EVALUATION OF ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA ISOLATES FROM CHRONIC WOUNDS ON PATIENTS ATTENDING MURANG'A LEVEL 5 HOSPITAL, KENYA

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DECLARATION AND APPROVAL

Declaration

This thesis is my original work and has never been presented for any academic award in any institution.

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DEDICATION

This thesis is dedicated to my lovely daughters, Celestine, Claire and Cynthia who had to bear with my tight schedule during my class days.

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ABSTRACT

Chronic wounds pose a serious public health risk. Therapy of chronic wound infections is significantly hampered by the unchecked and rapid spread of bacterial pathogens. A substantial amount of research has been done in developed countries on these chronic wounds. In Kenya there is a scarcity of the statistics on antimicrobial sensitivity and resistance of bacteria isolated from chronic wound infections. This study has determined the colonizing bacteria of chronic wounds and their antibacterial resistance pattern to most used antibiotics. Risk factors associated with chronic wounds was also evaluated. This analysis was carried out in Murang'a Level 5 Hospital which is located in rural area of Central region in Kenya. People in rural areas are presumed to be prone to wounds because of their lifestyles and nature of their works. It was a hospital-based cross-sectional study that was carried out from June 2023 to November 2023. Questionnaire was used to get social demographic characteristics and medical history from the patient. Swabs were aseptically picked from chronic wounds and transported in Amies transport media in a cooler box to Microbiology laboratory. Inoculation was done on Sheep Blood Agar and MacConkey Agar and incubated at 37^0c for 24 to 48 hours. Gram stain followed for the provisional isolate's identification. Further singling out was executed using a number of biochemical tests. The isolated microorganisms were evaluated for drug sensitivity and resistance using Kirby Bauer disk diffusion method on Mueller Hinton Agar. Descriptive statistics for frequency distribution of clinical bacterial pathogens and their antimicrobial susceptibility, were presented in tabular, graphical and chart form. The zone diameter breakpoints of all drug disks were evaluated in millimetres to determine whether they are sensitive or resistant. The potential association among variables was established using the Chi-square tests. P-value <0.05 was regarded to be a statistical significance association. The total swabs tested were 300 and positivity rate was 81.3%, *Staphylococcus aureus* being the most predominant bacteria infecting chronic wounds at 29.7%. *Pseudomonas aeruginosa* (16.3%) was second and most frequent in Gram negatives. *E. coli* had a prevalence of 15.2%. Levofloxacin was the most sensitive antibiotic to *S. aureus*, doxycycline and gentamicin followed closely. It was resistant to penicillin, co-trimoxazole and Augmentin. All Gram-negative bacteria in this study demonstrated high susceptibility to meropenem, piperacillin/tazobactam, gentamicin, imipenem, cefepime and ciprofloxacin in that order. They all showed resistance to ceftriaxone, Augmentin, co-trimoxazole and ampicillin. Of all *Staphylococcus aureus* isolated, 22.6% were methicillin resistance (MRSA) strains. Induced clindamycin resistance (ICR) strains were 5.95% and 2.38% had both MRSA and ICR strains. In all Enterobacteriaceae isolated, 19 (16.23%) are Extended spectrum beta-lactamase (ESBL) producing strains. *E. coli* accounted for 10 ESBL strains while the rest 9 ESBLs were from *Klebsiella pneumoniae.* Frequence monitoring of antimicrobial susceptibility pattern is necessary to curb the spread of antibacterial resistance. Microbiological results should be embraced into consideration when prescribing antibiotics whenever possible. The findings generated from this study will be forwarded for adoption to the health care practitioners for effective management and treatment of chronic wound infections.

Key words: Chronic wounds, Bacteria isolates, Antimicrobial resistance and susceptibility

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LIST OF ABBREVIATIONS AND ACRONYMS

ABHR Alcohol-based hand rub

AMR Antimicrobial resistance

AMS Antimicrobial sensitivity

API Analytical profile index

BMI Body mass index

CDC Centres for Disease Control and Prevention (USA)

CLSI Clinical and laboratory standards institute

CP Carbapenemase-producing

CPE Carbapenemase-producing Enterobacteriaceae

ESBL Extended spectrum beta-lactermase

EQAS External quality assurance system

GoK Government of Kenya

HAI Health care-associated infection

HCW Health care worker

ICR Inducible clindamycin resistance

IDDS Infectious diseases detection and surveillance

IPC Infection prevention and control

IPCAF Infection prevention and control assessment framework

IPCAT Infection prevention and control assessment tool

MAC MacConkey

MHA Muller Hinton agar

MOH Ministry of health

MRSA Methicillin resistant *Staphylococcus aureus*

MSSA Methicillin sensitive *Staphylococcus aureus*

NMRL National microbiology reference laboratory

PPE Personal protective equipment

SBA Sheep blood agar

SOP Standard operating protocols

USA United States of America

WASH Water, sanitation, and hygiene

DEFINITION OF OPERATIONAL KEY TERMS

Antimicrobial stewardship: A clear set of guidelines that support and promote the responsible use of antibiotics.

Primary health care facilities: These facilities offer family planning, antenatal care, outpatient medical expertise, and health services for mothers, newborns, and children. Examples of these facilities are dispensaries, health centres, and small Level 4 hospitals.

Primary-level hospital: Internal medicine is the primary specialty offered by these facilities. In addition, they provide general practice, pediatrics, general surgery, and obstetrics and gynecology. These facilities offer standard laboratory services for primary pathological analysis, not specialized services.

Secondary-level hospital: In these facilities patients get the care of specialists like oncologist, dermatologist, haematologist and pathologist. The bed capacities range from 200 to 800 beds These hospitals are frequently mentioned as Level 5 hospital.

Tertiary-level hospital: These facilities have staff with advanced training and diagnostic tools. A few instances are PCR machines, specialized imaging units, and intensive care units. They can accommodate between 300 to 1500 patients. They are commonly referred as a teaching hospital, university, or regional reference hospital, they are commonly referred as.

Personal protective equipment: These are biosafety garments that act as a protection to the health care workers or other persons from contacting infections or harmful substances. These PPEs usually consist of gloves, masks, googles, gowns and gumboots.

Standard operating procedure: This is a document consisting an outline of set of step-by-step instructions composed by the head of department or an institution to support employees in doing regular activities as effectively and efficiently as possible.

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CHAPTER ONE

INTRODUCTION

1.1. Background to the study

Healthy, intact skin controls microbial populations on its surface and protects epidermis and dermis tissues from colonization and invasion by potential microbes (Swaney et al., 2021). When underlying tissue is exposed due to degradation of the skin's integrity (i.e., a wound), it offers moist, warm and full of nourishment habitat that is favourable for microbial proliferation and colonization. Exposed from an intact skin envelope, these wounds can be traumatised again and advance in size. They may acquire microbial infections leading to sepsis, necrosis and amputation of the affected extremity (Kahraman et al., 2019). Chronic wound infections are a grievous headache globally. They are considered chronic wounds when healing fails to proceed in an orderly normal way and the functional integrity of the skin is not achieved in approximately 4 weeks (Eriksson et al., 2022). The circulatory insufficiency and infections play a major role to nonhealing chronic wounds. Infections originates from microbial proliferation in the wound base proceeding to a prolonged extreme inflammatory response, delayed collagen synthesis and epithelialization (Barchitta et al., 2019). The diversity and abundance of microbes in any chronic wound will be determined by factors such as the extent of tissue blood flow, location, depth, wound type and the antibacterial potency of the patient immune response (Patel et al., 2022).

