hypertension.

4) **Offloading and Pressure Redistribution:**

Offloading is critical for the management of pressure injuries and neuropathic ulcers, aiming to reduce pressure and shear forces on vulnerable areas and promote tissue perfusion and healing. Offloading devices, such as specialized footwear, custom orthotics,

foam padding, and pressure-relieving mattresses, help redistribute pressure away from bony prominences and high-risk areas.

5) **Surgical Interventions**:

Surgical interventions may be indicated for select cases of chronic wounds, particularly those associated with underlying pathology such as osteomyelitis, abscess formation, or vascular compromise. Surgical options include wound debridement, tissue reconstruction, skin grafting, flap reconstruction, vascular interventions, and amputation in cases of limb-threatening ischemia or infection.

6) Prevention and Patient Education:

Prevention plays a critical role in reducing the incidence and recurrence of chronic wounds, particularly in high-risk populations. Patient education, lifestyle modifications, and early intervention strategies are essential components of wound prevention efforts. Patient education is paramount in promoting self-care behaviors, optimizing wound healing, and preventing complications. Patients should receive education on wound care principles, including proper wound cleansing, dressing application, offloading techniques, nutritional support, smoking cessation, and foot care in patients with diabetes. It also involves Pressure injury prevention strategies, focusing on minimizing pressure and friction on vulnerable areas, maintaining skin integrity, and promoting mobility and repositioning in bedridden nursing care (Sen 2021).

In 2014, the Wound Care Society of Kenya (WCSK) held a symposium to discuss wound care management and emerging technologies in wound treatment, like Maggot Debridement Therapy (MDT). However, an increase in the number of patients who have chronic wounds as a result of; trauma, burns, skin cancers, infections, and underlying

medical conditions such as diabetes and obesity were the major concern. Kenyatta National Hospital (KNH), one of the biggest referral hospitals in Kenya, was full of increasing referral cases against limited bed capacity in the Specialized Burns Unit (KNH, 2014).

There have been laudable steps and improvements in managing chronic wounds in Kenya. Wound dressing has evolved from the old cotton gauze to new composite materials embedded with compounds that accelerate wound healing, such as; antimicrobials and metal-based nanoparticle oxides. Future technologies look at enzyme biodegradable dressing materials and MDT, especially for deep and wide area wounds and molecular therapies such as; bioengineered allogeneic cellular, stem cell, cellular xenograft, and growth factors. (Ongarora, 2022). Besides these advancements, there are abound challenges, including; inaccessibility of these technologies, high cost of materials and drugs, limited integration, research and stakeholder collaborations. Chronic wounds limit individuals' ability to engage in daily activities, work, and social interactions. Reduced productivity and social participation among patients with chronic wounds can contribute to socioeconomic disparities and further worsen the overall burden of disease and even introduce new disease. The public health challenges associated with chronic wounds requires a multifaceted and collaborative approach that encompasses prevention strategies, early detection, evidence-based treatments, and comprehensive wound care management programs (Jones *et al.*, 2007).

2.3 Wound healing

Chronic wound healing is a complex process impaired in individuals with certain medical conditions, such as diabetes mellitus, infections and peripheral vascular disease. These wounds fail to progress through the normal stages of healing and often remain open for an

extended period, leading to significant morbidity and decreased quality of life for patients. The impaired healing in chronic wounds is associated with intrinsic and extrinsic factors. Intrinsic factors include advanced age, comorbidities like diabetes or immunosuppression, and genetic predispositions. Extrinsic factors involve poor wound care practices, inadequate blood supply, infection, and excessive inflammation. Persistent inflammation is a hallmark of chronic wounds and can lead to the overproduction of reactive oxygen species, impairing cell proliferation and migration. Additionally, biofilm, a structured microbial community, in chronic wounds further hinders healing by promoting infection and inflammation (Sen *et al.,* 2009; Guo & Dipietro. 2010). Laboratory investigations and clinical studies have provided information about both normal and impaired wound healing process. More recently, a great many research has been directed at understanding the critical factors that influence slow healing of wounds. While many studies are required to unravel more concerning this phenomenon. Studies may lead to therapeutics that will promote proper tissue repair and improve epithelialization and finally wound healing.

The complex wound healing process involves interaction between primary host immunity and other natural host factors. It is a sequence of events that happen in an orderly and timely manner. This process is described scientifically in three differential phases; The coagulation/inflammatory (hemostasis), the proliferation (fibroblastic)/tissue formation, and the maturation/remodeling phase. These phases are not separated, they overlap and influence each other, and sometimes they occur together. These phases initiate an acute wound that progresses through each stage towards successful epithelialization and wound closure (Mihai *et al.,* 2018; Mercandetti *et al.,* 2021).

Wound healing process may be impaired by several factors, working together or singly. According to Uccioli *et al.* (2015), chronic non-healing wounds have similar features; reduced blood flow due to affected blood vessels, high proteases, increased inflammatory markers, and slow cell growth. The common factors contributing to the pathogenesis of non-healing wounds include; infection, ischemia, metabolic conditions, immunosuppression, other underlying chronic conditions, and radiations. Polymicrobial bacterial infections that originate from skin microflora and the surrounding environment; complicate further the healing process of chronic wounds (Silva *et al.*, 2018).

Another essential aspect of chronic wound healing is the role of chronic inflammation. In routine wound healing, inflammation is an essential part of the process, as it helps to remove debris and pathogens and initiates the tissue repair cascade. However, in chronic wounds, inflammation becomes prolonged and dysregulated. This chronic inflammation inhibits the formation of new blood vessels and delays the formation of granulation tissue. A study by Jones *et al.* (2007) emphasized the importance of addressing chronic inflammation through advanced wound dressings and managing underlying comorbidities.

Impaired angiogenesis is another hallmark of chronic wound healing. The formation of blood vessels and capillaries is essential for supplying oxygen and nutrients to the healing tissue. However, in chronic wounds, angiogenesis is often compromised. Factors such as reduced growth factors, increased levels of anti-angiogenic factors, and aberrant signaling pathways contribute to this impaired angiogenic response. A study by Takahashi *et al.* (2021) highlighted the potential of therapeutic interventions, such as growth factors and hyperbaric oxygen therapy, in promoting angiogenesis and improving chronic wound healing outcomes.

Successful chronic wound healing requires the coordination of multiple cellular and molecular events. Chronic wounds often have an imbalance in the production and degradation of extracellular matrix components, further hindering the formation of new tissue. The dysregulation of growth factors, such as transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF), also contributes to the impaired healing process. Various treatment modalities developed to promote healing in chronic wounds include; advanced wound dressings, negative pressure wound therapy, and bioengineered tissue substitutes. Advanced wound dressings, such as hydrogels and collagen-based dressings, provide a moist environment, promote angiogenesis, and facilitate autolytic debridement. Negative pressure wound therapy promotes wound contraction and the formation of granulation tissue. Bioengineered tissue substitutes, such as skin equivalents and cell-based therapies, aim to replace the lost or damaged tissue and stimulate wound healing (Gurtner *et al.,* 2008; Guo & Dipietro. 2010; Nussbaum *et al.,* 2018).

Wound healing is a multifactorial process often impaired in individuals with underlying medical conditions. Understanding the pathophysiology of chronic wounds and the factors contributing to their delayed healing is crucial for developing effective treatment strategies. By targeting the underlying causes, such as inflammation and impaired cell proliferation, clinicians can employ various interventions to promote healing and improve the quality of life for patients with chronic wounds. Further research is needed to explore new therapeutic approaches and optimize existing treatment modalities to enhance chronic wound healing outcomes (Gottrup *et al.,* 2013).

2.4 Bacterial wound infections and biofilms

2.4.1 Bacterial wound infection

Bacterial infection is one of the factors contributing to non-healing and the development of chronic wounds by disrupting the wound-healing process. According to Gajula *et al.* (2020), bacteria interrupt cell migration and cause cell death in several ways. However, the belief theory is that different bacterial spp have different modes of action involving the elaboration of various bioactive factors that lead to the persistence of low-grade inflammation in the wound bed. Successful bacterial infection is when the bacteria establish a colony and forms a biofilm encased in an EPS layer. Several factors affect this process; selection pressures due to antimicrobial misuse, host immune system, age, and comorbid conditions of the patient; furthermore, they increase virulence and resistance to treatment.

2.4.2 Exopolysaccharides and Biofilms

The protective extracellular polymeric substance called Exopolysaccharide (EPS), helps bacteria to adhere to a suitable surface. Many bacterial species form EPS, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, are two common microbes responsible for wound biofilm, have been studied and documented extensively. To scientifically study and understand biofilms it is however unethical to experiment and generate biofilms in human. Most of the present knowledge has been derived from in vitro and animal studies. A meta-analysis studies and published data indicates more 70% prevalence of biofilms in chronic wounds, making these a significant threat to wound healing and therapy. Bacterial orientation in wound biofilm is that, the outer layers of a biofilm bacteria are mostly aerobic, are actively metabolizing, rapidly multiplying and they grow easily in cultures.

The deeply rooted bacteria which survive in low oxygen tension or anaerobic environment, they are usually less and remain dormant for long period of time. They are not easy grow in conventional cultures. The EPS layer provides a physical barrier to immune attack and drugs, the more the bacterial strains form one EPS, the more virulent the infection against the immune attack and antimicrobials. These resident bacteria strains in biofilm community communicate with one another through quorum sensing (QS) molecules. A Clinical appearance and presentation of infected chronic wound is a red, friable granulation tissue covered by a slimy layer that comes back after debridement or dressing, with increased exudate formation and evidence of a receding epithelial margin layer. Current studies consider biofilm as an independent factor causing delayed wound healing, Antimicrobial Resistance and wound chronicity antimicrobials. Results from these studies would yield more effective treatment strategies. Effects of biofilm on various phases of wound healing in vitro models have not been fully explained by research. To produce a clear picture of bacteria and immune cell interaction, and to understand in detail how it works. It is however documented that biofilms affect all phases of wound healing directly or indirectly. Biofilm affects all phases of wound healing; however, the most affected stage is the inflammatory phase. (Gajula *et al.,* 2020).

Other studies describe biofilm as a system of single planktonic cells or multicellular aggregates of bacteria with or without surface attachment. Biofilms colonize medical devices such as catheters and implants apart from the wound surface. These bacteria are either single-strain spp or polymicrobial wrapped in a self-produced extracellular polymeric substance (EPS) layer. EPS comprises water, extracellular DNA, exopolysaccharides, and proteins and sometimes includes the hosts, immunoglobulins,

proteins, and DNA. Biofilms possess features such as; antibiotic tolerance and gene expression that constantly change depending on host interactions. The EPS layer is a physical barrier against antibacterial agents and host immune response by blocking and reducing the diffusion of these agents into the biofilm (Versey *et al.,* 2021).

Biofilms have channeled structures that effectively allow for continuous multiplication and colonization of the embedded bacteria. Fully grown and matured strains shed and move from one colony to another. The biofilm formation process begins with reversible attachment by the planktonic bacterial cell to a surface; it then colonizes and becomes irreversibly attached, after which growth and cell division occur. Production of an EPS follows with the formation of water channels and, finally, secondary attachments, DNA adaptations, dispersions and joining of biofilm colonies. Other theories suggest that these bacteria biofilm colonies develop tactile responses that result in phenotypic changes upon encountering a surface. The mechanism of resistance of a biofilm to antimicrobials occurs in three ways. One is the resistance of the biofilm surface, where the antibacterial fail to penetrate the EPS membrane. Two, resistance within the biofilm microenvironment where there is a complex matrix of factors including PH changes, reduced oxygen and accumulating waste materials affecting the efficacy of the antimicrobial agent. Lastly, resistance at the individual strain cell, either by genetic changes or by persister cells (Donlan R.M. 2000). A biofilm is an ecosystem that enables the bacteria and the host to establish different social interactions, such as competition or cooperation (Mihai *et al.,* 2018).

2.4.3 Bacterial susceptibility testing

Susceptibility testing be performed against biofilm organisms to establish an effective treatment strategy against biofilm resistance. It arrives at specific treatments that target specific components of the biofilms, such as the extracellular polymer compounds or structures. Understanding the role of biofilms in infection is crucial in developing such antimicrobials. More studies investigating how biofilms respond to antimicrobial treatment are required to advise developing new effective. The commonly reported wound infections include; surgical site (SSI), acute soft tissue, bite wound, burn wound, and pyogenic wound. Infected wounds are painful, malodorous, and hypersensitive and always lead to discomfort and inconvenience for patients (Akhmetova *et al.,* 2016). Active wound possesses features favoring successful bacterial colonization, proliferation, and infection. They include; warmth, moisture, and nutrients. Wound infections occur during trauma, accident, burn, surgical procedures, and chronic illnesses like diabetes mellitus and leprosy. The Source of infection can be contamination from endogenous sources; for instance, the patient's nasopharynx and gastrointestinal tract, the surrounding skin, and the immediate environment (Bowler *et al.,* 2001). Skin serves as a first-line defence (innate immunity) in the battle against bacteria, however in an open wound, the integrity of the skin is already compromised by the cause giving the bacteria a chance to circumvent the innate immunity and establish an infection (Pallavali *et al.,* 2017). Furthermore, wound infections have resulted in considerable morbidity, mortality, prolonged hospitalization, and escalation of direct and indirect healthcare costs (Siddiqui *et al.,* 2010).

2.4.4 Factors associated with wound infection

Many factors are associated with the patient and the wound accelerating wound infections. Patient-related factors include; comorbidities, old age, drugs or chemical therapeutic compounds, immune disorders, psychosocial factors, hospitalization, smoking, alcohol, and drug abuse. Wound factors include; foreign objects, like catheters, dead tissue debris, ischemia, exudates, wound body location, cross-contamination during clinical procedures, and repeated trauma (Lazarus *et al.,* 1994; Bowler *et al.,* 2001). Moreover, bacterial wound infections can cause systemic complications, such as sepsis, cellulitis, and osteomyelitis, particularly in immunocompromised individuals (Anderson *et al.,* 2007). The impact of wound infections extends beyond physical health, affecting the patient's psychological well-being, quality of life, and ability to perform daily activities. Educating the patients and caregivers on proper wound care techniques, including regular dressing changes and recognizing signs of infection, can also help prevent bacterial wound infections (Negut *et al.,* 2018). Timely identification and management of risk factors, such as diabetes or peripheral vascular disease, to reduce the possibilities of infection.

The rate of wound infections has reduced with antibiotic prophylaxis treatment. However, numerous studies have highlighted the high prevalence of chronic wound infections in Kenya. Some of these studies revealed a high prevalence of 86% and 94% of pathogenic bacteria from wound swabs (Elamenya *et al.,* 2015; Mutonga *et al.,* 2019). Similarly, studies done in Ethiopia and Nigeria showed bacterial isolation rates of 87.3%, 86.1%,70.5%, and 70.0% from wound infection (Azene and Beyene, 2011; Pondei *et al.,* 2013; Mama *et al.,* 2014; Sisay *et al.,* 2019). Furthermore, another study conducted by Chang *et al.,* (2021) in rural Kenya found the rate of chronic wound infections high among

individuals with limited access to healthcare facilities. The study emphasized the urgent need for improved healthcare infrastructure and access to wound care services in rural areas to reduce the burden of chronic wound infections. The study also revealed that these wounds were associated with prolonged hospital stays and increased healthcare costs. Several factors contributed to the high prevalence of chronic wound infections in Kenya. Firstly, inadequate wound care practices, including poor hygiene and improper dressing techniques, contribute to the development and persistence of chronic wound infections. A study by Njoroge *et al*. (2021) identified that a lack of knowledge among healthcare providers and patients about proper wound care management was a significant contributing factor. Additionally, socioeconomic factors such as poverty and limited access to healthcare services aggravate the problem. People living in poverty often face challenges in accessing timely and appropriate wound care, leading to delayed treatment and an increased risk of infection.

Chronic wound infections have a significant impact on healthcare resources in Kenya. The prolonged healing time and recurrent infections associated with chronic wounds increase the demand for healthcare services, including hospitalizations, outpatient visits, and wound care supplies. A study by Chang *et al.,* (2021) estimated that the economic burden of chronic wound infections in Kenya was substantial, leading to increased healthcare costs and decreased productivity.

2.5 Bacteria species prevalence in wound isolates

The most common bacteria involved in wound infections include *Staphylococcus aureus*, *Streptococcus pyogenes,* and *Pseudomonas aeruginosa* (Gardner *et al.,* 2013). They also note inconsistencies in their findings concerning wound bacteria. It is therefore important to highlight the need to describe and differentiate wound bacteria with regard to their

etiology and other pathophysiological mechanisms to determine differences in their prevalence. In this way, the significance of the chronic wound bacteria can be compared and contrasted among different chronic wounds types. Furthermore, studies that take advantage of clinical data, that are taken for the purpose of patient therapy will have the greater potential of revealing links between bacterial variation and chronic wound outcomes especially in longitudinal study designs.