A chronic wound is one that has not healed in a timely and organized way to restore the skin's functional and anatomic integrity. Wound repairing in human body is a complex of physiological processes that involves simultaneous initiation of different cell types and signalling pathways in well collaborated rhythm (Wang et al., 2023). Early prophylactic antimicrobial treatment and debridement should be implemented to avoid the presence of invading materials and damaged tissues in traumatic wounds which are most likely to encourage bacterial multiplication even though the microbiota connected to surgical incisions that are clean are expected to be minimal (Patrulea et al., 2020). Wound

colonization involves polymicrobial which are potentially pathogenic (Sekyere & Mensah, 2019). The most common bacterial pathogens associated with colonization of chronic wounds infections include *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalacteae* and *Enterococci* (Tanih et al., 2015). Those were the most Gram positive bacteria that colonise chronic wounds. *Klebsiella pneumonia, Klebsiella oxytoca, Pseudomonas aeruginosa, Proteus species* and *Escherichia coli* (Musila et al., 2021) are among Gram-negative bacteria that colonise wounds to chronic state (Serra et al., 2015).

In case of infections, wound healing fails and the patient clearly decreases the quality of life as these wounds are associated with increased physical pain, suffers trauma, the cost of treatment goes up, and general management of wound practices becomes a draining burden of the resources. Microbial proliferation and colonization which leads to infections of the wound, is the primary reason why the wound is taking so long to heal (Clinton & Carter, 2015), resulting in a persistent, non-healing wound condition. This burdens the healthcare institutions with many affected individuals, extreme high costs and repercussions that include social economic complications, hospitalization, amputation, and occasionally an agonizingly early mortality (Wang et al., 2023). According to estimates, between 1% and 2% of individuals in affluent nations will acquire chronic wound infections at some point in their lives (Järbrink et al., 2016). About 5.7 million people, or 2% of the overall population in the US, suffer from chronic wound infections; microbes contribute for nearly 60% of these wounds definitely illustrating the magnitude of this menace (Kadam, 2019).

Antibiotic resistance is a grave public health concern as ineffective antimicrobials make the clinical management and prevention of chronic wound infections challenging (Church et al., 2021). In an interview done by Sir Alexander Fleming in 1945, who discovered penicillin, the first antibiotic, had given advanced warning concerning over using antibiotic which would results to the possible rise of bacterial resistance (Sohaili et al., 2024). Antimicrobial resistance is being recognised as a major worldwide health and economic implications with this prediction now transpiring, with magnitude

effects on productivity and health care cost, morbidity and mortality (Asghar et al., 2024). Especially due to the polymicrobial environment associated with chronic wound infections, which promotes the transfer of resistance genes between different bacteria, it has been estimated that there is a high chance of microorganisms to develop resistance in these favorable and conducive conditions. The uncontrolled and rapid proliferation of bacterial pathogens poses a significant challenge to the management of chronic wound infections (Roy et al., 2017). High mortalities and morbidities are caused by a higher prevalence of bacterial infections, shortage of diagnostic capability, and restricted access to second- and third-generation antibiotics in countries with low and middle income (Bernabe et al., 2017). The infection site and pathogen's virulence have a significant impact in determining the severity of the complication.

Gram positive bacterial pathogens (for example, MRSA/VISA (methicillin-resistant and VAN-intermediate *Staphylococcus aureus*), *Enterococcus faecium* and vancomycin (VAN)-resistant are of critical concern (Li et al., 2022). Children's meningitis, osteomyelitis, pneumonia, and sepsis are caused by these bacteria, which also show a high rate of resistance to the medications that the World Health Organization (WHO) recommends (David, 2017). Humans are frequently infected with Staphylococcus aureus, which causes infections of the skin and soft tissues (Okello et al., 2021), endocarditis, osteomyelitis, bacteraemia, and lethal pneumonia (Guo et al., 2020). *S. aureus* is categorized as either methicillin-resistant (MRSA) or methicillin-sensitive (MSSA*) Staphylococcus aureus*.

In Africa, Eighty percent of *S. aureus* infections are caused by MRSA, which has resistance to most widely administered antibiotics, including tetracycline (TET), aminoglycosides, macrolides, and fluoroquinolones (Sekyere & Mensah, 2019) (Yitayeh et al., 2021). Understanding drug resistance in MRSA and elucidating its drug resistance mechanisms are essential to the treatment of *S. aureus* infections. Antibiotics resistance in microflora *Eschirichia coli* is a possible proxy for resistance in other gut-dwelling pathogenic bacteria. (Tornberg-Belanger et al., 2022). AMR in commensal *E. coli*

is a contributing factor to the emergence of E. Coli infections that are resistant (Tawfick et al., 2022). Few studies have investigated and characterized antimicrobial resistant in commensal *E. coli* in Kenya. Furthermore, this burden will continue to rise due to rising rates in surgical procedures, cancers, diabetes, hypertension, and prolonged life spans (Wangai et al., 2019).

Many variables contribute to the development of a chronic wound. Age, trauma, blood perfusion, immunological suppression, and related morbidity are a few examples of factors that make healing more challenging. Other danger elements include gender, obesity and life style (Patel et al., 2022). Thus, understanding the risk factors linked to chronic wounds can support initiatives to lessen their frequency, which would in return lower morbidity and mortality rate which occur as a result of chronic wounds complications (DesJardins-Park et al., 2022). Wound infections due to surgery complications are classified into deep incisional wounds, incisional surgical wounds, and organ-specific infections (Ostaszewska, 2019). Even with the strict guidelines for preoperative planning, antimicrobial prophylaxis and surgical techniques, there is still a significant risk of postoperative wound infections to develop. This study has determined the colonizing bacteria in chronic wounds, antimicrobial resistance and sensitivity pattern to frequently used antibiotics and further determined the risk factors related to persistent wounds.

1.2. Statement of the problem

Among the major sources of morbidities and mortalities are chronic wounds. The control of colonizing bacteria of chronic wounds is becoming more difficult as a result of prevalent bacterial resistance to commonly used antibiotics (Fatima et al., 2021). Thus, chronic wound infections are an epidemic affecting a big proportion of the population in the world. While most of these infections have been documented elsewhere, very few evaluations have been done to identify the bacteria colonizing chronic wounds here in Kenya. New resistance mechanisms are developing and spreading globally due to the

fact that antibacterial resistance has increased to potentially hazardous levels worldwide (Ferri, 2017). Kenya is not exceptional to this hence the need to seal the gap through this study.

Testing for susceptibility of the antibiotics is essential when treating chronic wound in order to prevent antibiotics from becoming resistance. Treating patients for common infectious wounds has become more challenging, and in some cases impossible, as antibiotics lose their effectiveness (Roberts et al., 2017). While abundant data on antimicrobial susceptibility is available, no data is available on the same from chronic wound infections in a rural set up. Risk factors such as malnutrition (Sohaili et al., 2024), stress, old age, co-morbidities and metabolic syndromes are underlying conditions predisposing patients to chronic, non-healing wounds. Facts about risk factors that could possibly result in chronic wounds has not been documented in Kenya hence the need to establish them. The lack of adequate data addressing the antimicrobial resistance pattern of chronic wounds in Kenya makes it challenging to develop scientifically supported strategies for the control, prevention, and treatment of chronic wound infections.