These bacteria are part of the normal skin flora but can cause infections under favorable conditions. Several studies have shown that Methicillin-Resistant *Staphylococcus Aureus* (MRSA) the prevalent bacteria isolated from infected wounds cultures, especially in pus swabs, followed by Methicillin Susceptible *Staphylococcus Aureus* (MSSA) (Pondei *et al.*, 2013; Tsige *et al.*, 2020). wound infections, remain an important concern in the practice and management of surgical procedures. These infections have fur reaching consequences and theses are few examples; prolonged hospitalization, increased healthcare costs, Severe complications, fatalities and generally Low quality of life. Understanding the causes, risk factors, preventive measures, and treatment options that are associated with surgical site infections or post-operative infections is imperative for healthcare providers and patients to effectively tackle the challenge of post infections.

Post-operative infections and SSI occur when microorganisms, such as bacteria, viruses, or fungi, invade the surgical site during or after a surgical procedure. These infections are known as iatrogenic infections and the most common pathogens responsible for these infections include; *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. These pathogens are documented prevalent nosocomial infections but their prevalences differ among many studies (Ongarora. B 2022). These infections enter the

body through various routes such as; airborne transmission, direct contact with contaminated surfaces, or the patient's own skin or mucous membranes considering these infections are found as normal flora on skin surfaces and Gastrointestinal tract. Several factors contribute to the development of wound infections. Surgical wounds are particularly susceptible to infection due to the disruption of the skin, a first line defence against infections, exposing the wound to the external environment harboring sea of microbiome species. In addition, how procedure is done, presence of foreign bodies such as; implants or catheters, and the patient's overall health state and immune function like genetics and other ill-health factors all play important role in determining the risk and severity of the infection. Patient populations that are at a higher risk of developing wound infections include; individuals with weakened immune systems, such as elderly patients, those with chronic illnesses, or patients undergoing prolonged and multiple surgical procedure. Furthermore, patients who are obese, diabetic, or smokers also face an increased risk of wound infections due to impaired wound healing process and compromised blood circulation. (Njoroge *et al.*, 2021)

Preventing wound infections requires a collaborative approach involving many related professions and departments bringing diverse expertise, technology and research. Health care providers must be up to date with the current management patterns and shortcomings and must be able to tailor therapy with respect current techniques such as; surgical procedure technique, adherence to infection control protocols, prophylaxis therapy and optimization of the patient's health status. These measures include; screening patients for pre-existing infections and administer prophylactic antibiotics to reduce the risk of surgical site contamination, maintaining strict aseptic conditions in the operating room such as

proper hand hygiene, sterilization of surgical instruments, and use of sterile drapes and gowns, essential for minimizing the risk of microbial contamination. Caution must be exercised to minimize tissue trauma and avoid unnecessary exposure of internal organs to pathogens. There are advocated techniques such as minimally invasive surgery, which involve smaller incisions and reduced tissue manipulation, which reduce the risk of post-operative wound infections compared to traditional open surgeries. Furthermore, the judicious use of surgical drains, closure methods and dressing promote optimal wound healing and can also contribute to reducing the risk of infection. Wound management is a continuous process and not a one of therapy. Close monitoring of the surgical site for signs of infection, such as redness, swelling, warmth, or is important. In most cases due to cost implications and time, this stage is left to the patient and his or her care givers. This usually a contributor to wound infections especially in developing countries. Early detection and prompt intervention are advocated for preventing the spread of infection and minimizing potential complications, this requires availability of current equipments and personnel. Studies advocate for antibiotic therapy especially in procedure probable for post infection. In such cases, and including where infections do occur, timely administration of appropriate antimicrobial therapy which is based on culture and sensitivity results is essential for controlling the infection and preventing systemic spread which is the essence of this study (Pondei *et al.*, 2013; Tsige *et al.*, 2020).

Surgical site infection (SSI) is one the most common complication that is persistent in patients who underwent surgery in developing countries. The prevalence is even higher in low-income countries. Effective infection prevention activities implemented in all hospitals right from primary care level to national level have proved effective, however

SSI infections are still common. Improving surgical techniques, operating rooms, and providing antimicrobial prophylaxis are practiced mitigation measures. Other methods practiced include decontamination, proper nutrition, preoperative bathing, and decolonization with mupirocin treatment. In Ethiopia, a study found *Staphylococcus aureus* was the most common pathogen identified in SSI, followed by *Escherichia coli, Klebsiella species,* and Coagulase-Negative *Staphylococci* (Birhanu & Endalamaw, 2020). The other strains commonly isolated from chronic wounds include; *Streptococcus epidermidis, Enterococci, Pseudomonas aeruginosa, Escherichia coli* (*E. coli*), and *Streptococcus pyogenes*; they are the prevalent nosocomial infections, usually normal flora on skin surfaces, water, and faeces (Trøstrup *et al.,* 2013). Results of wound swab isolates in Nigeria showed a higher prevalence of Gram-negatives than Gram-positives; *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus species,* and Morganella morganii in decreasing order; were the prevalent strains (Omoyibo *et al.,* 2018).

In Kenya, several studies have focused on characterizing the bacteria strain prevalence in chronic wound infections. These findings underscore the importance of Gram-positive cocci and Gram-negative bacilli as major bacteria in chronic wound infections. Similar studies have also shown Gram-negatives as the predominant isolates than Gram-positives; S. *aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* in decreasing order are the prevalent isolates in diabetic foot ulcers (Mutonga *et al.,* 2019).

Post-operative infections have been the determinants of quality of life after surgery. They are associated with morbidity and mortality, and increased cost to health services around

the world. Prophylaxis therapy has significantly reduced these infections. However, sepsis in modern surgery continues to be a major problem for healthcare practitioners across the globe because of resistance. Patients that are undergoing surgical procedures are at risk of acquiring infections; before surgery, during surgery or after surgery. According to Dinda *et al.,* (2013), SSI is one of the serious causes of complications, constituting 20% of all of health care-associated infections. Similar to findings from other studies done in the region; prolonged hospitalization and dirty wounds were the risks associated with postsurgical infections and she found SSI infection rate at 7% in Agakhan University Hospital, in Nairobi, Kenya. Another study conducted in KNH, Nairobi Kenya, showed *Staphylococcus aureus, Enterobacter spp, Streptococcus faecalis, Streptococcus pyogenes*, and *Pseudomonas spp* as the prevalent SSI bacterial infections (Karimi *et al.*, 2008). Elamenya *et al.* (2015). They also showed *Staphylococcus aureus* and *Pseudomonas aeruginosa* prevalent in surgical wound infections in the pediatric ward. Further research and surveillance are needed to continuously monitor the changing epidemiology of bacteria strains in chronic wound infections in Kenya.

From these studies, no particular organism was specific to; wound type, age, and sex; however, two studies previously done in Nigeria had associated specific bacteria with types of wounds (Otokunefor and Datubo-Brown, 1990; Okesola and Kehinde, 2008). A study by Tipton *et al.,* (2020) describes how patient genetic variation influences the types of bacteria likely to infect an individual and affect wound healing. From these studies, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the prevalent bacterial isolates in wound infections. The fact that they are commonly found on skin as normal microflora makes them the most prevalent. The concern is that they are common in nature,

increasing prevalence, growing resistance to antimicrobials, and the ability to delay wound healing (Pastar *et al.,* 2013). Understanding the prevalence and distribution of bacterial strains associated with chronic wound infections is crucial for defining effective management and targeted therapeutic interventions. The emergence of antibiotic-resistant strains, including MRSA, poses a significant challenge for effective treatment.

2.6 Bacteria isolation methods

The value of infected wound sampling for culture and bacteriological assessments is to determine the cause of infection. Knowing the exact cause will inform subsequent treatment methods and provide long-term solutions to patients and the health care system. The relevant, easy, effective, and less time-consuming microbiological techniques are usually advocated to save time and for quicker specified management of wounds. According to Bowler *et al.,* (2001), culture and simple differential tests like Gram Staining is faster and inexpensive alternative tests worthy of consideration. Gram staining alone does not differentiate individual bacteria but provide two broad category distinction (by cell wall composition); Gram-positive and gram-negative bacteria groups. Diagnosis of bacterial strain infections is a serious problem that requires time, sophisticated equipment, and qualified professionals. The best method for determining bacterial bio-burden and bio-film in chronic wounds is tissue biopsy (Bill *et al.,* 2001). Other techniques, such as needle aspirate of wound fluid and wound swabs, are also preferred in the clinical setting to avoid invasiveness and damage to healing tissue and minimize cost (Gardner & Frantz, 2004). A study by Haalboom *et al.,* (2018) compared bacteria obtained from cultures taken by wound biopsy and wound swabs on the same patients and concluded that the same bacteria were grown.

Bacterial culture isolation methods play a crucial role in microbiology, allowing scientists and healthcare professionals to identify and characterize bacterial pathogens, study their growth patterns, and determine their susceptibility to antibiotics. The common aerobic bacteria isolation and identification process involves the following techniques: specimens are first cultured or inoculated on a suitable medium (5% Blood Agar, MacConkey agar, Deoxycholate Citrate Agar (DCA), or Cysteine Lactose Electrolyte-Deficient agar (CLED)) aerobically for 24 to 48 hours at 37°C and then conventional biochemical tests follows for identification. These tests include; tests for carbohydrate fermentation, methyl red, citric acid utilization, and hydrogen sulfide production. Most researchers omit the identification of anaerobic bacteria because of complicated challenges in the culturing process requiring expensive oxygen-free culture medium and equipment. Anaerobic bacteria are often less detrimental to the wound-healing process. However, they can be in immunocompromised patients (Bowler *et al.,* 2001). However, over the years, various techniques and methodologies have been developed to isolate and culture bacteria from diverse environmental samples, clinical specimens, and research materials. To understand and differentiate these methods as follow (Kowalski *et al.,* 2015);

a) **Conventional Culture Methods:**

Conventional culture methods involve inoculating a sample onto a selective or non-selective growth medium and incubating it under specific conditions to encourage bacterial growth. Selective media contain ingredients that inhibit the growth of certain bacteria while allowing the growth of others, facilitating the isolation of specific bacterial species. Non-selective media support the growth of a wide range of bacteria and are commonly used for the initial isolation of unknown organisms. Agar plates are the most commonly used solid

medium for bacterial culture, providing a solid surface for bacterial growth and colony formation.

b) **Enrichment Culture:**

Enrichment culture involves incubating a sample in a liquid medium that contains nutrients favorable for the growth of target bacteria while suppressing the growth of others. This method is particularly useful for isolating bacteria present in low numbers or those that require specific growth conditions. Enrichment culture can be employed to isolate bacteria from complex environmental samples, such as soil, water, and food, where the target organisms may be present in low abundance.

c) **Automated Culture Systems:**

Automated culture systems utilize advanced technology and robotics to streamline the process of bacterial culture and identification. These systems can accommodate large numbers of samples simultaneously, allowing for high-throughput processing and analysis. Automated culture systems often incorporate various detection methods, including optical, fluorescent, and impedance-based detection, to monitor bacterial growth and identify specific pathogens.

d) **Molecular Techniques:**

Molecular techniques, such as polymerase chain reaction (PCR), nucleic acid sequencing, and DNA microarrays, offer rapid and specific methods for bacterial identification and characterization. PCR-based methods amplify specific regions of bacterial DNA, allowing for the detection and identification of target pathogens with high sensitivity and specificity. Nucleic acid sequencing techniques, such as whole-genome sequencing (WGS), provide

comprehensive information about the genetic composition of bacterial isolates, enabling phylogenetic analysis and epidemiological investigations.

e) **MALDI-TOF Mass Spectrometry:**

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and accurate method for bacterial identification. MALDI-TOF MS analyzes the protein profiles of bacterial isolates and compares them to a reference database to identify the species. This technique has revolutionized bacterial identification in clinical microbiology laboratories, allowing for the rapid and accurate identification of pathogens directly from culture plates.

f) **Microfluidic Systems:**

Microfluidic systems employ miniaturized devices and channels to manipulate and analyze small volumes of liquid samples. These systems offer rapid, automated, and highly sensitive methods for bacterial culture, detection, and characterization. Microfluidic platforms can integrate various functions, including sample preparation, cell lysis, nucleic acid amplification, and detection, onto a single device, enabling point-of-care testing and remote monitoring of bacterial infections.

While these bacterial culture isolation methods have significantly advanced the field of microbiology, they also have certain limitations and challenges. Conventional culture methods can be time-consuming and labor-intensive, requiring skilled personnel and specialized equipment. Enrichment culture may overlook fastidious or non-culturable bacteria, limiting the diversity of isolates obtained. Automated culture systems and molecular techniques require substantial upfront investment and ongoing maintenance costs. Additionally, the accuracy and reliability of molecular and mass spectrometry-based

methods depend on the quality of reference databases and the expertise of laboratory personnel.

In conclusion, bacterial culture isolation methods continue to evolve and diversify, driven by advances in technology, automation, and molecular biology. While each method has its own set of advantages and limitations, their collective use has revolutionized our ability to study and understand bacterial pathogens, diagnose infectious diseases, and develop targeted therapies and interventions. As technology continues to advance, future innovations in bacterial culture isolation methods hold the promise of further enhancing our ability to combat infectious diseases and safeguard public health. According to Noor & Khetarpal (2020), it is necessary to note that conventional biochemical identification tests often produce inconclusive results in situations where specific bacterial species and strains should be isolated and identified. It then calls for sophisticated molecular methods like rRNA and PCR sequencing, which are expensive and scarcely available.

2.7 Antimicrobial susceptibility and resistance

The primary objective of antimicrobial susceptibility testing is to determine the minimum inhibitory concentration (MIC) of an antimicrobial agent required to inhibit the growth of a specific pathogen. The MIC is the lowest concentration of an antimicrobial drug that prevents the visible growth of the organism after overnight incubation. MIC concentration is then determined; by testing the pathogen against several antibiotic concentrations. Several methods are available for conducting antimicrobial susceptibility testing, including disk diffusion, broth dilution, and automated systems. The disk diffusion method involves placing antibiotic disks onto an agar plate inoculated with the test organism. After incubation, inhibition zones around the disks are measured and interpreted according to established guidelines (Bayot & Bragg 2022).

Broth dilution methods involve testing the organism's growth in liquid media containing various concentrations of antibiotics. The lowest concentrations of the drug that inhibits visible bacterial growth are referred to as the MIC (Andrews, J. M. 2001). Automated systems, such as the Vitek and Phoenix systems, use predefined panels of antibiotics and provide rapid and accurate results. Interpretation of antimicrobial sensitivity testing results is based on established breakpoints, which define the susceptibility or resistance of the organism to the tested antibiotic. These breakpoints are determined by regulatory agencies and scientific organizations, such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The susceptibility breakpoints, are usually categorized as; susceptible (S), intermediate (I), and resistant (R). Susceptible organisms respond to the antimicrobial agent, while resistant organisms are not inhibited by achievable drug concentrations. Intermediate susceptibility indicates a potential response to higher drug concentrations or when the drug concentrates in specific body sites.

However, it is crucial to note that antimicrobial susceptibility testing should be interpreted, in conjunction with clinical factors, such as the site of infection, patient characteristics, and pharmacokinetic/pharmacodynamic properties of the antibiotic. Additionally, the emergence of multidrug-resistant organisms, such as methicillin-resistant Staphylococcus aureus (MRSA) and carbapenem-resistant Enterobacteriaceae (CRE), has posed significant challenges in treating infections and highlights the need for ongoing surveillance and research. Antimicrobial sensitivities play a critical role in guiding antibiotic therapy by providing information on the susceptibility of pathogens to specific antimicrobial agents. These tests, performed using standardized methods and interpreted based on established

breakpoints, assist healthcare providers in selecting appropriate antibiotics for the treatment of infectious diseases. Ongoing research and surveillance are essential to combat the emergence of antimicrobial resistance and to ensure the effectiveness of antimicrobial therapy in the future (Vaillant *et al.*, 2022; Bayot & Bragg 2022).

According to Munita & Arias (2016), to understand the mechanisms of Antibiotic Sensitivities, one must understand how bacteria respond to antimicrobials. Antibiotic resistance occurs when bacteria evolve and develop mechanisms to counteract the effects of antibiotics. Various resistance mechanisms have been identified, including;

1. Drug Targets: Antibiotics exert their therapeutic effects by targeting specific bacterial structures or processes. For instance, β-lactam antibiotics like penicillin inhibit cell wall synthesis by binding to penicillin-binding proteins (PBPs). Alterations in PBPs, such as mutations or decreased expression, can lead to reduced antibiotic binding and result in resistance. The identification of drug targets and their susceptibility to antibiotics aids in determining sensitivity.
2. Antibiotic Efflux Pumps: Efflux pumps are membrane proteins responsible for actively removing antibiotics from bacterial cells. These pumps can confer resistance by expelling antibiotics before they reach their intended targets. Mutations or overexpression of efflux pump genes can enhance efflux pump activity, leading to decreased intracellular antibiotic concentrations and reduced sensitivity.
3. Enzymatic Modification: Some bacteria produce enzymes that modify antibiotics, rendering them inactive or less effective. For example, β-lactamases are enzymes that hydrolyze β-lactam antibiotics, including Penicillins and cephalosporins.

Bacteria carrying β-lactamase genes are resistant to these antibiotics. Detection of such enzymes provides critical information on the susceptibility of bacteria to β-lactam agents.

4. Alterations in Antibiotic Uptake: Changes in bacterial membrane permeability or the expression of transport proteins involved in antibiotic uptake can affect sensitivity. Porin proteins, located in the outer membrane of Gram-negative bacteria, regulate the entry of antibiotics. Mutations in porin genes can reduce permeability, limiting antibiotic access and leading to decreased sensitivity.
5. Ribosomal Modifications: Antibiotics such as macrolides and tetracyclines target bacterial ribosomes and inhibit protein synthesis. Alterations in ribosomal components, such as rRNA methylases or mutations in ribosomal protein genes, can confer resistance to these antibiotics. The assessment of ribosomal modifications assists in determining antibiotic sensitivities.

Bayot & Bragg (2022) explained the Clinical Implications of Antibiotic Sensitivities, which is one of the essences of this study. The following are some of these implications;

1. Optimizing Treatment Selection: Knowledge of bacterial sensitivities to different antibiotics allows clinicians to choose the most appropriate therapeutic agent. This information aids in tailoring treatment regimens to maximize efficacy while minimizing the development of resistance. Effective antibiotic stewardship programs rely on accurate and timely antibiotic sensitivity testing.
2. Guiding Empirical Therapy: In situations where immediate antibiotic therapy is required before culture results are available, knowledge of local antibiotic

sensitivities and resistance patterns helps guide empirical treatment decisions. This reduces the risk of inadequate therapy and improves patient outcomes.

3. Preventing Spread of Resistance: Identification of antibiotic sensitivities contributes to infection control practices, such as implementing appropriate isolation measures for patients with drug-resistant bacteria. Prompt recognition and containment of resistant strains help prevent their dissemination within healthcare settings and the community.
4. Monitoring Resistance Patterns: Regular monitoring of antibiotic sensitivities provides valuable data on the prevalence and trends of resistance in specific bacterial populations. This information assists in the development of local and national guidelines for empirical therapy and guides public health interventions to combat antibiotic resistance.

To understand the importance of sensitivity data, we must understand the implication of antimicrobial resistance. The emergence and spread of antibiotic resistance pose a formidable threat to public health, rendering many once-effective antibiotics ineffective against bacterial pathogens. Antibiotic resistance occurs when bacteria evolve mechanisms to withstand the effects of antibiotics, limiting treatment options and increasing the risk of treatment failure, prolonged illness, and mortality. Antibiotic resistance arises through several mechanisms, including genetic mutations, horizontal gene transfer, and selective pressure exerted by antibiotic exposure. Bacteria can acquire resistance genes through mutations in their own genetic material or by acquiring genetic material from other bacteria through processes such as conjugation, transformation, and transduction. Once bacteria develop resistance, they can proliferate and spread rapidly, leading to the emergence of

multidrug-resistant strains that are resistant to multiple classes of antibiotics (Dinda *et al.*, 2013).

Several factors contribute to the emergence and spread of antibiotic resistance, including inappropriate antibiotic use, overprescribing by healthcare providers, misuse of antibiotics in agriculture and livestock farming, poor infection control practices, lack of access to clean water and sanitation, and global travel and trade. Overuse and misuse of antibiotics, including incomplete courses of treatment and inappropriate use for viral infections, contribute to the selective pressure that drives the evolution of resistant bacteria. In settings where antibiotics are readily available over the counter or without a prescription, the risk of inappropriate use and emergence of resistance is particularly high (Sen 2021).

The consequences of antibiotic resistance are far-reaching and profound, affecting individuals, communities, healthcare systems, and economies worldwide. Infections caused by resistant bacteria are associated with higher morbidity and mortality rates, longer hospital stays, increased healthcare costs, and decreased treatment options. Patients with resistant infections are at greater risk of treatment failure, complications, and death, highlighting the urgent need for effective interventions to combat antibiotic resistance. Antibiotic resistance also undermines the effectiveness of critical medical interventions, including surgery, chemotherapy, organ transplantation, and care for premature infants and patients with compromised immune systems. Infections caused by drug-resistant pathogens pose significant challenges for healthcare providers, who must navigate limited treatment options, implement infection control measures, and prevent the spread of resistant strains within healthcare facilities.

Antibiotic resistance is a global health crisis that transcends national borders and affects populations across all continents. The interconnectedness of modern society, facilitated by global travel, trade, and migration, contributes to the rapid spread of resistant bacteria and resistance genes between countries and regions. Developing countries face unique challenges in combating antibiotic resistance, including limited access to healthcare, inadequate infrastructure, weak regulatory frameworks, and high burden of infectious diseases. Addressing antibiotic resistance requires a coordinated and multifaceted approach that encompasses surveillance, research, innovation, education, regulation, and stewardship. Surveillance systems play a critical role in monitoring the prevalence and spread of resistant bacteria, tracking trends in antibiotic use, and informing public health interventions. Research efforts focus on understanding the molecular mechanisms of resistance, developing new antibiotics and alternative therapies, and identifying strategies to preserve the effectiveness of existing antibiotics (Cantón *et al.*, 2002).

Antibiotic stewardship programs promote the judicious use of antibiotics to optimize patient outcomes, minimize the emergence of resistance, and preserve the effectiveness of antibiotics for future generations. These programs involve implementing evidence-based guidelines, conducting antimicrobial susceptibility testing, promoting antimicrobial stewardship education and training for healthcare providers, and engaging patients and families in discussions about antibiotic use and resistance. Infection prevention and control measures are essential for reducing the transmission of resistant bacteria and preventing healthcare-associated infections. These measures include hand hygiene, environmental cleaning, isolation precautions, antimicrobial stewardship, vaccination, and surveillance of healthcare-associated infections. By implementing comprehensive infection prevention

strategies, healthcare facilities can mitigate the risk of transmission of resistant bacteria and protect patients, healthcare workers, and the community (Lin *et al.,* 2020).

Antibiotic resistance represents a complex and multifaceted challenge that requires collective action and global cooperation to address effectively. By raising awareness, promoting responsible antibiotic use, investing in research and innovation, strengthening healthcare systems, and implementing evidence-based interventions, we can mitigate the impact of antibiotic resistance and preserve the effectiveness of antibiotics for current and future generations. The time to act is now, as the consequences of inaction are dire and threaten the foundations of modern medicine and public health. Through collaborative efforts and sustained commitment, we can confront the global threat of antibiotic resistance and safeguard the health and well-being of individuals and communities worldwide (Llor & Bjerrum, 2014).

Developed countries like the United Kingdom, Indonesia, Australia, Belgium, and the Netherlands have not reported microbial resistance to macrolides. However, some countries like Poland, Hong Kong, Italy, Portugal, and Spain have registered high levels of macrolide resistance. Furthermore, Telithromycin resistance is low in Australia, Indonesia, Hungary, Austria, Germany, the Netherlands, Portugal, Sweden, and Switzerland. Regardless of the methicillin susceptibility declining, resistance to linezolid, teicoplanin, or vancomycin was not apparent globally (Cantón *et al.*, 2002).

In Bulgaria, Gram-negative isolates in patients with chronic vascular wounds had significant resistance to the beta-lactam antibiotics (Tzaneva *et al.,* 2016). According to Cantón *et al.,* (2002), Gram-positive anaerobes are sensitive to most antibiotics; However, Gram-negative anaerobes are resistant to ampicillin and cefazolin. *Streptococcus pyogenes*

spp establishes a biofilm in wound infections, which promotes higher mutation rates. It also makes it resistant to antimicrobials, including silver sulphadiazine and the host defence system (Trøstrup et al., 2013).

In Nigeria, several studies registered multiple antimicrobial resistance among wound swab isolates. All the isolates were resistant to cloxacillin, amoxicillin-clavulanate, ampicillin, cloxacillin, cefuroxime, and ceftazidime. Ceftriaxone and imipenem were the only antimicrobials that exhibited Moderate sensitivity. (Pondei *et al.,* 2013; Omoyibo *et al.,* 2018). Similar studies in Ethiopia revealed that all MRSA isolates were 100% resistant to Penicillins and β-lactam antibiotics with high multidrug resistance. Ciprofloxacin and gentamicin were relatively effective in treating wound infections with poly-microbial aetiology (Mama *et al.,* 2014; Sisay *et al.,* 2019; Tsige *et al.,* 2020).

Antimicrobial sensitivity results of studies carried out at Kenyatta National Hospital (KNH) and Moi Teaching and Referral Hospital (MTRH) (Kenya) revealed that MRSA is a multidrug-resistant organism (MDR) (Akoru *et al.,* 2016; Mutonga *et al.,* 2019). Elamenya *et al.* (2015) showed a breakdown of individual bacterial sensitivities against several antibiotics. Only imipenem and ceftriaxone registered minimal activity against all the bacteria. A similar study at Moi Teaching and Referral Hospital (MTRH) (Kenya) revealed that MRSA was only susceptible to Linezolid, Vancomycin and Fucidic acid, which are expensive are rarely available to the patients (Akoru *et al.,* 2016).

Understanding antibiotic sensitivities is crucial for effective management of bacterial infections. The mechanisms underlying antibiotic sensitivities provide insights into the development of resistance and guide treatment decisions. By optimizing treatment selection, guiding empirical therapy, preventing the spread of resistance, and monitoring

resistance patterns, antibiotic sensitivities play a pivotal role in combating the global challenge of antibiotic resistance (AMR).

2.8 Use of antibiotics

The World Health Organization advocates for the rational use of antibiotics, which includes; prescribing antibiotics for the correct indication, duration, timing, and dosages. Inappropriate usage of antibiotics leads to the development of AMR, a phenomenon that has manifested in several studies worldwide (Llor & Bjerrum, 2014; Lin *et al.,* 2020). Inappropriate use involves not following WHO guidelines and hospital-laid SOPs in antimicrobial use. (WHO 2020). Effective use of antibiotics, both in treatment and prophylaxis, has significantly reduced the rate of wound infection and colonization and promoted faster healing (Lin *et al.,* 2020).

Antibiotics have revolutionized modern medicine, saving countless lives by effectively treating bacterial infections. However, the widespread and often indiscriminate use of antibiotics has led to a global crisis of antibiotic resistance. Lin *et al.,* (2020), highlights the importance and the principles of rational use of antibiotics in line with WHO guidelines. Below are some the key values antimicrobial resistance;

1. Preserving Antibiotic Efficacy: The primary goal of rational antibiotic use is to maintain the effectiveness of existing antibiotics. Overuse or misuse of antibiotics can lead to the development of resistance, rendering these life-saving drugs ineffective against bacterial infections. By utilizing antibiotics only when it is necessary, at appropriate doses and durations, we can extend their effectiveness, ensuring that they remain a viable treatment option for years to come.
2. Minimizing Side Effects: Antibiotics are not without risks. They can cause adverse reactions and disrupt the delicate balance of the human microbiome, leading to

conditions such as Clostridium difficile infections. By employing a rational approach, healthcare providers can minimize the unnecessary exposure of patients to antibiotics, reducing the incidence of side effects and improving patient safety.

3. Reducing Healthcare Costs: Inappropriate antibiotic use contributes to escalating healthcare costs. Overuse drives up expenses through longer hospital stays, increased diagnostic tests, and the need for more expensive second-line antibiotics. By adhering to rational prescribing practices, healthcare systems can optimize resource allocation, ensuring that antibiotics are used wisely and cost-effectively

The quality of antimicrobial agents plays a pivotal role in modern medicine, serving as indispensable tools in the prevention and treatment of bacterial, fungal, viral, and parasitic infections. From antibiotics to antifungals and antivirals, these medications have revolutionized healthcare and saved countless lives. However, the effectiveness of antimicrobials hinges not only on their pharmacological properties but also on their quality. Ensuring the quality of antimicrobial agents is imperative to safeguard patient safety, mitigate antimicrobial resistance, and uphold public health standards. This comprehensive analysis explores the various dimensions of antimicrobial quality, including regulatory frameworks, manufacturing standards, quality control measures, and challenges in maintaining quality across different regions and healthcare settings (Lin *et al.,* 2020).

Governments and regulatory agencies worldwide have established stringent frameworks and standards to govern the manufacturing, distribution, and use of antimicrobial agents. In the United States, the Food and Drug Administration (FDA) oversees the approval and regulation of antimicrobial drugs, ensuring that they meet rigorous safety, efficacy, and quality standards before entering the market. Similarly, the European Medicines Agency

(EMA) regulates antimicrobial agents within the European Union, while other countries have their own regulatory bodies tasked with overseeing drug quality and safety.

International organizations such as the World Health Organization (WHO) and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) provide guidelines and recommendations to harmonize regulatory practices and promote global standards for antimicrobial quality. These guidelines cover various aspects of drug development, manufacturing processes, and quality control measures, aiming to facilitate uniformity and consistency in drug regulation across different regions. (DelTacca *et al.,* 2009; Hobeika *et al.,* 2020).

Manufacturing antimicrobial agents involves complex processes that demand adherence to stringent quality standards and good manufacturing practices (GMP). GMP encompasses a set of guidelines and protocols designed to ensure the consistency, purity, and potency of pharmaceutical products throughout the manufacturing process. Key principles of GMP include proper facility design, equipment maintenance, personnel training, raw material sourcing, quality control testing, and documentation practices. Manufacturers of antimicrobial agents are required to adhere to GMP guidelines and undergo regular inspections by regulatory authorities to verify compliance with quality standards. Failure to meet GMP requirements can result in product recalls, regulatory sanctions, and reputational damage for pharmaceutical companies. Therefore, maintaining high standards of manufacturing excellence is essential to uphold the integrity and quality of antimicrobial agents (Hobeika *et al.,* 2020).

Quality control measures play a critical role in verifying the quality, purity, and potency of antimicrobial agents throughout the production process. Analytical techniques such as

high-performance liquid chromatography (HPLC), mass spectrometry, spectroscopy, and microbiological assays are commonly employed to assess the identity, strength, and purity of active pharmaceutical ingredients (APIs) and finished dosage forms. For antimicrobial drugs, microbiological testing is particularly important to evaluate their efficacy against target pathogens and ensure freedom from contamination. Microbiological assays assess factors such as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and susceptibility profiles to ascertain the antimicrobial activity of drugs against specific microbial strains (DelTacca *et al.*, 2009; Ryu and Kim, 2017; Ordonez *et al.,* 2019; Hobeika *et al.,* 2020.).

In addition to laboratory testing, manufacturers implement robust quality assurance protocols to monitor product quality at every stage of the supply chain, from raw material procurement to finished product distribution. This includes rigorous sampling, testing, and validation procedures to detect and address any deviations from established specifications. Despite advancements in regulatory oversight and manufacturing technologies, ensuring the quality of antimicrobial agents remains a complex and multifaceted challenge. Several factors contribute to the complexity of maintaining antimicrobial quality, including:

1. Counterfeit and Substandard Drugs: The proliferation of counterfeit and substandard antimicrobial drugs poses a significant threat to patient safety and public health. Counterfeit medications often contain incorrect dosages, inactive ingredients, or no active ingredient at all, compromising their efficacy and safety. Substandard drugs, on the other hand, may contain insufficient quantities of active ingredients or fail to meet quality specifications, leading to treatment failures and the emergence of drug-resistant pathogens.

2. Global Supply Chain Issues: The global nature of pharmaceutical manufacturing and distribution presents challenges in ensuring the integrity and traceability of antimicrobial agents across diverse supply chains. Raw materials sourced from different regions, outsourcing of manufacturing processes, and complex distribution networks increase the risk of quality lapses and supply disruptions.
3. Antimicrobial Resistance (AMR): The emergence of antimicrobial resistance represents a major public health crisis that threatens the efficacy of existing antimicrobial agents. Misuse, overuse, and inappropriate prescribing practices contribute to the development of drug-resistant pathogens, rendering once-effective treatments ineffective. Addressing AMR requires a multifaceted approach that encompasses antimicrobial stewardship, infection prevention and control measures, and the development of novel antimicrobial agents.