1.3. Purpose of the study

This study has determined colonizing bacteria of the chronic wounds, antimicrobial susceptibility and resistant pattern to most prescribed antibiotics. Risk factors associated with chronic wounds was also established.

1.4. Objectives of the study

1.4.1. General Objective

To evaluate the antimicrobial resistance pattern of bacteria isolates from chronic wounds on patients attending Murang'a level 5 hospital.

1.4.2. Specific Objectives

i. To identify bacteria species that colonizes the chronic wounds on patients attending Murang'a Level 5 Hospital.

ii. To establish antimicrobial susceptibility patterns of bacteria isolates from chronic wounds on patients attending Murang'a Level 5 Hospital.

iii. To identify the risk factors associated with chronic wound infections in patients attending Murang'a Level 5 Hospital.

1.5. Research Questions

i. What are the bacteria species that colonize the chronic wounds on patients attending Murang'a Level 5 Hospital?

ii. What are the antimicrobial susceptibility patterns of bacteria isolates from chronic wounds on patients attending Murang'a Level 5 Hospital?

iii. What are the risk factors associated with chronic wound infections in patients attending Murang'a Level 5 Hospital?

1.6. Justification of the study

Chronic wound colonization involves an array of potentially pathogenic microbes (Aslam, 2018). In case of infection, general wound management procedures become more resource-demanding, treatment costs increase, and the patient experiences increased trauma (Valentine, 2017). Prompt management of the wound infections is paramount to curbing infection-related complications. Thus, this study has provided important information of the colonizing bacteria in chronic wounds to all health care stake holders for effective wound treatment and management.

A pandemic of nosocomial wound infections has major health repercussions because of the rising rate of bacterial infections and the increasing resistance to antibiotics. Antimicrobial resistance (AMR) in

commensal bacteria in established wound infections is a potential surrogate marker of resistance in other pathogenic bacteria (Mardourian et al., 2023). Testing for antibiotic susceptibility is crucial for modifying the regimen of treatment for chronic wound infections preventing the development of further complications arising from resistant bacterial strains. The findings of this investigation will be helpful in informing on the antimicrobial susceptibility pattern providing knowledge to health care practitioners as they provide prescriptions for antibiotics to treat persistent wound infections.

Progression of wounds to infected states entails an array of factors (Zaidi & Sharma S., 2022a). It has been observed that the size, kind, location and depth of the chronic wounds, as well as the total degree of pathogenicity exhibited by the microbials involved, promotes chronic wound infections (Rankin et al., 2016). Therefore, basic surveillance is desperately needed to provide evidence-based information on potential risk factors for chronic wound infections. When public health offficers are formulating mitigating strategies, this knowledge is very essential.

1.7. Scope of the study

This research envisioned to investigate colonizing bacteria from chronic wounds of patients attending Murang'a level 5 hospital, Kenya. Bacteria were isolated from the different types of chronic wounds; surgical, bites, cellulites, abscess, burns and trauma in patients being attended to at the hospital. The bacteria isolates were examined for patterns of resistance and susceptibility to antibiotics and the risk factors predisposing patients to chronic wounds were also evaluated.

1.8. Study limitations

The study concentrated on the persistence bacterial infections of the wound and the isolates' patterns of resistance to antibiotics. The antimicrobial resistance genes, and genotypes were not established due to the financial strains which is also limiting the geographic size to be covered. The obligate anaerobes strains were not captured due to lack of carbon dioxide gas jar.

1.9. Delimitations

Due to the large numbers of antimicrobial agents globally, the number of antimicrobials tested was limited to those prescribed/recommended by the physicians at Murang'a level 5 hospital as per the ministry of health (MOH) and government of Kenya (GoK) guidelines.

1.10. Assumptions of the study

During the investigation, the following presumptions were made:

i. The research participants reside in Murang'a County.

ii. The study participants had not been exposed to antimicrobial therapy at the time of study.

iii. The wound infection was exclusively caused by bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

An extensive array of microorganisms can infect and colonize exposed subcutaneous tissue because it provides a favorable environment (Kahraman et al., 2019). Controlling the population of microbes dwelling on the exterior of the skin and preventing potential microbes from colonizing and invading underlying tissue are the main functions of intact skin. The most favorable conditions for microbial development arise when the host's immunity is impaired (Swaney et al., 2021). Chronic wound infections provide a significant obstacle to recovery and can negatively affect both the patient's quality of life and the rate at which the wound heals. Infections can cause wounds to become more sensitive, odorous and unpleasant, which can cause patients greater distress and inconvenience (Choudhari et al., 2022). It's most likely that these wound contaminants originate from:

1. Exogenous – these are chronic wounds associated with organisms from the environment or those caused by burns, bites from animals and humans, severe injuries, and foreign substances in the skin.
2. Endogenous – these chronic wounds are associated with microbiome of mucous membrane, mouth cavity, the genitourinary mucosa and gut's typical microbiome. Most of microorganisms are supplied by these sites. A dental infection, septic arthritis, osteomyelitis, appendicitis, cholecystitis, cellulitis, and sinusitis can all be associated to an abscess. A number of these infections are acquired through nosocomial following medical surgeries, invasive procedures and others from hematogenous spread from primary site of infection.
3. The skin's surrounding environment, which includes skin *Diphtheroid*, *Micrococci*, and *Staphylococcus epidermidis*, among other common flora.

There is ultimate four phases which contribute to the intricate physiological progression of wound repairing, that is inflammation, proliferation, migration, and the development of new tissue (Oliveira et al., 2022). For the normal physiological mechanism to lead to a healthy healing phase with little to no scarring, the wound recovery process needs a good, healthy environment. Using microbiologically proved antibiotics to treat any microbial infection and sterilizing the injured tissue is one of the most crucial ways to maintain the healing process (Negut Irina et al., 2018). When a wound cannot heal to the final stage because of an interference with any of the wound healing processes, bacterial infections occur resulting in colonized chronic wounds (Yi-Fan Liu et al., 2022). According to research conducted by the National Institutes of Health (NIH), chronic wounds are defined as wounds that remain open for longer than a month and have not healed normally (Kangal et al., 2022). According to published research, a chronic condition is indicated if the surface area of a wound does not decrease by approximately 15 percent within a week or by 50 percent within a month (Sisay et al., 2019). Chronic wounds have a variety of etiologies, all of which cause strains to the medical system. Multidrug-resistant (MDR) bacteria that colonize wounds increases morbidity and mortality and it's truly alarming the global wellness (Neopane et al., 2018). Patients with suppressed immune systems and chronic illnesses, such as diabetes, infected wounds are a major cause of death to them and poses a substantial clinical and financial challenge to both patients and healthcare system.

2.2 Chronic wounds classification

Based on their origin, chronic wounds are divided into four main types according to the Wound Healing Society. These include diabetic ulcers, venous ulcers, pressure ulcers, and arterial ulcers (Frykberg & Banks, 2015). Additional significant causes of wounds are lymphoedema, malignancy and sickle cell disease. Injuries from interpersonal conflicts, road accidents, burns, surgical wounds, bites and cellulitis are examples of wounds caused by trauma. Among the dermatological disorders that can result in persistent wounds are systemic lupus erythematosus and pyoderma gangrenosum

(Shanmugam & Victoria K, 2016). Prior research has indicated that wounds resulting from hypertension, diabetes, venous disorders, and surgical site infections are most susceptible to pathogenic bacterial colonization in addition to primary cutaneous skin infections (K. Rahim et al., 2017) . Approximately 15% of nosocomial infections are post-surgical wounds, which pose a significant challenge in terms of treatment due to their resistance to many antibiotics (Schweizer et al., 2019). It's critical to categorize chronic wounds for patient clinical therapy. Understanding the underlying etiology and mechanism of the wound's persistence should serve as a starting point for the therapeutic strategy.