Ensuring the quality of antimicrobial agents is essential to safeguard patient safety, preserve treatment efficacy, and combat the growing threat of antimicrobial resistance. Regulatory frameworks, manufacturing standards, quality control measures, and ongoing surveillance efforts are instrumental in upholding the integrity and reliability of antimicrobial drugs. However, addressing challenges such as counterfeit drugs, global supply chain issues, and antimicrobial resistance requires collaborative efforts from governments, regulatory agencies, healthcare providers, pharmaceutical manufacturers, and other stakeholders. By prioritizing quality assurance and adherence to best practices, the global community can mitigate the risks associated with poor antimicrobial quality and advance the goal of safe and effective antimicrobial therapy for all (Ordonez *et al.,* 2019; Hobeika *et al.,* 2020).

The WHO (2020), emphasized rational use of antibiotics an urgent global health priority. Health care providers have a duty to protect this invaluable resource and ensure that antibiotics remain effective weapons in the fight against bacterial infections. In collaboration with other stake holders, they are required to implement the principles of rational antibiotic use. Some of these principles include (Leekha *et al*., 2011);

1. Accurate Diagnosis: Proper diagnosis is crucial for rational antibiotic use. Healthcare providers should employ evidence-based guidelines and clinical expertise to differentiate between bacterial and viral infections. Antibiotics are ineffective against viral infections, and prescribing them unnecessarily contributes to resistance. Diagnostic tools such as laboratory tests can aid in accurate identification of the causative pathogen, enabling targeted antibiotic therapy.
2. Antibiotic Stewardship Programs: Healthcare facilities should establish robust antibiotic stewardship programs. These programs promote the appropriate use of antibiotics, incorporating strategies such as guidelines, education, and surveillance. By fostering a culture of responsible antibiotic use among healthcare providers, stewardship programs help prevent unnecessary prescriptions, monitor antibiotic resistance patterns, and improve patient outcomes.
3. Individual Responsibility: Patients also play a vital role in the rational use of antibiotics. It is crucial to educate the public about the importance of completing prescribed antibiotic courses and avoiding self-medication. Patients should not demand antibiotics for viral illnesses or use leftover antibiotics from previous treatments. Empowering individuals with knowledge about the risks of antibiotic

resistance can foster responsible behavior and reduce the burden of unnecessary antibiotic consumption.

4. Targeted Therapy: Tailoring antibiotic therapy to specific pathogens is essential in rational antibiotic use. This involves selecting the most appropriate antibiotic based on the susceptibility profile of the causative bacteria. Healthcare providers should consider local antibiotic resistance patterns and update their prescribing practices accordingly. Narrow-spectrum antibiotics should be favored over broad-spectrum antibiotics whenever possible, as they target specific bacteria, minimizing collateral damage to beneficial bacteria.
5. Dose Optimization and Treatment Duration: Administering antibiotics at appropriate doses and for the correct duration is critical. Under-dosing may not effectively eradicate the infection, leading to treatment failure and the development of resistance. Conversely, unnecessarily prolonged courses expose patients to antibiotics for an extended period, increasing the risk of adverse effects and promoting resistance. By optimizing dosing regimens and adhering to evidence-based treatment durations, healthcare providers can strike a balance between efficacy and minimizing resistance development.

Antibiotic selection; is based on its effectiveness to eradicate or prevent the infecting organism with minimal or no harmful effects on the patient. However, combination therapy is currently the advocated treatment measure to combat the challenges of increasing AMR and mutations (Coates *et al.,* 2020). Similarly, in wounds already with established multi-bacterial biofilm infection, broad-spectrum antibiotic combinations are recommended for effective treatment (Bowler *et al.,* 2001). A study on *Klebsiella pneumonia*

carbapenemases (KPC) by Hirsch & Tam (2010) emphasizes combination therapy to treat such infection because it is highly resistant, and its optimal therapy option is unknown. International bodies like the Wound Healing Society (WHS), working groups on diabetic foot ulcers, and chronic wound care groups recommend drug combination therapy (Sen, 2019). These are some of the approved drug combinations: Ampicillin with sulbactam, ticacillin with a clavulanate, amoxicillin with clavulanate, clindamycin with quinolone, second/third-generation cephalosporins with quinolone, and metronidazole with quinolone (Howell-Jones *et al.,* 2005).

Some of the studies done at the Kenyatta National Hospital (Kenya) have demonstrated extensive use of these drugs for prevention and treatment besides other antibiotics: ceftriaxone, cefuroxime, flucloxacillin, gentamicin, ciprofloxacin, ceftazidime, Cloxacillin, anti-tuberculosis, chloramphenicol, erythromycin, and amoxicillin-clavulanate.(Karimi *et al.,* 2008; Elamenya et al., 2015; Mutonga *et al.,* 2019).The prevalence of wound infection and antimicrobial resistance was still high despite the wide use of antibiotics.

2.9 Economic and social burden of chronic wounds

Chronic infected wounds pose a significant economic and social burden on individuals, healthcare systems, and society. These wounds, characterized by delayed healing and persistent infection, have far-reaching implications beyond healthcare. The significant economic and social impacts of chronic infected wounds include; financial costs, reduced quality of life, and the need for effective management strategies. The economic burden of chronic infected wounds is substantial and multifaceted. It encompasses direct healthcare costs, indirect costs associated with lost productivity, and the expenses related to wound care management. The direct costs of treating chronic infected wounds include

hospitalization, surgical interventions, diagnostic tests, and antimicrobial therapies. A study by Rice *et al.* (2014) estimated that the annual cost of managing infected wounds in the United States alone exceeds $20 billion.

Chronic wounds represent a significant public health concern due to their prevalence, impact on quality of life, and economic burden on healthcare systems worldwide expressed by many studies. These wounds, which fail to progress through the normal stages of healing within a predictable timeframe, often result from, infections and underlying health conditions and can lead to serious complications if left untreated. Understanding the public health impact of chronic wounds is crucial for developing effective prevention and management strategies to improve patient outcomes, quality of life and reduce healthcare costs (Nussbaum *et al.*, 2018).

Chronic wounds encompass a diverse array of health conditions, including pressure ulcers, diabetic foot ulcers, venous leg ulcers, and arterial ulcers. While the etiology of these wounds may vary, common underlying factors such as impaired circulation, neuropathy, inflammation, and tissue ischemia contribute to their development and hinder the natural healing process. Additionally, lifestyle factors such as smoking, poor nutrition, and inadequate wound care can exacerbate chronic wounds and impede their resolution. Understanding these conditions and factors separately is the primary goal. This study narrows it to infections. According to epidemiological studies, millions of people worldwide suffer from chronic wounds, with prevalence rates increasing with age and the presence of comorbidities such as diabetes, peripheral vascular disease, and immobility. As the global population ages and rates of chronic diseases continue to rise, the burden of chronic wounds on public health systems is expected to escalate in the coming years.

The impact of chronic wounds extends beyond physical discomfort and impaired mobility, affecting various aspects of patients' lives, including psychological well-being, social interactions, and overall quality of life. Individuals with chronic wounds often experience pain, depression, anxiety, and feelings of isolation, which can significantly diminish their ability to perform daily activities and engage in meaningful relationships. Furthermore, chronic wounds may impose financial hardships on patients and their families due to the costs associated with wound care supplies, medications, and frequent healthcare visits (Chang *et al.,* (2021).

From a public health perspective, the management of chronic wounds poses several challenges including; resource constraints, limited access to specialized wound care services, and gaps in healthcare provider education and training. In many regions, disparities in wound care access and outcomes persist, particularly among underserved populations and those residing in rural or remote areas. Addressing these disparities requires a multifaceted approach that emphasizes early detection, timely intervention, and patient-centered care (Chang *et al.,* (2021).

Preventing chronic wounds is a key priority in public health efforts aimed at reducing the burden of wound-related complications and improving patient outcomes. Strategies for preventing chronic wounds include promoting healthy lifestyle behaviors, such as smoking cessation, regular physical activity, and maintaining a balanced diet to support optimal wound healing. Additionally, early identification and management of risk factors, such as diabetes, peripheral artery disease, and pressure injuries, are essential for preventing the development of chronic wounds in high-risk individuals. In healthcare settings, implementing evidence-based practices for wound assessment, treatment, and prevention

is critical for reducing the incidence of chronic wounds and minimizing their impact on patients and healthcare systems. This includes adopting standardized protocols for wound care, promoting interdisciplinary collaboration among healthcare providers, and leveraging technology and innovation to enhance wound management techniques and improve patient outcomes (Jones *et al.*, 2007).

In addition to direct healthcare costs, chronic infected wounds impose a significant economic burden through productivity losses. Individuals suffering from chronic infected wounds often experience declining work productivity, increased absenteeism, and job loss. This results in reduced income and increased reliance on social welfare programs. A study by Morris *et al.,* (2023) demonstrated that chronically infected wounds led to a 45% reduction in employment among affected individuals, exacerbating the economic impact on individuals and society.

Furthermore, managing chronically infected wounds requires extensive resources, such as wound dressings, specialized equipment, and skilled healthcare professionals. These costs contribute to the overall economic burden, especially considering the long duration often associated with chronic infected wounds. Effective wound care management and prevention strategies are crucial for reducing the economic impact and promoting better outcomes (Jones *et al.*, 2007).

The social burden of chronically infected wounds is equally significant, affecting individuals' quality of life and overall well-being. The physical pain and discomfort experienced by those with infected chronic wounds can lead to a reduced ability to perform daily activities and engage in social interactions. It can result in social isolation, diminished self-esteem, and increased psychological distress.

Individuals with infected chronic wounds often experience a decreased quality of life, leading to restrictions in mobility, limitations in participation in hobbies and recreational activities, and compromised independence. The psychological impact of infected chronic wounds can manifest in depression, anxiety, and a higher risk of developing mental health disorders. A study by Zhu *et al.,* (2022) found that individuals with chronic infected wounds had significantly lower health-related quality of life scores than those without such wounds.

Moreover, infected wounds can lead to stigmatization and societal discrimination. These wounds' visible nature can result in embarrassment, shame, and social exclusion. Furthermore, further, exacerbate the psychological impact and hinder the affected individuals from seeking appropriate medical care and support. Addressing the social burden of infected chronic wounds requires a comprehensive approach that enhances patient well-being, reduces stigma, and promotes community awareness and education.

Effective management strategies are imperative to mitigate the economic and social burden of chronic infected wounds. Prevention measures play a vital role in reducing the incidence of these wounds. Public health campaigns and education programs should emphasize the importance of proper wound care, hygiene practices, and early detection of infections. Timely and appropriately managing acute wounds can prevent their progression to chronicity and reduce the burden on healthcare systems.

Furthermore, healthcare professionals should adopt evidence-based guidelines for wound management, incorporating the principles of wound bed preparation, infection control, and appropriate dressing selection. Multidisciplinary wound care teams, including nurses, physicians, and specialized wound care clinicians, can provide comprehensive care and

optimize patient outcomes. Integrating telemedicine and telehealth technologies can enhance accessibility to wound care expertise, particularly in remote or underserved areas. Investment in research and innovation is crucial for developing advanced wound care products and therapies. These include the development of antimicrobial dressings, bioengineered skin substitutes, and new approaches to wound healing, such as growth factors and stem cell-based therapies. These advancements can reduce healing time, prevent infections, and improve patient outcomes, ultimately alleviating the economic and social burden of chronic infected wounds (Chang *et al.,* (2021).

Chronic infected wounds impose a substantial economic and social burden on individuals, healthcare systems, and society. The costs associated with their management, coupled with the impact on productivity and quality of life, underscore the urgency of addressing this issue. By implementing prevention strategies, adopting evidence-based wound care practices, and investing in research and innovation, healthcare systems can work towards reducing the burden of chronic infected wounds. It is essential to prioritize developing and implementing comprehensive management approaches to improve patient outcomes, enhance quality of life, and alleviate the economic and social consequences of chronic infected wounds (Chang *et al.,* (2021).

In conclusion, chronic wounds represent a significant public health challenge with far-reaching implications for individuals, communities, and healthcare systems worldwide. By raising awareness of the burden of chronic wounds, advocating for equitable access to wound care services, and investing in research and education initiatives, public health efforts can help alleviate the suffering associated with chronic wounds and promote better health outcomes for all. Furthermore, most of the Literature highlighted has demonstrated

that bacteria disrupt the wound-healing process. The effect of untreated wound infection is crucial for individual health, public health, and the whole health system. Additionally, increasing AMR is spotlighted extensively. The resistance patterns of these bacteria vary among regions, antimicrobials used, bacteria species or strain, and infection type (single or multi-organism). Biofilm formation is one among many contributors to AMR. Managing and reducing AMR requires high collaborations and research comprising several disciplines in the health sector. Information synthesized from this literature demonstrates gaps in findings like; the type of wound infection, bacterial strain prevalence, drug resistance, biofilm formation, and integration of technology in management of infected wounds. Therefore, calling for more studies to clarify these observations; to augment the WHO antimicrobial stewardship programs. Apart from the ability to penetrate tissue, low toxicity, and minimal allergic reactions: the choice of antimicrobials should also be made based on their existing sensitivity patterns within the particular geographical area (Zhu *et al.*, (2022).

2.10 Conceptual framework

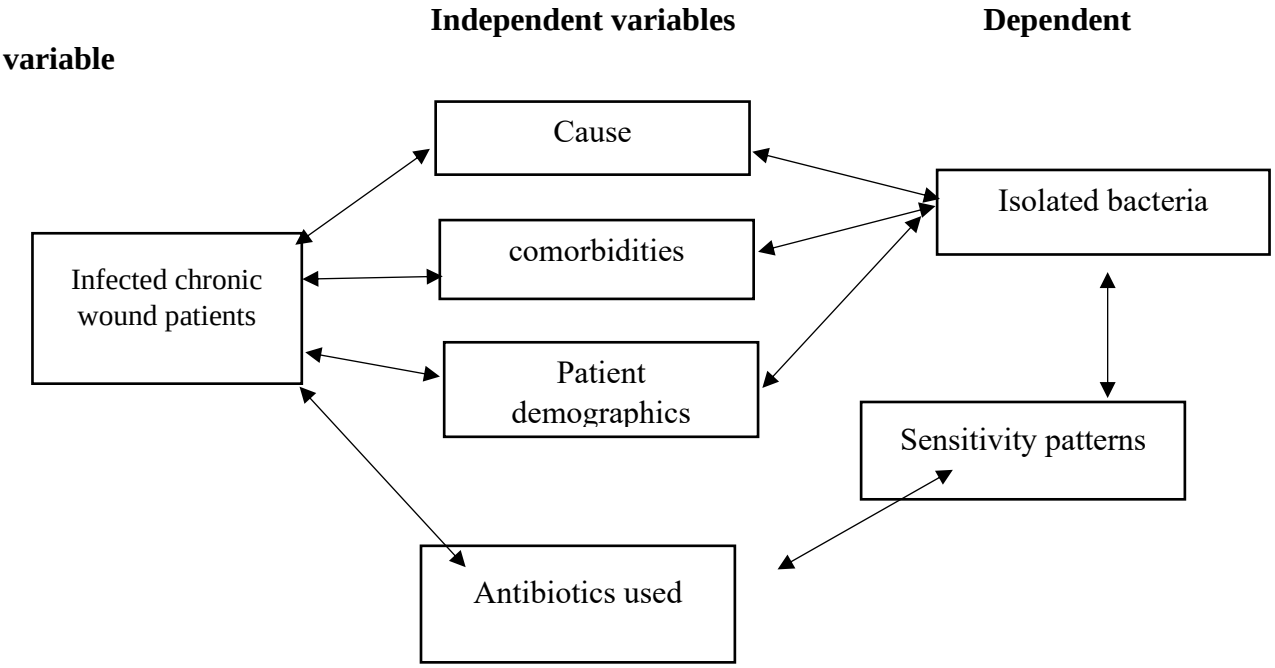

Figure 1: Conceptual framework

CHAPTER THREE

3.0 METHODOLOGY

3.1 Setting

Moi Teaching and Referral Hospital (MTRH), Eldoret Hospital, Reale Hospital, and St. Luke's Hospital in Eldoret Town, Kenya. These Hospitals; have established operational Microbiology Laboratories with qualified Laboratory Technicians who perform wound swab cultures and sensitivity tests. The Eldoret town is the headquarters of the Uasin-Gishu County Government, located west of the Great Rift Valley. It is the 5th most populated urban town in Kenya after Nairobi, Mombasa, Nakuru, and Ruiru.

3.2 Study population

The study population of interest were individuals with severe wound infections, which wound swab culture and sensitivity tests were performed for clinical purposes. Identified by patient file, records and microbiology laboratory data on culture and sensitivity tests.

3. 3 Study design

A cross-sectional study design was used to examine the microbiology laboratory data on culture and sensitivity tests and patient records of chronic wound swab culture and sensitivity test results.

3.4 Sample size determination

The previous studies done in Kenya, at Kenyatta National Hospital (KNH) by Elamenya *et al.,* (2015) and Mutonga *et al.* (2019), which reported prevalence of 86% and 94% respectively of pathogenic bacteria from wound swabs. Taking an average prevalence of 90% from these studies and assuming an absolute/precision error of 5% and 0.05 significance level, the sample size was calculated using the Fischer's formula bellow.

$$n = \frac{Z^2\, p\,(1 - p)}{e^2}$$

Where (e) is the margin of error, (n) is the sample size, (Z) is the standard deviation at a 95% confidence interval, and (P) Proportion of the target population with infected wounds taken from previous studies prevalence.

$$n = \frac{1.96^2 \times 0.90(1-0.90)}{0.05^2}$$

Therefore, the sample size for this study was 138.

3.5 Sampling procedure

The patient files and records that indicated bacterial culture and sensitivities tests done; for clinical purposes were selected by convenience sampling, whereby files were sampled and recorded first in order of the year the tests were conducted. More recently collected and recorded files were the first to be sampled. Sequential sampling of the laboratory data on chronic wound culture and sensitivities, beginning with the most recent data.

The sampling order depended on the number of chronic wound patients these hospitals admitted. Beginning with the one with the highest numbers, the samples obtained were therefore, proportionate to the patient numbers. Therefore, unequal sampling was adopted, provided the samples added up to the overall sample size of 138. Furthermore, recently conducted microbial wound culture and sensitivity data in the selected hospitals were considered a priority.

3.6 Data collection

Each patient record had its data abstraction form with provision for patient demographic, history of antimicrobials used, cause of the wound, comorbidities, and culture sensitivity test results data required for analysis, as shown in appendix1.Bacteria types isolated, antibiotics and sensitivity patterns were the primary variables, while comorbidity, cause of the chronic wound, and notable demographic factors were the secondary variables.

3.7 Pilot work

A pilot study was done by interviewing two qualified laboratory technicians from each of the four Hospitals; using a Questionnaire (Appendix 2). The questions in the questionnaire were meant to ascertain; the validity, reliability, and neutrality of the data. The questionnaire was to help determine if the stipulated microbiological procedures were adhered to during the collection of specimens, storage, transportation, culture, isolation, identification, sensitivity tests, tabulation and interpretation of results.

3.8 Ethical issues and approvals

Moi University/Moi Teaching and Referral Hospital Ethics and Review Board (Institutional Research and Ethics Committee (IREC)) granted ethical clearance for this study (Appendix 3). Patient consent was waived because the study was not dealing with the patients directly. However, approvals from the Hospital management were sought, which required studying patient hospital culture and sensitivity test records and the laboratory data on culture and antimicrobial sensitivities. Patient confidentiality and privacy were maintained at all times by keeping the information and data collected under lock and key and codes used in places of patient names and Hospital record numbers. The raw data collected were archived in retrieval custody under principal investigator (PI). The raw data will be destroyed by shredding and incineration after 5 years.

3.9 Data analysis

The data were analyzed using the statistical software STATA Version 16 with the help of a biostatistician. Simple frequencies and percentage frequencies of the number of chronic wounds among the baseline socio-demographic variables were calculated and tabulated. The bacterial prevalence was also analyzed and expressed in percentage frequencies from

the most to the least prevalent. The susceptibility of individual bacteria types against several antibiotics was tabled and expressed in percentage frequencies from most to least susceptible. For the inferential statistics, Canonical correlation analysis was used to test for the independence between the independent variables (Gender, Age in years, cause of wound and comorbidities) against the dependent set of variables (the number and type of bacteria isolated). The null hypothesis was that the regression coefficients (except for the intercepts) were all equal to zero, equivalent to the null hypothesis that the first set of variables is independent of the second set using the Wilks lambda test statistic for canonical correlation. The P-values that were less than 0.05 were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic features

4.1.1 Sample distribution

The findings from the pilot work assured that study results were valid and with minimal confounding factors. Data Validity was enhanced and ascertained by confirming that qualified and certified professionals performed the above procedures using correct and approved tools. The pilot study also confirmed consistency in all the microbiological processes, which ascertained the validity and reliability of the data. Using the same materials for every specimen collection, storage, and transportation, and at the same time, these specimens were analyzed and interpreted in the same way.

A total of 138 files were analyzed; out of which 104 (75.4%) were retrieved from Moi Teaching and Referral Hospital (MTRH), While 17 (12.3%), 11 (7.9%), and 6 (4.3%) from Eldoret Hospital, St. Luke's Hospital, and Reale Hospital in Uasin Gishu County respectively (Table 4.1.1). The patient files sampled were recorded between 2016 and 2022. The oldest was June 2016, while the latest was April 2022. Out of the total sampled files, 77 (56%) were male while 61 (44%) were female with male to female ratio of (1.2: 0.8) (Table 2).

Table 1: Sample distribution among the four selected hospitals

Variable	Frequency(n)	Percent (%)
Distribution (Hospital)		
Moi Teaching and Referral Hospital	104	75.4%
Eldoret Hospital	17	12.3%
St Luke's Hospital	11	7.9%
Reale Hospital	6	4.3%
Total	138	100%

4.1.2 Age and sex

Their ages ranged from 3 to 81 years, with a mean age of 33.2 years (Standard Deviation; 7.3), and the majority were between the ages of; 30 to 40 years (Table 4.1.2). There was a greater frequency of wound infection in the age group 31 to 40 (22.5%) in both males and females, but there was a high number in males. However, there seems to be a higher number of infections in productive age bracket, as shown in Table 2. From the inferential statistic relations, there was no association between sex and the bacteria isolated (p=0.2709), and no relationship between age in years and the number of bacteria isolated (p=0.2539).

Table 2: Distribution among Age in years and Sex

Variable	Age group in years	Frequency(n)	Percent (%)
1. Age			
	0-10	17	12.3%
	11-20	26	18.8%
	21-30	19	13.8%
	31-40	31	22.5%
	41-50	17	12.3%
	51-60	16	11.6%
	61-70	7	5.1%
	71-80	4	2.9%
	81-90	1	0.7%
		138	100%
2 Sex			
	Male	77	55.8%
	Female	61	44.2%
		138	100%

4.1.4 Cause of wound and comorbidities

Trauma/ accidents and surgical were the majority (36.2% and 23.9% respectively) causes of the wounds, with 65% of them having various comorbidities (Table 4.2.1). Diabetes and hypertension were the leading comorbidities among cancer, anaemia, HIV/AIDS and bone diseases. There was no association between comorbidities and number of bacteria isolated (P=0.3168).

Table 3: Distribution among comorbidities and cause of wound

Variable		Frequency(n)	Percent (%)
4. Comorbidities			
	Yes	90	65.2%
	No	48	34.8%
		138	100%
5. Cause of the Wound			
	Trauma/Accident	50	36.2%
	Surgical	33	23.9%
	Burns	15	10.9%
	Bed sores	23	16.7%
	Lesion	11	8.0%
	Bite	6	4.3%
		138	100%

4.1.5 Prevalence of bacteria types isolated

Following the criteria for sample collection, all the samples yielded a significant bacterial growth indicative of wound infections. Six samples (4%) indicated polymicrobial wound infections whereby more than one bacterium was co-isolated. Two of these were *Staphylococcus aureus*/*Enterococcus faecalis*, two *Staphylococcus aureus*/*pseudomonas aeruginosa*, one *Staphylococcus aureus*/*Proteus mirabilis*, and one *Pseudomonas aeruginosa*/*Escherichia coli*/*Staphylococcus aureus* infections. The total number of bacteria isolated from the wound swabs were 144 with an arithmetic mean of 8. Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* being the most prevalent at 29.2% and 20.1%, respectively. Table 4.3.1 summarizes the prevalence of these bacteria strains from the most to the least.

Table 4: Bacterial strain prevalence

Strain	**Frequency (n)**	**Percent (%)**
Staphylococcus aureus	42	29.2%
Escherichia coli	29	20.1%
Enterococcus faecalis	12	8.3%
Pseudomonas aeruginosa	11	7.6%
Acinetobacter baumanii	10	6.9%
Proteus mirabilis	9	6.3%
Streptococcus pyogenes	7	4.9%
Klebsiela pneumoniae	5	3.5%
Staphylococcus epidermidis	4	2.8%
Enterobacter cloacae	3	2.1%
Enterococcus gallinarum	2	1.4%
Proteus vulgaris	2	1.4%
Enterococcus avium	2	1.4%
Staphylococcus warneri	1	0.7%
Staphylococcus hominis	1	0.7%
Citrobacter koseri	1	0.7%
Morganella morganii	1	0.7%
Staphylococcus intermedius	1	0.7%
Acinetobacter lwofii	1	0.7%
	144	100%

4.1.6 Distribution of the bacteria strain isolates and cause of the wounds

The isolated strains are mixture of both Gram-positive and Gram-negative, however, majority are Gram-negative pathogens. Trauma/Accidents were the leading cause of chronic wounds (36.2%) (Table 4.2.1), with the highest number of organisms isolated (54 (37.5%)), followed by surgical, bed sores, burns, lesions and bites respectively. (Table 4.4.1). There was no dependence between the cause of the wound and the number of bacteria isolated (p=0.1504).

Table 5: The number and species of bacteria strains isolated against the cause of the wounds

	Cause						
Bacterial strain	**Trauma/ Accident**	**Surgical**	**Burns**	**Bed sores**	**Lesion**	**Bite**	**Total**
Staphylococcus aureus	13	8	6	6	5	4	42
Escherichia coli	16	7	1	4	1	0	29
Enterococcus faecalis	3	4	2	3	0	0	12
Pseudomonas aeruginosa	6	0	1	1	3	0	11
Acinetobacter baumanii	6	4	0	0	0	0	10
Proteus mirabilis	0	0	3	5	0	1	9
Streptococcus pyogenes	4	0	1	1	1	0	7
Klebsiela pneumoniae	3	2	0	0	0	0	5
Staphylococcus epidermidis	0	0	0	1	3	0	4
Enterobacter cloacae	2	1	0	0	0	0	3
Enterococcus gallinarum	0	2	0	0	0	0	2
Proteus vulgaris	0	2	0	0	0	0	2
Enterococcus avium	0	2	0	0	0	0	2
Staphylococcus warneri	0	0	1	0	0	0	1
Staphylococcus hominis	0	0	0	1	0	0	1
Citrobacter koseri	0	0	0	1	0	0	1
Morganella morganii	0	1	0	0	0	0	1
Staphylococcus intermedius	0	0	0	0	0	1	1
Acinetobacter lwofii	1	0	0	0	0	0	1
Total	**54**	**33**	**15**	**23**	**13**	**6**	**144**

4.1.7Antibiotics used to manage chronic wounds in the selected hospitals

The antibiotics prescribed to treat wound infections, are summarized in Table 4.5.2, from the most prescribed to the least. Ceftriaxone was the most prescribed parenteral antibiotic, while clindamycin was the most prescribed oral antibiotic. Ceftriaxone was the leading prescribed antibiotic in each of the selected hospitals. Tigecycline was the only antibiotic used in one hospital; Moi Teaching and Referral hospital among the four selected hospitals.

Most of these antimicrobials, were prescribed at empiric stage before the specific therapy. From the 138 files sampled, 13 (9.4%) did not indicate antibiotics used, while 125 (90.6%) showed extensive antibiotics prescribed. The highest number of antibiotics per patient was four (Table 4.5.1).

Table 6: Number of antibiotics prescribed per patient

Number of antibiotics per patient	**Frequency (n)**	**Percent (%)**
1	27	19.6%
2	38	27.5%
3	41	29.7%
4	19	13.8%
Not indicated	13	9.4%
	138	100%

Table 7: Prevalence of antibiotics prescribed

Antimicrobial	Frequency (n)	Percent (%)
Ceftriaxone	53	16.4%
Clindamycin	31	9.6%
Metronidazole	29	9.0%
Flucloxacillin	26	8.0%
Meropenem	25	7.8%
Amoxicillin/clavulanic	21	6.5%
Azithromycin	19	5.9%
Cefuroxime	16	5.0%
Ciprofloxacin	16	5.0%
Linezolid	15	4.6%
Nitrofurantoin	14	4.3%
Cefazolin	14	4.3%
Piperacillin/tazobactam	11	3.4%
Amikacin	9	2.8%
Vancomycin	7	2.2%
Levofloxacin	7	2.2%
Cefepime	5	1.5%
Ampicillin	3	0.9%
Tigecycline	2	0.6%
	323	100%

4.1.8 Bacterial susceptibility patterns and response

The sensitivity data obtained from the four Hospitals; were conducted on the different generic antibiotic brands available in these Hospitals. Susceptibilities of only 19 antimicrobial agents against the isolated strains; were obtained, as summarized in Tables 4.6.1 to 4.6.6 below.

Table 8: Antibiotic susceptibility patterns for S. aureus, E. coli, E. faecalis, P. aeruginosa, A. baumanii and P. mirabilis against; Ceftriaxone, Clindamycin, Metronidazole, Flucloxacillin, Meropenem, Amoxicillin/clavulanic, Azithromycin, Cefuroxime and Ciprofloxacin

Strain	Pattern	Antibiotics								
		CRO	CLN	MTZ	FLX	MEM	AMC	AZM	CFX	CIP
S. aureus	S	6(14%)	13(31%)	0(0%)	0 (0%)	16(38%)	2(5%)	8(19%)	11(26%)	11(26%)
	I	3(7%)	8(19%)	0(0%)	5(12%)	9(21%)	3(7%)	2(5%)	9(21%)	9(21%)
	R	33(79%)	21(50%)	42(100%)	37(88%)	17(41%)	37(88%)	32(76%)	22(53%)	22(53%)
E. coli	S	2 (7%)	4(14%)		0(0%)	19(66%)	7(24%)		2(7%)	17(59%)
	I	9(31%)	4(14%)		2(7%)	5(17%)	2(7%)		8(27%)	0(0%)
	R	18(62%)	21(72%)		27(93%)	5(17%)	20(69%)		19(66%)	12(41%)
E. faecalis	S	0 (0%)	0(0%)	0(0%)	2(17%)	2(17%)	3(25%)	2(17%)	0(0%)	4(33%)
	I	2(17%)	1(8%)	0(0%)	3(25%)	4(33%)	4(33%)	1(8%)	0(0%)	0(0%)
	R	10(83%)	11(92%)	12(100%)	7(58%)	6(50%)	5(42%)	9(75%)	12(100%)	8(67%)
P. aeruginosa	S	3(27%)	2(18%)		0(0%)	7(64%)	0(0%)	0(0%)	0(0%)	6(55%)
	I	0(0%)	0(0%)		0(0%)	2(18%)	3(27%)	0(0%)	0(0%)	0(0%)
	R	8(73%)	9(82%)		11(100%)	2(18%)	8(73%)	11(100%)	11(100%)	5(45%)
A. baumanii	S	0(0%)			0(0%)	1(10%)	0(0%)		0(0%)	2(20%)
	I	1(10%)			0(0%)	1(10%)	0(0%)		0(0%)	0(0%)
	R	9(90%)			10(100%)	8(80%)	10(100%)		10(100%)	8(80%)
P. mirabilis	S	2(22%)	1(11%)			7(78%)	0(0%)	5(56%)	4(44%)	4(44%)
	I	2(22%)	2(22%)			0(0%)	1(11%)	0(0%)	1(11%)	0(0%)
	R	5(56%)	6(67%)			2(22%)	8(89%)	4(44%)	4(44%)	5(56%)

S-Sensitive; I-Intermediate; R- Resistant; CRO- Ceftriaxone; CLN- Clindamycin; MTZ-Metronidazole; FLX-Flucloxacillin; MEM-Meropenem; AMC- Amoxicillin/clavulanic; AZM-Azithromycin; CFX-Cefuroxime; CIP-Ciprofloxacin.