2.3. Burden of wound infections

A retrospective study conducted in 2018 amongst Medicare beneficiaries' findings from the United States of America (USA) revealed that 8.2 million persons had wounds, either infected or open clean wound (Sen & Chandan K, 2021). This posed a great economic burden as the amount that Medicare paid for the cost of both persistence and acute wound treatment was $28.1–96.8 billion. Diabetic foot ulcers and surgical wounds had the highest costs, with outpatient wound care costs being higher compared to the inpatient costs (Olsson et al., 2019). Thus, the increased healthcare costs, the continuing global threat posed by obesity and diabetes, along with antimicrobial resistance, makes chronic wounds a major clinical, social, and financial burden. Considering all factors, it is estimated that 2% of Americans are victims of chronic wounds (Nussbaum et al., 2018). According to Wales research done by the National Health Service (NHS) in 2016, it incurred a 5.5% cost as a result of the 6% prevalence of chronic wounds (Phillips et al., 2016).

Diabetic foot ulcer prevalence was 6.3% worldwide, with Europe having a lower prevalence of 5.1% while North America had 13.0% (Jassim et al., 2023). Of all thirty-three countries sampled, the highest prevalence of patients with diabetic foot ulcers was from Belgium at 16.6% while the lowest was from Australia at 1.5%, with males being more susceptible to foot ulcers than females (4.5% vs. 3.5%)

(Raghav et al., 2018). Additionally, the prevalence of foot ulcers was higher in type 2 diabetes patients (6.4%) than in type 1 diabetic patients (5.5%).

Only a few African nations have some information on chronic wounds. A five-year study done in a Nigerian tertiary hospital with 509 patients with chronic wounds, found a percentage of 70.1% (Kisoi & Stella K, 2021). To my understanding, no published information exists regarding the trend of antibiotic resistance in chronic wound infections in Kenya.

2.4 Bacteria colonizing chronic wounds

When the host's natural immune system is defeated or exceeded by the virulence factor that one or more bacteria in a wound express, an infection develops. A sequence of local and systemic host reactions is triggered by the subsequent invasion and spread of microorganisms in living cells (Rachel Pei-Hsuan et al., 2019). While most wounds are triggered by a combination of aerobics and anaerobics, the most commonly identified cause of prolonged wound repair and infection is aerobics bacteria like beta hemolytic *Streptococci*, *P. aeruginosa* and *S. aureus* (Percival et al., 2018). One particular instance is *S. aureus*, which has been determined to be the bacteria with the greatest risk of infections resulting from trauma, surgery, and burn injuries. Additionally linked to wound infections are bacterial pathogens such as *Escherichia coli*, *Enterococcus faecium*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, and *Enterobacter species* (Patrulea et al., 2020). The afore mentioned pathogens are termed *"ESKAPE*" pathogens and they are multidrug resistant (Sainz-Mejías et al., 2020). *Pseudomonas aeruginosa and Staphylococcus aureus* can develop biofilms making their treatment difficult as bacteria with biofilms are highly tolerant to antimicrobial agents (Sandoval-Motta & Aldana, 2016).

2.5 Antimicrobial resistance and susceptibility of bacteria isolates

The best way to treat wounds and prevent them from progressing to chronic wounds state is to diagnose them early and give the patient right antibiotics based on their wound causative (Schweizer et al.,

2019). In Sub-Sahara Africa including Kenya, the primary causes of antimicrobial resistance include inadequate access to suitable treatments, overuse of antimicrobial agents and a lack of microbiology diagnostic laboratories to test antibiotics for susceptibility (Mnyambwa et al., 2021). The majority of persistence infections associated with wound are treated empirically with antimicrobials, primarily purchased over-the-counter without a prescription (Sekyere & Mensah, 2019). The empirical treatment administered is still relevant for assessing the progress of wound healing. Many research carried out in advanced economies have reported on the growing antibiotic resistance among pathogenic bacteria causing chronic wound infections, which is a grave issue (Frieri et al., 2017).

Microbes develop antibiotic resistance as a biological process in response to the selection pressure exerted by antimicrobial agents. Clinically, non-compliance with recommended antibiotic medication is the primary cause of the development of antibiotic resistance (Ayukekbong et al., 2017). Consequently, it encourages random changes in chromosomes or regulatory genes, resulting in novel mutant pathogens that have selective pressure when antimicrobials are present. Kenya, an East African nation, suffers several challenges since almost 43% of its people are impoverished. The nation faces significant health challenges, such as high rates of chronic infectious wounds together with the burden of maternal and infant mortality (Ogugua et al., 2024). Thus, the growth in resistance to first-line routinely used medications and the rise in infections from microorganisms that are extremely harmful to people's health are causes of alarm. Worrisomely, organisms that are extended spectrum lactamase-producing and carbapenem-resistant Enterobacteriaceae (CRE), are on the rise in Kenya and are associated with poor clinical outcomes in invasive chronic wound infections (Celestin, 2020). A recent study found that 7–17% of hospitalized patients had infections caused by these bacteria, and 39% of isolates met the criteria for "difficult to treat resistance."

This condition is causing an urgent public health problem that requires attention. This is exacerbated by the dearth of regular monitoring of the data and technical constraints that significantly restrict the use of local data on antibiotic consumption and resistance (Kariuki et al., 2022). If empirical therapy

is supported by evidence, such as the knowledge of common pathogenic organisms and their susceptibility to antibiotics, medication resistance can be reduced (Mohamed et al., 2019). This study established the leading pathogen causing infections in chronic wounds and evaluated their antimicrobial sensitivity patterns in Murang'a, Kenya. The findings will help physicians and clinician in making informed decisions while prescribing antibiotic therapy.

2.6 Risk factors for chronic wounds

Acute clean wounds do not pose a significant problem in a healthy population. Dangers arise when these wounds get infected. Using immunosuppressive drugs, smoking, advanced age, hypovolemia, obesity, diabetes, use of steroid, and Patients who have concurrent infections at different sites are at a higher risk of developing wound infections (Alsharari et al., 2021). These underlying conditions among others such as metabolic disorders and stress make people more vulnerable to long-term, non-healing wounds (Golubnitschaja et al., 2021).

2.6.1 Comorbidities

Individuals with obesity and diabetes have an increased risk of getting persistence wounds. In the diabetic population, probabilities of getting ulcers of the foot are high and have a neuropathic genesis (Ousey et al., 2018). An estimated 4–10% of people globally get foot ulcers each year, with 15%–25% of diabetic patients at risk of developing one. Individuals who have dementia, diabetes, stroke, or reduced mobility are more susceptible to pressure ulcers (PU) (Lee et al., 2016). Likewise, extended periods of inactivity in the critical care unit may result in the pressure ulcers development.

2.6.2 Age

Chronic wounds are prevalent in the elderly people. It was reported that three percent of United State population over 65 years have open wounds (Sen, 2019). According to US Census Bureau reports, there will be over 90 million senior population in the country by 2060, suggesting that chronic wounds will remain a problem for a long time (Bureau & U.C, 2022). Furthermore, as people age, pressure ulcers become more common and can get exacerbated by dehydration, starvation, and inadequate skin

perfusion (Zaidi & Sharma S., 2022b). As people age, diabetes becomes more common. It affects 4% of individuals between the ages of 18 and 44, 17% of adults between the ages of 45 and 64, and 25% of elderlies over 65 (Shah & Garg, 2015). In developing nations, diabetes commonly onset between the ages of 45 and 64. It is projected that by 2030, developing countries would have 82 million people over 65 years with diabetics more than developed countries at 48 million.