Table 9: Antibiotic susceptibility patterns for S. pyogenes, K. pneumoniae, S. epidermidis, E. cloacae, E. gallinarum and P. vulgaris against; Ceftriaxone, Clindamycin, Metronidazole, Flucloxacillin, Meropenem, Amoxicillin/clavulanic, Azithromycin, Cefuroxime and Ciprofloxacin

Strain	Pattern	Antibiotics								
		CRO	CLN	MTZ	FLX	MEM	AMC	AZM	CFX	CIP
S. pyogenes	**S**	3(43%)	2(29%)		4(57%)	5(71%)	3(43%)	1(14%)	3(43%)	5(71%)
	I	0(0%)	1(14%)		2(29%)	0(0%)	1(14%)	1(14%)	2(29%)	0(0%)
	R	4(57%)	4(57%)		1(14%)	2(29%)	3(43%)	5(71%)	2(29%)	2(29%)
K. pneumoniae	**S**	2(40%)	1(20%)			0(0%)	0(0%)	1(20%)	2(40%)	2(40%)
	I	0(0%)	1(20%)			1(20%)	0(0%)	0(0%)	1(20%)	1(20%)
	R	3(60%)	3(60%)			4(80%)	5(100%)	4(80%)	2(40%)	2(40%)
S. epidermidis	**S**	0(0%)	1(25%)		0 (0%)	2(50%)	0(0%)	1(25%)	1(25%)	2(50%)
	I	1(25%)	1(25%)		0(0%)	1(25%)	1(25%)	1(25%)	1(25%)	1(25%)
	R	3(75%)	2(50%)		4(100%)	1(25%)	3(75%)	2(50%)	2(50%)	1(25%)
E. cloacae	**S**	0(0%)	0(0%)		0 (0%)	1(33%)				0(0%)
	I	0(0%)	0(0%)		1(33%)	0(0%)				1(33%)
	R	3(100%)	3(100%)		2(67%)	2(67%)				2(67%)
E. gallinarum	**S**	0(0%)	0(0%)		0(0%)	1(50%)	0(0%)	0(0%)	0(0%)	0(0%)
	I	0(0%)	0(0%)		0(0%)	0(0%)	1(50%)	0(0%)	0(0%)	0(0%)
	R	2(100%)	2(100%)		2(100%)	1(50%)	1(50%)	2(100%)	2(100%)	2(100%)
p. vulgaris	**S**	0(0%)	0(0%)			0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	I	0(0%)	0(0%)			1(50%)	0(0%)	0(0%)	0(0%)	0(0%)
	R	2(100%)	2(100%)			1(50%)	2(100%)	2(100%)	2(100%)	2(100%)

S-Sensitive; I-Intermediate; R- Resistant; CRO- Ceftriaxone; CLN- Clindamycin; MTZ-Metronidazole; FLX-Flucloxacillin; MEM-Meropenem; AMC- Amoxicillin/clavulanic; AZM-Azithromycin; CFX-Cefuroxime; CIP-Ciprofloxacin.

Table 10:Antibiotic susceptibility patterns for E. avium, S. warneri, S. hominis, C. koseri, M. morganii, S. intermedius and A. lwofii against; Ceftriaxone, Clindamycin, Metronidazole, Flucloxacillin, Meropenem, Amoxicillin/clavulanic, Azithromycin, Cefuroxime and Ciprofloxacin

Strain	Pattern	Antibiotics								
		CRO	CLN	MTZ	FLX	MEM	AMC	AZM	CFX	CIP
E. avium	**S**	0(0%)	0(0%)		0(0%)	2(100%)	1(50%)	0(0%)	0(0%)	0(0%)
	I	0(0%)	0(0%)		0(0%)	0(0%)	1(50%)	0(0%)	0(0%)	0(0%)
	R	2(100%)	2(100%)		2(100%)	0(0%)	0(0%)	2(100%)	2(100%)	2(100%)
S. warneri	**S**	0 (0%)	0 (0%)		0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	I	0(0%)	0(0%)		0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)
	R	1(100%)	1(100%)		1(100%)	0(0%)	1(100%)	1(100%)	1(100%)	0(0%)
S. hominis	**S**	0(0%)	0(0%)		0(0%)	1(100%)	0(0%)		0(0%)	0(0%)
	I	0(0%)	0(0%)		0(0%)	0(0%)	0(0%)		0(0%)	0(0%)
	R	1(100%)	1(100%)		1(100%)	0(0%)	1(100%)		1(100%)	1(100%)
C. koseri	**S**	0(0%)	0(0%)		0(0%)	1(100%)	0(0%)	0(0%)	0(0%)	0(0%)
	I	0(0%)	0(0%)		0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	R	1(100%)	1(100%)		1(100%)	0(0%)	1(100%)	1(100%)	1(100%)	1(100%)
M. morganii	**S**	0(0%)	0(0%)			0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	I	1(100%)	0(0%)			1(100%)	0(0%)	0(0%)	0(0%)	1(100%)
	R	0(0%)	1(100%)			0(0%)	1(100%)	1(100%)	1(100%)	0(0%)
S. intermedius	**S**	0(0%)	0(0%)		0(0%)	0(0%)	0(0%)		0(0%)	0(0%)
	I	0(0%)	0(0%)		0(0%)	1(100%)	0(0%)		0(0%)	1(100%)
	R	1(100%)	1(100%)		1(100%)	0(0%)	1(100%)		1(100%)	0(0%)
A. lwofii	**S**	0(0%)	0(0%)			0(0%)	0(0%)		0(0%)	0(0%)
	I	0(0%)	0(0%)			0(0%)	0(0%)		0(0%)	1(100%)
	R	1(100%)	1(100%)			1(100%)	1(100%)		1(100%)	0(0%)

S-Sensitive; I-Intermediate; R- Resistant; CRO- Ceftriaxone; CLN- Clindamycin; MTZ-Metronidazole; FLX-Flucloxacillin; MEM-Meropenem; AMC- Amoxicillin/clavulanic; AZM-Azithromycin; CFX-Cefuroxime; CIP-Ciprofloxacin.

Table 11: Antibiotic susceptibility patterns for S. aureus, E. coli, E. faecalis, P. aeruginosa, A. baumanii and P. mirabilis against; Linezolid, Nitrofurantoin, Cefazolin, Piperacillin/tazobactam, Amikacin, Vancomycin, Levofloxacin, Cefepime, Ampicillin and Tigecyline

Strain	Pattern	Antibiotics									
		LZD	NFN	CZO	PIP	AMK	VAN	LVX	CPM	AMP	TGL
S. aureus	S	40(94%)	42(100%)	0(0%)	9(21%)	39(92%)	39(93%)	0(0%)	16(38%)	0(0%)	40(95%)
	I	1(3%)	0(0%)	0(0%)	12(29%)	2(5%)	3(7%)	0(0%)	9(21%)	0(0%)	2(5%)
	R	1(3%)	0(0%)	42(100%)	21(50%)	1(3%)	0(0%)	42(100%)	17(41%)	42(100%)	0(0%)
E. coli	S		24(83%)		5(17%)		29(100%)	9(31%)	5(17%)	0(0%)	
	I		5(17%)		6(21%)		0(0%)	3(10%)	0(0%)	0(0%)	
	R		0(0%)		18(62%)		0(0%)	17(59%)	24(83%)	29(100%)	
E. faecalis	S	11(92%)	12(100%)	1(8%)	0(0%)	0(0%)	7(58%)	5(42%)	2(17%)	7(58%)	12(100%)
	I	0(0%)	0(0%)	2(17%)	3(25%)	6(50%)	4(33%)	2(17%)	4(33%)	3(25%)	0(0%)
	R	1(8%)	0(0%)	9(75%)	9(75%)	6(50%)	1(8%)	5(42%)	6(50%)	2(17%)	0(0%)
P. aeruginosa	S		2(18%)	4(36%)	4(36%)	10(91%)	3(27%)	3(27%)	5(45%)	0(0%)	
	I		2(18%)	0(0%)	1(9%)	0(0%)	2(18%)	2(18%)	3(27%)	0(0%)	
	R		7(64%)	7(64%)	6(55%)	1(9%)	6(55%)	6(55%)	3(27%)	11(100%)	
A. baumanii	S				0(0%)	1(10%)	5(50%)		5(50%)	1(10%)	
	I				0(0%)	3(30%)	0(0%)		1(10%)	0(0%)	
	R				11(100%)	6(60%)	5(50%)		4(40%)	9(90%)	
P. mirabilis	S	6(67%)	5(56%)	0(0%)	4(44%)	5(56%)	1(11%)	3(33%)	1(11%)	0(0%)	
	I	1(11%)	0(0%)	0(0%)	0(0%)	1(11%)	3(33%)	0(0%)	1(11%)	2(22%)	
	R	2(22%)	4(44%)	9(100%)	5(56%)	3(33%)	5(56%)	6(67%)	7(78%)	7(78%)	

S-Sensitive; I-Intermediate; R- Resistant; LZD- Linezolid; NFN- Nitrofurantoin; CZO- Cefazolin; PIP- Piperacillin/tazobactam; AMK- Amikacin; VAN- Vancomycin; LVX- Levofloxacin; CPM- Cefepime; AMP- Ampicillin; TGL- Tigecycline.

Table 12: Antibiotic susceptibility patterns for S. pyogenes, K. pneumoniae, S. epidermidis, E. cloacae, E. gallinarum and P. vulgaris against; Linezolid, Nitrofurantoin, Cefazolin, Piperacillin/tazobactam, Amikacin, Vancomycin, Levofloxacin, Cefepime, Ampicillin and Tigecycline

Strain	Pattern	Antibiotics									
		LZD	NFN	CZO	PIP	AMK	VAN	LVX	CPM	AMP	TGL
S. pyogenes	S	6(86 %)	5(71 %)	3(43 %)	4(57 %)	3(43 %)	7(100 %)	5(71 %)	4(57 %)	3(43 %)	
	I	1(14 %)	1(14 %)	1(14 %)	2(29 %)	2(29 %)	0(7%)	2(29 %)	0(0%)	0(0%)	
	R	0(0%)	1(14 %)	3(43 %)	1(14 %)	2(29 %)	0(0%)	0(0%)	3(43 %)	4(57 %)	
K. pneumoniae	S		0(0%)	0(0%)	1(20 %)	1(20 %)		3(60 %)	0(0%)	0(0%)	5(100 %)
	I		2(40 %)	0(0%)	1(20 %)	2(40 %)		1(20 %)	2(40 %)	1(20 %)	0(0%)
	R		3(60 %)	5(100 %)	3(60 %)	2(40 %)		1(20 %)	3(60 %)	4(80 %)	0(0%)
S. epidermidis	S	4(100 %)		0(0%)	1(25 %)		3(75 %)	2(50 %)		0(0%)	3(75 %)
	I	0(0%)		1(25 %)	0(0%)		1(25 %)	1(25 %)		0(0%)	1(25 %)
	R	0(0%)		3(75 %)	3(75 %)		0(0%)	1(25 %)		4(100 %)	0(0%)
E. cloacae	S					1(33 %)		1(33 %)	1(33 %)	0(0%)	2(67 %)
	I					1(33 %)		0(0%)	0(0%)	0(0%)	1(33 %)
	R					1(33 %)		2(67 %)	2(67 %)	3(100 %)	0(0%)
E. gallinarum	S	2(67 %)		0(0%)		0(0%)	0(0%)	0(0%)		0(0%)	
	I	1(33 %)		0(0%)		0(0%)	0(0%)	0(0%)		1(50 %)	
	R	0(0%)		2(100 %)		2(100 %)	2(100 %)	2(100 %)		1(50 %)	
p. vulgaris	S	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	
	I	1(50 %)	1(50 %)	0(0%)	0(0%)	1(50 %)	0(0%)	0(0%)	1(50 %)	0(0%)	
	R	1(50 %)	1(50 %)	2(100 %)	2(100 %)	1(50 %)	2(100 %)	2(100 %)	1(50 %)	2(100 %)	

S-Sensitive; I-Intermediate; R- Resistant; LZD- Linezolid; NFN- Nitrofurantoin; CZO- Cefazolin; PIP- Piperacillin/tazobactam; AMK- Amikacin; VAN- Vancomycin; LVX- Levofloxacin; CPM- Cefepime; AMP- Ampicillin; TGL- Tigecycline.

Table 13: Antibiotic susceptibility patterns for E. avium, S. warneri, S. hominis, C. koseri, M. morganii, S. intermedius and A. lwofii against; Linezolid, Nitrofurantoin, Cefazolin, Piperacillin/tazobactam, Amikacin, Vancomycin, Levofloxacin, Cefepime, Ampicillin and Tigecycline

Strain	Pattern	Antibiotics									
		LZD	NFN	CZO	PIP	AMK	VAN	LVX	CPM	AMP	TGL
E. avium	**S**	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	0(0%)	0(0%)	
	I	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	1(50%)	0(0%)	
	R	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)	0(0%)	1(50%)	2(100%)	
S. warneri	**S**	1(100%)		0(0%)			0(0%)	0(0%)		0(0%)	
	I	0(0%)		0(0%)			1(100%)	0(0%)		0(0%)	
	R	0(0%)		1(100%)			0(0%)	1(100%)		1(100%)	
S. hominis	**S**	1(100%)		0(0%)			1(100%)	0(0%)		1(100%)	
	I	0(0%)		0(0%)			0(0%)	0(0%)		0(0%)	
	R	0(0%)		1(100%)			0(0%)	1(100%)		0(0%)	
C. koseri	**S**		0(0%)	0(0%)		0(0%)				0(0%)	
	I		1(100%)	0(0%)		1(100%)				0(0%)	
	R		0(0%)	1(100%)		0(0%)				1(100%)	
M. morganii	**S**		0(0%)	0(0%)	0(0%)	1(100%)		0(0%)	0(0%)	0(0%)	
	I		1(100%)	0(0%)	0(0%)	0(0%)		1(100%)	0(0%)	0(0%)	
	R		0(0%)	1(100%)	1(100%)	0(0%)		0(0%)	1(100%)	1(100%)	
S. intermedius	**S**	1(100%)		0(0%)			1(100%)	1(100%)		0(0%)	
	I	0(0%)		1(100%)			0(0%)	0(0%)		0(0%)	
	R	0(0%)		0(0%)			0(0%)	0(0%)		1(100%)	
A. lwofii	**S**				1(100%)	0(0%)		1(100%)	0(0%)	1(100%)	
	I				0(0%)	1(100%)		0(0%)	1(100%)	0(0%)	
	R				0(0%)	0(0%)		0(0%)	0(0%)	0(0%)	

S-Sensitive; I-Intermediate; R- Resistant; LZD- Linezolid; NFN- Nitrofurantoin; CZO- Cefazolin; PIP- Piperacillin/tazobactam; AMK- Amikacin; VAN- Vancomycin; LVX- Levofloxacin; CPM- Cefepime; AMP- Ampicillin; TGL- Tigecycline.

4.1.9.Susceptibilities of different bacterial strains to different antibiotics

4.1.9.1 Staphylococcus aureus

The 42 isolates of *Staphylococcus aureus* were 100% susceptible to Nitrofurantoin. There were high susceptibilities of; 95%, 94%, 93% and 92% with Tigecycline, Linezolid, Vancomycin and Amikacin, respectively. There was absolute resistance to Metronidazole, Cefazolin, Levofloxacin and Ampicillin. Notable resistance of; 88%, 88%, 79%, 76%, and 63% with Amoxicillin/clavulanate, Flucloxacillin, Ceftriaxone, Azithromycin, and Cefuroxime, respectively. High resistance to methicillin antibiotics describes the presence of MRSA strains.

4.1.9.2 Escherichia coli

Escherichia coli isolates were only susceptible to Vancomycin. High susceptibilities were also exhibited with Nitrofurantoin (88%) and Meropenem (66%). Out of the 29 isolates: 29(100%), 27(93%), 24(83%), 21(72%), and 20(69%) were resistant to; Ampicillin, Flucloxacillin, Cefepime, Clindamycin, and Amoxicillin/clavulanic, respectively.

4.1.9.3 Enterococcus faecalis

Of all the antibiotics tested, *Enterococcus faecalis* was highly susceptible to Tigecycline, Nitrofurantoin and linezolid. Out of the 12 isolates, 7(58%) were susceptible to Vancomycin and Ampicillin. All the 12 isolates were resistant to Cefuroxime and Metronidazole.

4.1.9.4 Pseudomonas aeruginosa

There were 11 *Pseudomonas aeruginosa* isolates, 10(91%) were susceptible to Amikacin and 7(64%) to Meropenem. Flucloxacillin, Azithromycin, Cefuroxime, and Ampicillin showed absolute resistance, followed by Clindamycin (82%), and Ceftriaxone and Amoxicillin/clavulanic (73%).

4.1.9.5 Acinetobacter *baumanii*

Out of the 10 *Acinetobacter baumanii* isolates, 70% were susceptible to Tigecycline followed by Cefepime and Levofloxacin at 50%. There was absolute resistance with; Flucloxacillin, Amoxicillin/clavulanic, and Cefuroxime, followed by Ceftriaxone and Ampicillin at 90%, Ciprofloxacin and Meropenem at 80% and Piperacillin Tazobactam at 70%.

4.1.9.6 *Proteus mirabilis*

The highest susceptibility of *Proteus mirabilis was* seen with Meropenem at 78%, followed by Linezolid at 67%, and Nitrofurantoin, Amikacin, and Azithromycin at 56%. Highest resistance to; Cefazolin (100%), Amoxicillin/clavulanic (89%), Cefepime, and Ampicillin at 78%.

4.1.9.7 *Streptococcus pyogenes*

Streptococcus pyogenes isolates were only susceptible to Vancomycin. High susceptibilities were also seen with; Linezolid (86%), Nitrofurantoin, Meropenem, Ciprofloxacin, and Levofloxacin (71%). Out of the 7 isolates, highest resistance was only seen with Azithromycin (71%), followed by Ceftriaxone, Clindamycin, and Ampicillin at 57%.

4.1.9.8 Klebsiela pneumoniae

The five *Klebsiela pneumoniae* isolates were susceptible to Tigecycline at 100%, followed by Levofloxacin at 60%. There was absolute resistance to Cefazolin and Amoxicillin/clavulanic. High resistance to; Ampicillin, Meropenem and Azithromycin at 80%, followed by Ceftriaxone, Clindamycin, Nitrofurantoin and Piperacillin Tazobactam at 60%.

4.1.9.9 Staphylococcus epidermidis

Staphylococcus epidermidis was 100% susceptible to Linezolid, followed by Tigecycline and Vancomycin at 75%. All *Staphylococcus epidermidis* isolates were resistant to Flucloxacillin and Ampicillin. Ceftriaxone, Amoxicillin/clavulanic, Cefazolin and Piperacillin Tazobactam also registered high resistance of 75%.

4.1.9.10 *Enterobacter cloacae*

Only 2 (67%) of the three isolates of *Enterobacter cloacae* were susceptible to Tigecycline. All the isolates were 100% resistant to; Ceftriaxone, Clindamycin, and Ampicillin, while 67% were resistant to; Flucloxacillin, Meropenem, Ciprofloxacin, and Ampicillin.

4.1.9.11 *Enterococcus gallinarum*

The two isolates of *Enterococcus gallinarum* were 100% resistant to; Ceftriaxone, Clindamycin, Flucloxacillin, Azithromycin, Cefuroxime, Ciprofloxacin, Cefazolin,

Amikacin, Vancomycin, and Levofloxacin. One (50%), was susceptible to Meropenem and Linezolid. Amoxicillin/clavulanic and Ampicillin expressed intermediate sensitivities of 50%. All the isolates

4.1.9.12 *Proteus vulgaris*

The two isolates of *Proteus vulgaris,* did not show any susceptibility to any of the antibiotics tested. Intermediate sensitivities were observed with Meropenem, Linezolid, Nitrofurantoin, Amikacin, and Cefepime. All the isolates were 100% resistant to: Ceftriaxone, Clindamycin, Amoxicillin clavulanic, Azithromycin, Cefuroxime, Ciprofloxacin, Cefazolin, Piperacillin/tazobactam, Vancomycin, Ampicillin, and Levofloxacin.

4.1.9.13 *Enterococcus avium*

Enterococcus avium isolates were all susceptible to Meropenem, Nitrofurantoin and linezolid. It showed intermediate sensitivity to Piperacillin/tazobactam, but the two isolates were 100% resistant to; Vancomycin, Ampicillin, Cefuroxime, Ceftriaxone, Clindamycin, Azithromycin, Ciprofloxacin, Cefazolin, and Amikacin.

4.1.9.14 *Staphylococcus warneri*

The *Staphylococcus warneri* isolate was only susceptible to Linezolid from the antibiotics tested. Intermediate to Meropenem, Ciprofloxacin and Vancomycin. While absolute resistance was seen with; Flucloxacillin, Ampicillin, Clindamycin, Ceftriaxone, Amoxicillin/Clavulanic, Azithromycin, Cefuroxime, Cefazolin, and Levofloxacin.

4.1.9.15 *Staphylococcus hominis*

Staphylococcus hominis isolate was found susceptible to; Meropenem, Vancomycin, linezolid, and Ampicillin. It was 100% resistant to Flucloxacillin, Clindamycin, Ceftriaxone, Amoxicillin/clavulanic, Cefuroxime, Ciprofloxacin, Cefazolin, and Levofloxacin.

4.1.9.16 *Citrobacter koseri*

The one isolate of *Citrobacter koseri* was only susceptible to Meropenem and intermediate to Nitrofurantoin and Amikacin. However, it was resistant to: Ceftriaxone, Clindamycin, Flucloxacillin, Amoxicillin/clavulanic, Amoxicillin clavulanic, Azithromycin, Cefuroxime, Ciprofloxacin, Cefazolin, and Ampicillin.

4.1.9.17 *Morganella morganii*

Morganella morganii isolate was only susceptible to Amikacin and intermediate to; Meropenem, Ceftriaxone, Ciprofloxacin, Nitrofurantoin, and Levofloxacin. 100% resistance was observed with the following antibiotics: Clindamycin, Flucloxacillin, Amoxicillin/clavulanic, Azithromycin, Cefuroxime, Cefepime, Cefazolin, Ampicillin and Piperacillin/tazobactam.

4.1.9.18 Staphylococcus intermedius

Staphylococcus intermedius isolate expressed susceptibility to Linezolid Vancomycin and Levofloxacin among all the antibiotics tested. Intermediate to; Meropenem, Ciprofloxacin, and Cefazolin. However, the following antibiotics were 100% resistant: Flucloxacillin, Clindamycin, Ceftriaxone, Ampicillin, Amoxicillin/Clavulanic, and Cefuroxime.

4.1.9.19 *Acinetobacter lwofii*

The one isolate of *Acinetobacter lwofii* was susceptible to Piperacillin/tazobactam, Levofloxacin, and Ampicillin. Intermediate to; Ciprofloxacin, Amikacin and Cefepime. While 100% resistant to Ceftriaxone, Meropenem, Flucloxacillin, Amoxicillin clavulanic, Amoxicillin/clavulanic, and Cefuroxime.

CHAPTER FIVE

5.0 DISCUSSION AND LIMITATIONS

5.1 Discussion

5.1.1 Demographics

The highest prevalence of bacterial wound contamination resulting from Trauma/accidents and surgical wounds; is suggestive of open wound contaminations before or in the hospital setting. There was no significant association between the number of bacteria isolated and the comorbidities, however all six patients that exhibited polymicrobial bacterial infections had underlying health conditions. These results agree with the facts from previous studies, linking chronic wounds to comorbidities and polymicrobial infections (Sen, 2021).

Several studies have shown that chronic wounds are affected by bacteria at a community level or the hospital level, hindering their healing process (Omoyibo *et al.,* 2018; Kadam *et al.,* 2019). In this study, these pathogens are suspected to be nosocomial, non-nosocomial and possible iatrogenic wound infections. However, nosocomial were the majority, with *Staphylococcus aureus* as the predominant strain and *Escherichia coli* as an emerging nosocomial strain. The data obtained suggest high infections in male subjects between the ages; of 0-10 years and 31-40 years than in females. The number of infected wounds was high between ages 30-40 years, even though there was no significant association between age in years, gender, and incidence of wound infection, consistent with the observation of a similar retrospective study by Pondei *et al.,* (2013).

This study has shown low polymicrobial isolates (4%), similar to prospective studies done by Omoyibo *et al.,* (2018) and Upreti *et al.,* (2018). However, review articles done by; Bowler et al. (2001), Versey *et al.,* (2021) and a retrospective meta-analysis study by Sisay

et al., (2019) reveal a higher percentage of polymicrobial isolates than single-strain isolates. This difference, could be because of the specimen collection technique, culturing techniques or regional microbial load difference due to geographical and patient characteristics. Specimen collection techniques have been the subject of controversy regarding the diversity of microorganisms obtained. Some studies have argued that, by use of molecular methods rather than culture, a higher number of bacteria will be identified (Rhoads *et al.,* 2012). Versey *et al.,* (2021) argues that, even though wound swabs are preferred culture collection methods because of their non-invasiveness and can be performed many times for longitudinal studies compared to tissue biopsies, it collects low biomass and cannot capture the diversity of bacteria in deeper tissues. However, Travis *et al.,* (2020) compared bacteria isolates sampled by swabs and tissue biopsy from diabetic foot ulcers (DFU) using RNA sequencing technique and found no significant difference in the overall bacterial count of pathogenic species isolated. In addition, Haalboom *et al.,* (2018) also confirm that swabs and tissue biopsies produce similar culture results when taken from the same wound. These studies recommend a non-inversive swab technique that follows the Levine technique to collect the culture samples.

5.1.2 Bacterial species prevalence

Gram-positive *Staphylococcus spp* bacteria were the most common pathogens isolated. It is the abundant in human skin and mucous membranes as normal flora in humans. It was also common in all polymicrobial infections, and arguably MRSA strains, because of their absolute resistance to Beta-Lactam antibiotics (Tsige *et al.,* 2020). *E. Coli* strains have shown high prevalence in this study and it is considered an emerging nosocomial strain. *E. Coli* is a normal flora pathogen in human Gastrointestinal tract (GI).

The prevalent bacterial strains obtained from this study correlate with the results of prospective studies done in Kenyatta Hospital (KNH) (Elamenya *et al.,* 2015; Mutonga *et al.,* 2019) but differ from the findings of a similar retrospective study done in Nigeria by Pondei *et al.* (2013), and other prospective studies by (Pallavali *et al.,* 2017; Omoyibo *et al.,* 2018) which showed *Pseudomonas aeruginosa* as a predominant strain. Mutonga *et al.,* (2019) revealed a high prevalence of nosocomial infections, which agrees with the finding of this study. The most difficult nosocomial bacteria to manage in hospitals is *Pseudomonas aeruginosa* (Oladeinde *et al.,* 2013)

5.1.3Antimicrobial used to manage chronic wound infections

The Results of this study have shown that 90.6% of the patients were on antibiotics, both prophylaxis and treatment. Similar to other studies conducted in Kenya (Karimi *et al.,* 2008; Elamenya *et al.,* 2015; Mutonga *et al.,* 2019), the data obtained; indicated that many patients were on prescribed antibiotic therapy. In this study, some view files revealed that the patients had taken over-the-counter (OTC) antibiotics before seeking hospital therapy. It is evident from this study that the antimicrobials used where generics and easily available in the market. This augmented by the culture and sensitivity tests, which showed generic antibiotics were tested. Studies have shown a tremendous improvement in managing chronic wounds in Kenya (Ongarora, 2022). However, gaps still exist that require research, for example, the quality of generic antibiotics in the market and increasing antimicrobial resistance (AMR) today.

In wound management, the most desired antibiotic is the one that augments the wound-healing process and able to break through biofilm barrier. There are many concerns and questions about the efficacy and quality of generic antibiotics. The innovator brand enjoys

a patency period of 20 years before anyone else is allowed to produce a replica drug (generic) into the market, according to U.S. Food and Drug Administration (FDA). Generic drugs must have the same quality, safety, efficacy and effectiveness aspects as the innovator brand. Generics should contain the same active ingredient to produce similar clinical outcomes before they are allowed into the market. However, the generic manufacturers are not required to have or repeat clinical trials for approval, unlike the innovator. The common question is, why are the generic brands cheaper than the innovator if they should produce the same clinical outcomes? Generics compete through costs for the market share. For drug costs to reduce significantly, concerns and questions about quality, safety, and efficacy arise. It is, therefore, an area which more research must be devoted because it is one of the reasons for antimicrobial resistance (AMR) (Desai *et al.,* 2019).

Desai *et al.,* (2019) substantiated that authorized generics (AG) are only effective if they match the innovator in terms of; safety, quality, and efficacy dictated by factors like; active and inactive ingredients, manufacturing processes, packaging and storage, packaging materials, clarity and level of particulate matter, and many others that must meet the standards of the US FDA. A study in India by Pathak *et al.,* (2017) confirmed that generic Amoxicillin capsules were not bioequivalent to branded Amoxicillin capsules by measuring their Minimum Inhibitory Concentrations (MIC). There are also concerns about reliance on clinical signs and symptoms in disease prognosis by doctors, pharmacists, and patients, contributing to the increase in the over-the-counter (OTC) purchase of antibiotics, which in turn adds to the bigger problem of AMR. Serena *et al.,* (2022) confirmed that reliance on clinical signs and symptoms in assessing infected chronic wounds would lead to the haphazard use of antibiotics.

Other studies (DelTacca *et al.,* 2009; Ryu and Kim, 2017; Ordonez *et al.,* 2019; Hobeika *et al.,* 2020) have raised concerns about the safety, effectiveness, and therapeutic equivalence of generic antibiotics sold OTC and in Hospitals. Notwithstanding the extensive use of antibiotics, the prevalence of infection remains high. The following are contributing factors to this phenomenon; poor choice of antibiotics, misuse and overuse of antimicrobials and non-compliance to dose adherence by the patients leading to high bacterial resistance (Llor & Bjerrum, 2014; Lin *et al.,* 2020).

5.1.4 Susceptibilities of the antibiotics used to manage chronic wound infections

In-vitro antibiotic susceptibilities of the isolated strains through minimum inhibitory concentrations (MIC), the Kirby Bauer disc diffusion method showed significant antibiotic resistance. Most isolates manifested multiple drug resistance, with less than half showing susceptibilities of above 50% of the antibiotics tested. More than 50% of the isolates are 100% resistant to commonly used antibiotics; Flucloxacillin, Cefuroxime, Amoxicillin, cefazolin, and Ampicillin, a cause for concern.

The susceptibility pattern of *Staphylococcus aureus* against; Cefazolin, Ampicillin, Levofloxacin, Metronidazole, Ceftriaxone, Azithromycin, Flucloxacillin, and Amoxicillin/clavulanic acid are similar to those of Akoru *et al.,* (2016) and Mutonga *et al.,* (2019). It shows that this strain has developed resistant proteins that lower its affinity to Beta-lactam antibiotics. Sensitivity patterns of other *Staphylococcus spp* were similar to *S. aureus.* However, they are less predominant compared to *S. aureus.*

Escherichia coli showed a similar resistance pattern to those found by Elamenya *et al.,* (2015). However, in this study sensitivity of *E. coli* to fluoroquinolones is decreasing. *E. coli* being human intestinal normal flora, the frequency of exposure to antibiotics or

reduced affinity of existing Penicillin Binding Proteins (PBP) through the development of other insensitive proteins are among factors that could have contributed to its resistance pattern.

The sensitivities of *Enterococcus spp* were similar to the findings of Elamenya *et al.*, (2015), which showed high sensitivities to penicillin antibiotics. However, in this study, *E. faecalis* was resistant to; Metronidazole, Cefuroxime, Clindamycin, Ceftriaxone, Cefazolin, and Piperacillin/tazobactam. All the *Enterococcus* strains were sensitive to Nitrofurantoin, Meropenem, Vancomycin, and Linezolid and are known to be a common cause of SSI in humans.

Some studies (Pondei *et al.,* 2013; Pallavali *et al.*, 2017; Omoyibo *et al.*, 2018) have shown *P. aeruginosa* as the prevalent strain in infected wounds, unlike this study. *P. aeruginosa* is a multidrug-resistant strain (MDR), according to Mama *et al.,* (2014) and Pallavali *et al.*, (2017), which agrees with the findings of this study. Overuse and misuse; of Quinolones, Cephalosporins, and Penicillins could be one of many factors that contribute to the high and increasing resistance of *P. aeruginosa*. Amikacin and Meropenem showed some positive susceptibility towards *P. aeruginosa*.

A. baumanii was the predominant *Acinetobacter spp* and responsible for most Acinetobacter infections. It is one of the emerging multidrug-resistant strains in nosocomial diseases. It was only susceptible to Tigecycline and resistant to beta-lactam antibiotics. High resistance to commonly used Penicillins suggests that *A. baumanii* produces beta-lactamase enzyme, and its capability of forming a biofilm colony makes some of its virulent factors. *A. lwoffii,* a rare and less common human skin's normal

flora; the one isolate could have been a result of cross-contamination during specimen collection. Unlike *A. baumanii, A. lwoffii* was less resistant to beta-lactam antibiotics.

*Proteus spp p*redominant strain was *P. mirabilis,* responsible for more than 90% of proteus infections (Elamenya *et al.,* 2015). *P. vulgaris* is an opportunistic infection in humans; found in soil, water, and faecal matter. Unlike other studies that showed high susceptibility of *P. mirabilis* to Amoxicillin/clavulanic acid, Ceftriaxone, and Imipenem (Karimi *et al.,* 2008; Elamenya *et al.,* 2015), this study shows high resistance of *Proteus spp* and Urease-producing non-lactose fermenter *Morganella morganii* (normal flora in human, animal, and reptilian intestines) to beta-lactam antibiotics. Similarly, Karimi *et al.,* (2008) and Elamenya *et al.,* (2015) also demonstrated high sensitivities of *S. pyogenes* to Amoxicillin/clavulanic acid, contrary to the findings of this study. The reasons for this geographical difference may be related to differences in several factors including, patient characteristics, source of infection, weather and the environment, specimen types, prior antibiotic use, and regulation of over-the-counter antibiotics.