2.6.3 Obesity

Too much fat accumulation block some of the body's vital functions, making people sick and raising the possibility of developing more health issues. Obesity in children is linked to a higher risk of fractures and a tendency to have breathing issues (Rankin et al., 2016), hypertension, psychosocial impacts, insulin resistance and cardiovascular diseases. It has become a global pandemic health issue. Obese children tend to continue being overweight even in their adulthood predisposing them to an ally of health risks including non-healing wounds (Yachmaneni et al., 2023). Obesity in adults is linked to a number of problems, including impaired cutaneous wound healing after surgery. Obesity raises the risk of infection-related consequences significantly (Thelwall et al., 2015). One of the main reasons obese patients have higher infection rates is because their adipose tissue has decreased vascularization. The amount of host immune cells that can reach the colonized site is restricted by poor perfusion.

2.6.4 Malnutrition

Malnutrition which happens when the body is depleted of vitamins, minerals, and other nutrients necessary to sustain healthy tissue and organ functions, is another systemic cause of poor wound healing. It happens to those who are both undernourished and overfed individuals (Elof Eriksson et al., 2022). Normal collagen synthesis, for instance, requires sufficient protein consumption and cofactors like vitamin A and C in order to produce new extracellular matrix. Zinc is equally necessary for the production of extracellular matrix (Balasundaram & Krishna S, 2024). Wound healing is metabolically demanding as energy is required to encourage cell motility, division, differentiation, chemotactic reactions (cytokine response and growth factors), as well as cellular repair (Wangai et al., 2019).

Nutritional deficits and malnourishment are common in patients with chronic wounds hence delayed wound healing.

2.6.5 Stress

Psychosocial stress is among the key determinants to wound healing. Stress impairs cellular immunity (WYNN et al., 2019), compromising wound healing. It has been shown that there is a direct correlation between psychological stress and health, which includes, but not limited to, impaired functions of immunity system and slow healing surgical wound outcomes (Valentina S Lucas, 2015). In the human anatomy, skin is the biggest organ, it produces vitamins, regulates body temperature, and shields the body from infections, fluid loss, UV rays, and mechanical injury. Many sensory nerves that provide information about pressure, temperature, pain, and pleasure are found in large quantities in the skin (Joyce Y. Kim & Harry Dao, 2024). Because of the skin's essential protective and regulatory roles, wound healing in time is very important. It has been demonstrated that psychological stress has a deleterious direct and indirect effect on wound healing. By modifying the humoral, neural and immune systems, it causes certain physiological alterations. According to (Yi-Fan Liu et al., 2022), stress induces immune dysregulation through the endocrine systems and central nervous. Studies based on observation have demonstrated that anxiety and nervousness before surgery may interfere with the natural healing of wounds, leading to chronic state (Words, 2018).

Table 2.1: Summary of risk factors associated with wound healing.

Category/level	Risk Factors
1) General factors that do not directly cause wounds but facilitate tissue damage	• Social factors like social groups, marital status and stress. • Race, gender and age as demographic factors. • Co-morbidities e.g diseases and related medications. • General health factors e.g., nutritional status.
2) Local factors that don't directly cause damage but impede wound closure and healing.	• Affected site. • Wound size and depth

	• Wound period. • Skin microbiota.
3) Systemic factors that cause trauma, and affect wound closure	• Endocrinological system, • Renal system, • Connective system, • Immune and Nervous system • Muscular system (sarcopenia).
4) Molecular/cellular factors that either directly cause damage or enable factors affecting wound closure.	• Genetic factors e.g., transcription and translation of genes and mutation. • Histones and methylation (epigenetic factors). • Proliferation and migration • Intracellular phenomena (diformation, stress on the endoplasmic reticulum, autophagy, apoptosis, and mitochondrial stress). • Signaling components, such as ions, proteins, and gradients System components (responses, controls, and adaptations)

2.7. Theoretical Framework

Wound management consists of 3 key processes (Chen et al., 2015) as demonstrated below in Figure 2.1

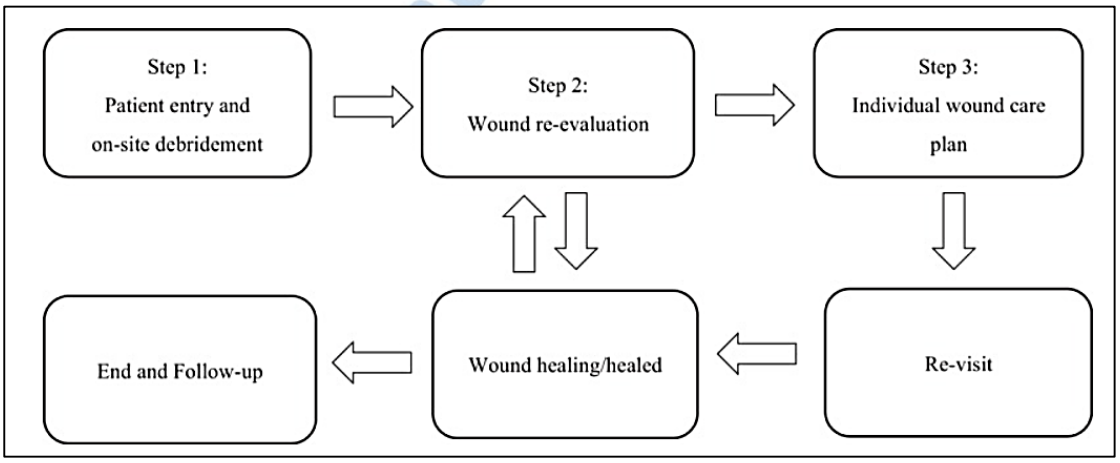

Figure 2.1: Wound management's theoretical framework

1) **Patient entry with onsite wound debridement:** Referrals for patients come from both inpatient admissions and outpatient clinics. Wound care centers, orthopaedic surgery clinics, etc. are examples of outpatient clinics. The resident physician is in charge of initiating wound care for in-house admissions.
2) **Wound re-assessment** is conducted following completion of the first visit and debridement. During this step, neurologic, vascular, tissue infection/inflammation and wound size/edges are evaluated.

3) Wound care plan involves setting up a customized wound management regime in context of the results of the reevaluation of the wound. Depending on the problems associated to the patient, other professions including physical and occupational therapists may help with the wound management plan.

2.8. Factors associated with wound healing and infection

Delays in wound healing are associated with inflammation, poor mobility, frailty and the incidence of metabolic conditions like diabetes. Stress, mental status (compliance) and a limited metabolic capacity also has an adverse effect on the mechanisms involved in wound healing (Zhao et al., 2017). This is shown in figure 2.2 below.

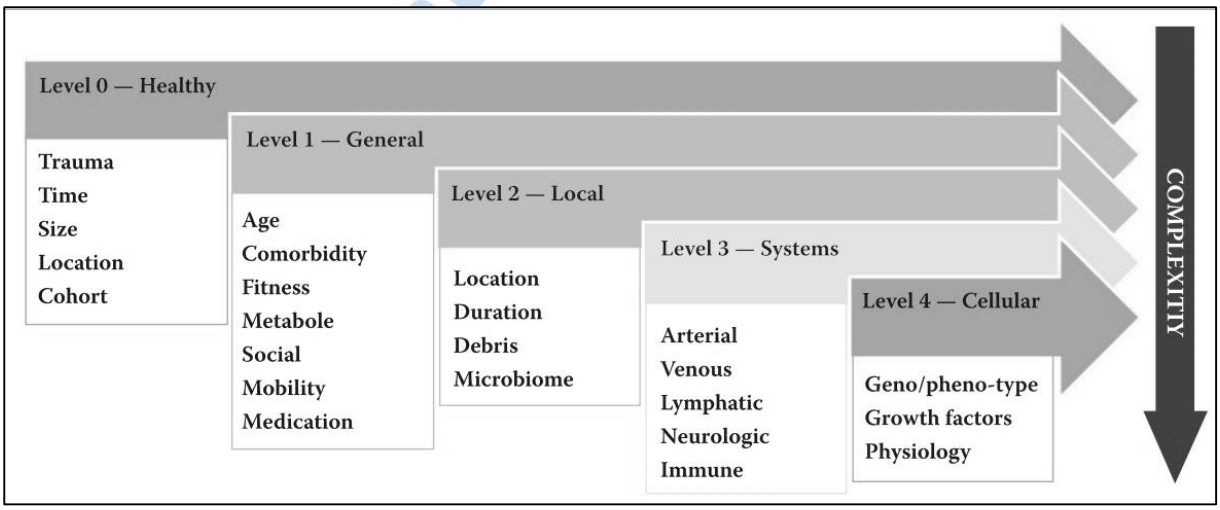

Figure 2.2: Factors associated with wound healing and infection

- Level 0 entails the ‘normal’ healing process and is used as a gauge for normal wound healing.
- Level 1 wounds heal over a slightly longer time compared to level 0 as general patient variables such as age, stress, nutritional status and metabolic syndromes impede the progress of wound healing.
- Level 2 pathology is secondary due to primary events that initiated the wound. Thus, level 2 variables result from the wound rather than causing it. These include wound location, wound size, affected tissue, structures involved (e.g., open fracture) and the individual’s microbiome (antibiotic exposure and impaired immune system).
- Level 3 variables cause wounds either directly or indirectly due to the impaired physiological systems. For instance, compromised homeostasis is the cause of pressure ulcers, diabetic foot ulcers, and leg ulcers. An impaired cardiovascular system affects tissue perfusion. The lymphatic, neurological, renal and endocrine systems are associated with impaired wound healing. Medical interventions that are aimed at treating the impaired systems promote wound healing.
- Level 4 pathology of wound healing is because of dysfunction at the molecular and cellular levels. Proliferation, cell division, cellular senescence, neutrophil migration, and epigenetics (Yang et al., 2020), hypoxia and reperfusion injury are some of the factors associated with delayed wound healing. Dysregulation of local signals, growth factors and oxidative stress are implicated.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area/site

This research was carried out at Murang'a Level 5 Hospital over a period of 6 months. This is a public hospital in Murang'a County, Central Kenya region located at latitude 0.7180443636466275 and longitude 37.16071899705351 (plus code 75J6+M7 Murang'a). It has a high population of 942,581 (CHS, 2017). Murang'a Level 5 Hospital is the referral hospital that delivers medical services including laboratory services to both local and external referral cases from within and outside the county. It is among the country's largest and most well-equipped hospital. It has a bed capacity of three hundred and thirty excluding ICU which has thirty-five beds in its capacity. An average of nine hundred patients per day are served in this hospital. Its infrastructures include a laboratory for clinical microbiology, parasitology, serology, haematology, chemistry and cytology test analysis. All these amenities make the hospital the best option to carry out the research work.

3.2. Study design

It was a cross-sectional, descriptive study done in a hospital with the aid of the questionnaire as the data collection tool. A consecutive random sampling method was used to obtain wound swabs from patients who showed their consent by signing.

3.3. Study population

Patients with visible chronic wound infections attending Murang'a Level 5 hospital were given general information about the study's risks and benefits, confidentiality, and their freedom to decline participation, as part of the consent process. Patients were then enrolled in the trial after giving written and signed agreement.

3.4. Sample size determination

This study sample size was approximated based on the formula for qualitative variable (Charan & Biswas, 2013) to determine the proportion of participants with chronic wound infections.

$$Sample\ size\ (n) = \frac{Z^{2\cdot}P(1-p)}{d^2}$$

Where:

n is the number of subjects required

z^2= Is the standard normal variant (at 5% type 1 error (P<0.05) it is 1.96 and at 1% type 1 error (P<0.01). As in many studies P values are considered significant when below 0.05, hence 1.96 is used in the formula.

p = Expected proportion in population based on previous studies.

d = Absolute error or precision i.e., 0.05

Thus:

$$Sample\ size = \frac{1.96^2 x 0.22(1-0.22)}{0.05^2} = 263$$

Thus, 263 participants were required. To account for dropouts, a 15% margin was permitted allowing for a total of 300 (SD±2) participants to be recruited.

3.5. Sampling procedure

This was a consecutive random sampling technique where patients presenting with visible chronic wounds at the Murang'a level 5 hospital were considered to take part in the research.

3.6. Inclusion and exclusion criteria

3.6.1. Inclusion criteria

Clients with visible wounds which have stayed over 4 weeks, equal or above the legal age of 18 years, with prior written consent were incorporated into the research. Minors (below eighteen years old) were recruited in the research only after a signed consent was obtained from their adult guardians.

3.6.2 Exclusion criteria

Clients who were lacking any visible wounds were excluded in this study, who declined to give a prior written consent or were minors without a consenting adult guardian.

3.7. Laboratory techniques

3.7.1 Specimen collection and processing

Cleaning of the wound was done using saline to get to the wound base. Wound swabs were sampled by qualified medical personnel following the standard operating procedures on sample collection at Murang'a Level 5 Hospital, placed in a container which was sterile and had amies transport media for preservation of fastidious organism. Labelling of the swabs was done with the same identification unique number as on the patient's consent form. Three hundred swabs were collected. Out of these, sixteen swabs were from children below 18 years while two hundred and eighty-four were from adults. They were then placed in a cooler box that had a temperature of 8^0c and below for preservation of the organisms. Swabs were delivered to the Medical Microbiology laboratory in an hour or two after collection for identification analysis, culture and susceptibility testing. At every stage, precautions were taken to prevent cross-contamination. In the Laboratory, to differentiate Gram positive from Gram negative bacilli or cocci, Gram stain test was carried out.

Social demographic data of the patients was captured using structured questionnaire forms. It included age, gender, weight, occupation, the wound's cause or genesis and its endurance and whether they are formally or informally employed. Also, it captured whether they have taken antibiotics in the last 3 weeks.

3.7.2 Identification of colonizing bacteria

The culture media both MacConkey and sheep blood agar were prepared as indicated in the appendix I, autoclaved, transferred to Petri dishes and left to cool. Sheep blood agar is enriched type of media suitable for most organisms. The MacConkey agar (MAC) is a differential and selective type of culture media used to inhibit growth of the Gram-positive colonies and differentiate lactose from non-lactose

fermenters organisms. To create single colonies, the inoculum was applied then spread across using a sterile wire loop. Each sample cultured on sheep blood agar and MAC agar was aerobically incubated for 24 hrs at 37°C. In case there is no growth obtained, blood agar media was re-incubated to a maximum of 48 hrs. Gram stain and a number of biochemical tests were used to identify the bacterial isolates including API 20 E.