Klebsiella pneumoniae was the only strain of *Klebsiella spp* isolated. It is one of the emerging nosocomial pathogens, a normal flora in the skin, mouth and intestines (Patilaya *et al.,* 2019). The sensitivity pattern of *Klebsiella pneumoniae* was similar to those found by Patilaya *et al.,* (2019) and Jiang *et al.,* (2020) but different from other studies that have illustrated high susceptibility to beta-lactam antibiotics (Pondei et al., 2013; Elamenya *et al.,* 2015). Continuous exposure and overuse of these antibiotics could be among the factors that explain the difference in resistance because it was susceptible to Tigecycline which is not commonly available and less prescribed.

Other Important resistant strains that were also isolated were the peritrichous flagellated *Enterobacter cloacae* and *Citrobacter koseri,* members of normal flora in the human gut and emerging nosocomial pathogens. They were resistant to Beta-lactams and Macrolides, which agrees with the results of Davin-Regli and Pages (2015), and they also associate the resistance of *E. cloacae* with the production of constitutive AmpC β-lactamase enzymes.

5.1.5 Antimicrobial resistance (AMR) and public health

Resistance of Hospital-Acquired Infections (HAIs), evident from this study, pose a significant threat to patient safety leading to increased morbidity, mortality, and healthcare costs. Infection prevention and control (IPC) programs play a crucial role in mitigating the spread of infections within healthcare settings. Magill *et al.,* (2014), provide importance of IPC in mitigating the threats of AMR and Biofilms. These infections contribute to patient morbidity and mortality, prolong hospital stays, increase healthcare costs, and pose a significant challenge to the delivery of quality healthcare. Infection prevention and control (IPC) programs are critical in reducing the transmission of infections within healthcare settings.

A multidisciplinary approach involving healthcare professionals from various disciplines is essential for effective IPC. Infection prevention and control committees should be established within healthcare facilities, comprising representatives from nursing, medicine, microbiology, epidemiology, pharmacy, and environmental services. This multidisciplinary collaboration ensures the development and implementation of comprehensive IPC policies and guidelines based on the latest evidence and best practices.

Robust surveillance systems are vital for early identification, monitoring, and prevention of HAIs. Surveillance enables the detection of trends, outbreak identification, and evaluation of the effectiveness of IPC interventions. The National Healthcare Safety Network (NHSN) in the United States and similar systems in other countries provide standardized surveillance protocols for tracking HAIs. Accurate and timely data collection allows healthcare facilities to implement targeted interventions and measure their impact on reducing infections.

Hand hygiene is recognized as the single most important measure in preventing the transmission of healthcare-associated pathogens. Healthcare workers must perform hand hygiene before and after patient contact, after exposure to bodily fluids, and after touching patient surroundings. Alcohol-based hand sanitizers are preferred for routine hand hygiene, while soap and water are necessary when hands are visibly soiled or contaminated. Ongoing education and training programs, along with easy access to hand hygiene facilities, promote compliance among healthcare workers.

Environmental surfaces can harbor pathogens and contribute to the spread of infections. Effective environmental cleaning and disinfection are crucial components of IPC programs. Regular cleaning of high-touch surfaces, such as bedrails, doorknobs, and bedside tables, using appropriate disinfectants, reduces the risk of transmission. Additionally, adherence to proper cleaning techniques for medical equipment and instruments is essential to prevent cross-contamination.

Antimicrobial stewardship programs (ASPs) are integral to IPC efforts, aiming to optimize antimicrobial use, reduce resistance, and minimize the occurrence of HAIs. ASPs involve guidelines for appropriate antibiotic prescribing, monitoring of antibiotic use, and

education of healthcare providers. By implementing ASPs, healthcare facilities can promote the judicious use of antimicrobials, reducing the risk of infections caused by multidrug-resistant organisms.

Continuous education and training are vital components of IPC programs. Healthcare workers should receive regular training on hand hygiene practices, proper use of personal protective equipment (PPE), and adherence to standard precautions. Educational initiatives should also address the importance of IPC practices among patients and visitors to encourage their active participation in infection prevention efforts.

Hospital infection prevention and control is of paramount importance to ensure patient safety and minimize the burden of HAIs. A comprehensive IPC program, encompassing a multidisciplinary approach, robust surveillance systems, hand hygiene, environmental cleaning, antimicrobial stewardship, and education and training, can effectively reduce the transmission of infections within healthcare facilities. By prioritizing infection prevention and control measures, healthcare systems can provide safer environments for patients and enhance the overall quality of care. The results from this study provides an overview that, hospital infection prevention and control programs still require improvement; highlighting key strategies, interventions, and best practices. The importance of a multidisciplinary approach, surveillance systems, hand hygiene, environmental cleaning, and antimicrobial stewardship are emphasized. By implementing comprehensive IPC measures, healthcare facilities can effectively reduce the incidence of HAIs, AMR and promote patient safety.

5.2 Study Limitations

From the pilot survey, this study could not control possible confounders that could have resulted during specimen collection and analysis of the raw data and the brands of the antibiotics used. Therefore, these isolated cases below are the limitations highlighted.

1. The culture and sensitivity tests of some of the antimicrobials were not available in all of the four selected Health facilities.
2. Not all of the isolated strains were tested against all the antibiotics used in all of the four selected Health facilities.
3. Could not control confounders arising from specimen collection and culture process, like normal flora contamination and culture of TB organism, as the primary data was collected by different person not privy to this study.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study showed that chronic wounds are colonized and infected with bacteria mostly from endogenous and exogenous sources, consequently affecting the wound healing processes. The cause of the wound has no relation to the type and number of bacteria isolated. However, Trauma/ Accident wounds, proved to be the leading infected wounds because they were the most prevalent. Patients who had pre-existing health conditions were at higher risk of acquiring multiple wound infections because of their weakened immune system. In addition, difficult-to-treat nosocomial strains were the majority of the bacteria isolated, a sign that many chronic wound patients overstayed in the hospital facility primarily because of the infected chronic wound and other comorbidities.

We conclude that the bacterial strain and species prevalence is related to individual strain resistance and virulent factors. Availability as human normal flora also added to their prevalence. *Staphylococcus aureus* was the most prevalent pathogen due to its high virulent and resistant factors, one being the ability to neutralize antibiotic enzymatic activity. Gram-positive *Staphylococcus aureus* displayed characteristics of MRSA strain from its resistance patterns to beta lactam antibiotics, due to its beta-lactamase enzyme. *Escherichia coli* strain in this study was an unlikely pathogen to be among most prevalent strain. *E. coli* proved an emerging nosocomial bacteria strain, a Gram-negative, oxidase-negative facultative anaerobe *commonly* found in human gastrointestinal tract. *E. coli was* not prevalent in previous similar studies, however, being major human normal flora, human contamination of wound could have contributed to its prevalence. *P. aeruginosa* was the third prevalent strain because of mutations it has acquired after a prolonged patient stay in the hospital environment and frequent exposure to antibiotics. *P. aeruginosa* is a known and documented Hospital Acquired Infection (HAI), suggestive that most of its infections occurred in the Hospital facility and in this case, it has found its way has one of common pathogen in wound infection.

It was also evident that beta-lactams were the leading most prescribed antibiotics to manage chronic wound infections. From the antibiotic names registered in the patient file, most of those antibiotics were generic brands, that were easily available in the region. The quality, efficacy of these antimicrobials used is subject under question. From this study and several other studies, the quality of antimicrobials is paramount especially the generic brands and should match the original brand in terms of efficacy and safety.

The multiple drug resistance experienced by the *coccus spp* is because of their high affinity to form a biofilm. This is confirmed by the high number of the *coccus spp* isolates registered in this study. All the strains isolated showed a multi-drug resistance, including the widely used cephalosporins (Ceftriaxone, Cefazoline, and Cefepime) and Penicillins (Amoxicillin/clavulanate, Flucloxacillin, Piperacillin/tazobactam, and Ampicillin). However, a limited class of antibiotics such as; Glycylcycline (Tigecycline), Glycopeptides (Vancomycin), Oxazolidones (Linezolid), Macrodantin (Nitrofurantoin), and Aminoglycoside (Amikacin) that were hardly available and prescribed showed high susceptibilities to the isolated strains. This is explained that there was no effective implementation of rational use of antibiotics, while managing these chronic wound infections.

Policymakers, healthcare providers, and individuals must collaborate to implement and promote rational antibiotic use, ensuring a sustainable future where antibiotics remain effective in combating bacterial infections. We can slow down the emergence of antibiotic resistance, extend the lifespan of existing antibiotics, and safeguard the future of healthcare. It is also important to note that, quality of generic antibiotics in the market play a major role in effective management of infected chronic wounds, as well as Hospital IPC programs.

6.2 Recommendations

1. Based on the findings of this study, majority of the bacterial strains are resistant to penicillin antibiotics. Therefore, when managing chronic infected wounds Penicillins are not recommended, however it should be given based on the available culture and sensitivity test.

2. Creation of awareness and advice on health policies at primary health level is recommended. From the findings of this study a number of infections were hospital acquired. This calls for health facilities adopt guidelines and policies that work to prevent or minimize wound infections at the hospital facility. For example; Health facilities should have established temporal local antibiogram data closer to patient specificity to improve strict adherence to IPC in the Hospital facilities. Develop rigorous infection control guidelines that maintain an aseptic environment in the Hospital facilities to minimize the spread of virulent nosocomial pathogens infecting wounds.
3. Due to increasing mixed infections and Multi-Drug Resistant (MDR) strains, as evidenced from finding of this study, when managing chronic infected wound empirically where definitive therapy is a challenge, antibiotic combination therapy is recommended based on the established localized antibiogram. This will enhance antibiotic effectiveness and quality of health.
4. It is also evident from the finding of this study that the quality of antibiotics used is questionable. We recommend that Hospitals should formulate and adopt health policies that confirm off-patent (generic) antibiotics to merge the originator brand; in terms of quality, safety, and efficacy before authorizing usage in the facility. With the aid of sensitivity data and studies conducted on quality, efficacy, and safety of antibiotic generic brands in the market, Health facilities should update and improve policies and SOPs on the use of antibiotics to minimize AMR and improved patient-hospital costs.

5. The findings of this study also depicted usage of antibiotics over the counter (OTC) or without prescriptions. We recommend strict patient guidelines that ensure rational use of antibiotics and stewardship such that; there is strict adherence to dose, duration, timing and correct amounts to minimize misuse and overuse. Ministry of health to adopt policies that prohibit the sale of counterfeit antibiotics, and OTC antibiotics which encourage antimicrobial resistance.

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APPENDICES

APPENDIX 1: DATA ABSTRACTION FORM

Baseline characteristics

Patient File Code

(Initials)……Date…….

1. Gender---------- Male ☐ Female ☐

2. Age in years-----☐

Age group 0-18☐ 18-35☐ above 35☐

4. Ward……

5. What is the cause of the wound? Surgical ☐ Burns ☐ Bites (insect, animal or snake) ☐ Accident ☐

Others (specify)……

6. Other co-morbidities------No ☐Yes☐

If above is yes specify……..

7. What is the type of the chronic wound?

a) surgical ☐

b) ulcers ☐ (arterial, diabetic, trauma, venous, pressure)

Others

(specify)……

……

8. Antibiotics prescribed

……..

…….

1. IDENTIFIED BACTERIA

Table 2.1: (a) Gram positive bacteria

Bacteria Isolate	**(Tick where applicable)**
Staphylococcus spp	
Streptococcus pyogenes	
Enterococcus	
Acinetobacter baumanii	
Alcaligenes spp	
Listeria spp	
Bacillus spp	
Corynebacteria spp	
Others (list)	

Table 2.2: (b) Gram negative bacteria

Bacteria Isolate	**(tick where applicable)**
Pseudomonas aeruginosa	
Escherichia coli	
Klebsiella spp	
Proteus spp	

Chlamydia spp	
Yersinia spp	
Haemophilus spp	
Salmonella spp	
Enterobateriaceae spp	
Others(list)	

2. ANTIBIOTIC SENSITIVITY

Tale 2.3: Sensitivity pattern

Bacteria……

Antibiotic	**Resistant**	**Intermediate**	**Sensitive**
Amoxicillin-Clavulanate			
Cefuroxime			
Ceftriaxone			
Imipenem			
Ciprofloxacin			
Cefoxitin			
Cloxacillin			
Ceftazidime			
Ofloxacin			
Cefazolin			
Azithromycin			
Levofloxacin			

Doxycycline			
Metronidazole			
Clindamycin			
Others(list)			

Boaz Cheruiyot

Principal investigator

APPENDIX 2: PILOT STUDY QUESTIONNAIRE

Validity and reliability assessment

RESPONDENT NO: ☐ ONE ☐ TWO

HOSPITAL…….

(Tick mark for the right answer/s in the box)

QUESTIONS

Q1. How often are culture and sensitivity test conducted in the hospital?

☐ Daily

☐ Weekly

☐ Monthly

☐ Rarely

Q2. How often are chronic wound swab culture and sensitivity tests done?

☐ Daily

☐ Weekly

☐ Monthly

☐ Rarely

Q3. Who collects wound swab culture specimen?

☐ Investigating health practitioner

☐ Qualified laboratory technician

☐ Other (mention)……

Q4. Are there requirements and standard procedures provided for wound swab specimen collection?

☐ Yes

☐ No

Q5. Specimens are always confirmed to have been collected, stored and transported in the standard conventional procedures before culture?

☐ Yes

☐ No (Reasons)…….

Q6. List the microbial culture media that are available in the hospital?

……

……

Q7. These are the bacteria that are cultured and identified in the hospital?

☐ Aerobic bacteria

☐ Anaerobic bacteria

☐ All gram positives and gram negatives

☐ Atypical bacteria

☐ Other (mention)……...

Q8. Give reasons if not all the above bacteria are cultured and identified?

……

……

Q9. Standard conventional microbial culture and identification procedures followed for every specimen?

☐ Yes

☐ No (Reasons)…….

Q10. Are there any other non-conventional microbial culture and identification procedures practiced?

☐ Yes (illustrate)…….

☐ No

Q11. Sensitivity test done on all the antibiotics administered in the hospital or specific?

☐ All

☐ Specific (list and give reasons)……

……...

Q12. List all the conventional biochemical tests conducted in the hospital for bacterial identification?

……

……

Q13 list other non-conventional biochemical test conducted in the hospital for bacterial identification if any?

……

……

Q14. Are there some of the conventional bacterial identification tests that are not conducted in the hospital?

☐ Yes (list)…….

☐ No

Q15. Give reasons why some of these conventional bacterial identification tests are not conducted in the hospital?

……

……

THANK YOU FOR PARTICIPATING.

Boaz Cheruiyot

Principal investigator

APPENDIX 3: IREC APPROVAL

MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
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Tel: 33471/2/3

Reference: IREC/2021/202
Approval Number: 0004035

9th December, 2021

Cheruiyot Boaz Kipkurui,
Moi University,
School of Public Health,
P.O. Box 4606-30100,
ELDORET- KENYA.

Dear Mr. Cheruiyot,

BACTERIAL TYPE AND PREVALENCE IN CHRONIC WOUND SWAB ISOLATES AND THEIR ANTIMICROBIAL SENSITIVITY PATTERN IN UASIN GISHU COUNTY, KENYA

This is to inform you that ***MTRH/MU-IREC*** has reviewed and approved the above referenced research proposal. Your application approval number is ***FAN: 0004035.*** The approval period is **9th December, *2021- 8th December, 2022.***
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, Material Transfer Agreements (MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MTRH/MU-IREC***.
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 ***MTRH/MU-IREC*** 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 ***MTRH/MU-IREC*** within 72 hours.
v. Clearance for export of biological specimens must be obtained from ***MOH at the recommendation of NACOSTI*** for each batch of shipment.
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 ***MTRH/ MU-IREC.***

Prior to commencing your study; you will be required to obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and other relevant clearances from study sites including a written approval from the CEO-MTRH which is mandatory for studies to be undertaken within the jurisdiction of Moi Teaching & Referral Hospital (MTRH) and its satellites sites.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
09 DEC 2021
APPROVED
P. O. Box 4606 - 30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

APPENDIX 4: NACOSTI APPROVAL

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **548054**

Date of Issue: **13/January/2022**

RESEARCH LICENSE

This is to Certify that Mr.. boaz kipkurui cheruiyot of Moi University, has been licensed to conduct research in Uasin-Gishu on the topic: BACTERIAL TYPE AND PREVALENCE IN CHRONIC WOUND SWAB ISOLATES AND THEIR ANTIMICROBIAL SENSITIVITY PATTERN IN UASIN GISHU COUNTY, KENYA. for the period ending : 13/January/2023.

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