3.7.2.1. Gram stain

This is the most important and widely applied staining technique in bacteriology especially in microbiology related to medicine. It is typically the first test performed on bacteria in order to examine and identify them. By observing their staining characteristics and properties, pathogens are identified using this Gram staining method. The basis of their variation is in the differences of composition and structure of bacterial cell walls (Alsharari et al, 2021). Bacteria with thick peptidoglycan layer on their cell walls will appear violet or purple because it will retain the primary stain resisting decolorization. When decolorizing, bacteria with a thin peptidoglycan layer and poor cross-linkage lose their primary stain and acquire counterstain, which makes them appear red or pink (Kisoi, 2021).

Dried smears were heat fixed, flooded with crystal violet for the maximum of 1 minute and rinsed with water. Gram iodine was flooded then rinsed with clean water. Decolourization was done with acetone followed by counter staining with neutral red for a maximum of 2 minutes and rinsed with water. The smear was air dried and observed under the microscope. Gram negative bacteria turned reddish or pinkish, while Gram positive bacteria retained their crystal violet color.

3.7.2.2. Biochemical Tests

All procedures are illustrated in appendix I.

Indole test

This test was conducted on bacterial isolates to ascertain the organism's capacity to transform tryptophan into indole. Tryptophanase enzyme is responsible for an organism's capacity to separate indole from the amino acid tryptophan. When tryptophanase is present, tryptophan which is an amino acid goes through deamination and hydrolysis. Indole is produced when tryptophan undergoes reduction deamination through the intermediary molecule indole pyruvic acid (Clinton, A., & Carter, T. 2015). The appearance of crimson or reddish-violet color on the surface of the broth's alcohol layer indicates positive results while yellow color indicated negative results.

The Triple Sugar Iron test

It is a unique medium containing sodium thiosulfate, ferrous sulfate, phenol red, 1% lactose, 1% sucrose, and 0.1% glucose, among other sugars that combine to form a pH-sensitive dye. When all of these ingredients are mixed together and allowed to settle at an angle, an agar test tube with a slanted angle is created. The tilted architecture of this medium provides various surfaces that are exposed to oxygen-containing air either totally or partially, allowing for the determination of the fermentation patterns of various organisms. The TSI agar is intended to differentiate among various species based on differences in their hydrogen sulfide production and fermentation processes of carbohydrates (Eriksson, et al 2022). Carbohydrate fermentation is indicated by the production of gas and a change in the pH indicator's color from red to yellow. Because of the buildup of acid during fermentation, the pH falls. When acids are produced, the carbohydrate medium's orange-red color changes to yellow, and this is indicated by the addition of phenol red, an acid-base indicator. Hydrogen sulfide formation is detected by the medium's ferrous ammonium sulfate and sodium thiosulfate content, which results in the tube's butt turning black. After the limited glucose is depleted, the organisms will start utilizing

sucrose or lactose. The principal purpose of the test was to distinguish Members of the Enterobacteriaceae from different Gram-negative bacteria (Najma, et al, 2021). Additionally, it was used to distinguish between various Enterobacteriaceae based on how they fermented sugar.

Observed Results

• A red slant and yellow butt reaction, indicated an alkaline/acid reaction which was specific for the fermentation of dextrose only.

• The fermentation of glucose, lactose, and sucrose was shown by an acid/acid response (yellow slope, yellow butt).

• The absence of carbohydrate fermentation was demonstrated by an alkaline/alkaline response (red slant, red butt).

• The presence of H_2 was indicated by the black colour.

• Fissures or bubbles in the agar revealed the presence of gas (CO2 and H2).

Voges Proskauer

The VP test is a biochemical method used to determine whether bacteria can convert pyruvate into acetoin, also known as acetylmethylcarbinol, a neutral intermediate product. When KOH (potassium hydroxide) and oxygen (O2) are present, produced acetoin will oxidize to diacetyl (Binsuwaidan, et al, 2023). Therefore, in the presence of alpha-naphthol, the produced diacetyl will react with the guanidine component of peptone to form a polymer that can range in color from pink to red. It was carried out on the isolates in order to identify the pathways leading to the production of acytyl Methyl carbinol, an end product that's neutral reacting. Positive outcome was shown by a cherry red color over the medium's surface, whereas a negative result was shown by a yellow-brown color over the medium's surface or by the creation of the copper color.

Citrate Utilization

It was performed to evaluate how well an organism might use citrate as a fuel source. Inorganic ammonium salt is the only source of nitrogen in the media; citrate is the only source of carbon. Alkalinity is increased when bacteria metabolize citrate, converting the ammonium salt to ammonia. The PH shift caused the bromothymol blue indicator in the medium to change from green to blue.

Urease test

It was performed on the isolates to identify those possess urease enzyme. An enzyme called urease is present in certain bacteria, and when water is present, it can split urea and release carbon dioxide and ammonia. When ammonia, carbon dioxide, and water combine to form ammonium carbonate, the medium turns alkaline (Celestin, 2020). As a result, the indicator phenol red turns vibrant pink instead of its initial orange-yellow color.

Catalase test

The catalase test is essential for distinguishing between catalase-positive Micrococci and Staphylococci from catalase-negative Streptococci. This test is also useful in differentiating between obligatory anaerobes from aerobic species. Enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water (Pokharel, et al, 2019). A colony inoculum added to hydrogen peroxide caused oxygen bubbles to rapidly develop, indicating the presence of the enzyme in the isolated bacterial species. A weak or lack of bubble formation indicated the absence of catalase.

Coagulase test

It was performed to distinguish between S. aureus that produce coagulase enzyme from S. saprophyticus and S. epidermis which are coagulase negative. Coagulase is a thermostable thrombin-like protein that functions as an enzyme, splits fibrinogen to fibrin forming clumps or clots as the end product (David, et al, 2017). A drop of physiological saline, approximately 10 ul was placed on a slide.

To create a smooth, milky-colored suspension, using an inoculating loop, colonies from a fresh culture were collected and emulsified into the saline. A fresh human plasma drop was put to the slide. Agglutination indicated positive results while absence of agglutination indicated a negative test result (Alsharari et al, 2021).

Other biochemical tests carried out on Gram negative bacteria included API 20E, Oxidase, Sulphur reduction and Motility among others.

3.7.3 Antimicrobial susceptibility testing.

Antibacterial sensitivity testing of bacteria growth was analysed applying the Kirby–Bauer disk diffusion sensitivity testing (Hamdy Mohammed et al., 2016) on Muller Hinton Agar (Oxoid, UK). A pure colony was suspended in normal saline to produce a turbidity equivalent to MacFarland standard of 0.5 and uniformly spreaded on a Muller Hinton Agar (MHA). The selection of antibiotic agents was made using both widely accessible medications and drugs that clinicians commonly prescribe. The isolated and accurately identified bacteria were evaluated for sensitivity to ampicillin (10µg), penicillin (10 units), cefoxitin (30µg), amoxiclav (10µg), tetracycline (30µg), azithromycin (15µg), gentamicin (10µg), ciprofloxacin (5µg), cotrimoxazole (25µg), vancomycin (30µg), ciprofloxacin (5µg), levofloxacin (5µg), doxycycline (30µg), clindamycin (2µg), ceftriaxone (30µg), cefepime (30µg), ceftazidime (30µg), tobramycin (10µg), meropenem (10µg), aztreonam (30µg), amikacin (30µg), cefotaxime (30µg), cefuroxime (30µg) and piperacillin/tazobactam (100/10µg) Oxoid, UK. The MHA was then aerobically incubated at 37^0c for 18-24 hours. Susceptibility, intermediate and resistance information was regarded in terms of zone of inhibition (mm) based on the Clinical and Laboratory Standards Institute (CLSI) guideline (Yitayeh et al., 2021)(Karita et al., 2009).

3.7.4. Phenotypic confirmatory test for ESBL producing strains

It has been stated by Clinical and Laboratory Standards Institute (CLSI) that cephalosporin/clavulanate combination disk to be used to confirm the ESBL producing strains. Ceftazidime disk (30ug) with

clavulanate (10ug) and another one without clavulanate were used on Mueller Hinton agar. A 5mm difference between the zone diameters of ceftazidime disk and their corresponding ceftazidime/clavulanate disk was assumed to be phenotypic confirmatory test for ESBL production. Furthermore, Clinical and Laboratory Standards Institute reviewed that all organisms that test positive for ESBLs must be reported as resistance to all cephalosporins except (cefoxitin, cefotetan and cephamycin) and to aztreonam irrespective of routine susceptibility results.

3.7.5 Screening for MRSA strains

The gold standard for detecting MRSA in clinical specimens is Gram staining, culture and susceptibility testing, this is according to CLSI standard M100. Cefoxitin disk diffusion test was performed by placing cefoxitin (30ug) on MHA plate. Inhibition zone was evaluated after 24 hours of incubation at 37^0c. The cleared zone was translated as indicated in CLSI guidelines.

Sensitivity = or > 22mm

Resistant = or < 21mm

3.8. Quality control

Data on patient demographic and laboratory analyses was evaluated for accuracy, consistency, completeness. To ensure high quality research output, standard microbiological methods were observed to provide accurate and precise laboratory test results. American Type Culture Collection (ATCC) standard reference strains (E. coli ATCC-25922, E. coli ATCC 35218 for β-lactams, S. aureus ATCC-25923, and Pseudo aeruginosa ATCC 27853) obtained from National Microbiology Reference Laboratory (NMRL) were utilized to confirm the efficacy of the culture media and antibiotic drug disc potency. During the period of research, the principal investigator also analysed Kenya National External Quality Assessment scheme (KNEQAS) samples and she scored 100% on all samples inclusive of pus swab. Standard operating procedures were followed to avoid pre-testing, testing and post-testing mistakes. Murang'a Microbiology Laboratory is ISO 15189:2012 certified.

3.9. Data management

To avoid duplicating the data, a unique serial code was given to each study proforma. Every completed assessment form was secured with a lock. Data collected was entered in the Microsoft excel sheet and protected with secured password. When the study was completed, the information entry was cross checked with the hard copy for any inconsistencies and cleaned for missing values and erroneous entries.

3.10. Data analysis and presentation

The csv files were exported, and data analysed using GraphPad Prism 6 statistical software statistics for Windows. All p-value <0.05 were regarded to be significant in terms of statistics and all tests carried out were chi square two tailed. Basic descriptive statistics such as evaluation of colonizing bacteria isolates from chronic wounds were illustrated using tables, pie charts, and graphs accordingly. Zones of inhibition between the susceptible and resistant isolates of chronic wounds were evaluated using the student's t-test. The P-value for risk factors associated with chronic wound infections was determined using the Chi-square test. Predisposing variables like smoking, coexisting infections, diabetes, use of steroids, obesity and immunosuppressive medication use among others such as stress and metabolic syndromes escalating patients to chronic, non-healing wounds was evaluated from the questionnaire form. Age was presented as mean, mode and median.

3.11. Ethical considerations

Research was conducted in accordance with the Declaration of Helsinki. Permission to conduct research with human participants was acquired from the Institutional Research ethics Committee (IREC) of Mount Kenya University (MKU), The National Commission of Science, Technology and Innovation (NACOSTI), and the Murang'a Level 5 Hospital. All respondents and caregivers of participants under the legal age of eighteen provided written informed permission prior to the study. The data collected from this survey is kept private and used exclusively for the investigation. The attending physicians were informed of positive results for the appropriate treatment of the patients.

CHAPTER FOUR

RESEARCH FINDINGS AND DISCUSSION

4.1 Social demographics of the study subjects

The study had a total of 300 study participants constituting of 153 (51%) males and 147 (49%) females. The frequency of chronic wounds infections was determined as per gender of the patients as shown in the figure 4.1 below. More males had chronic wounds infection than females at 51%. However, the frequency of chronic wounds did not differ significantly with gender, CI= 95%, P>0.05

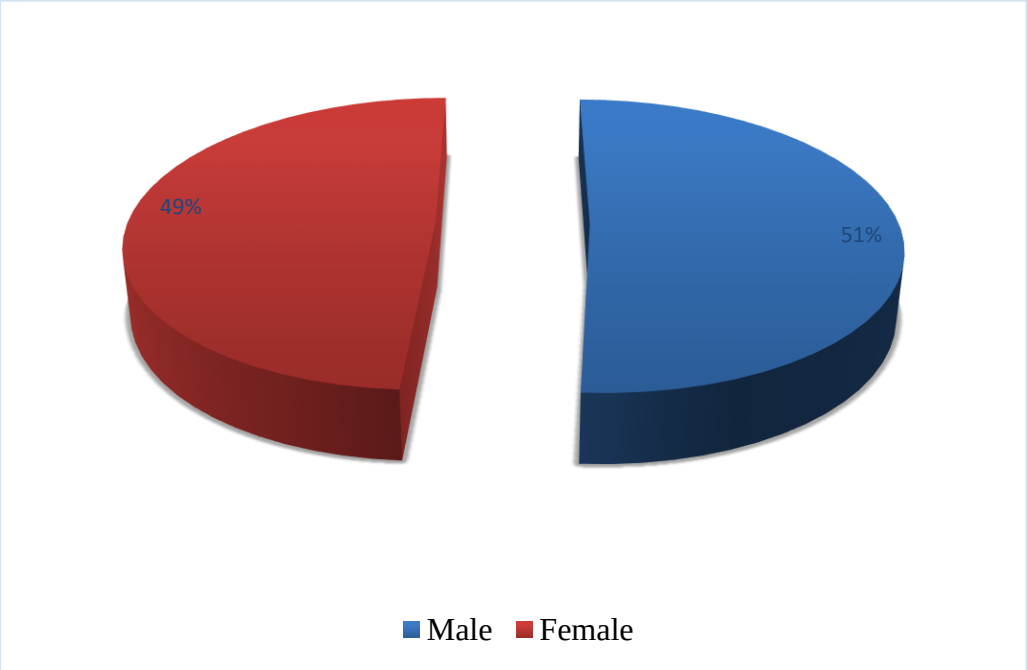

Figure 4.1: Prevalence of infectious chronic wounds in males and female.

Prevalence of infectious chronic wounds in patients attending Murang'a Level 5 Hospital was determined across age groups as shown in figure 4.2 below. The youngest participant had 2 months while the most aged participant had 92 years. These two participants were of the female gender. The

group of 31-40 years had the greatest prevalence on chronic wounds infections with 53 participants. The mode age was 40 years with 12 participants. Median age was 43 years while the mean was 45 years. However, the prevalence of chronic wounds did not differ significantly across the age groups, df =10, CI = 95%, P > 0.05

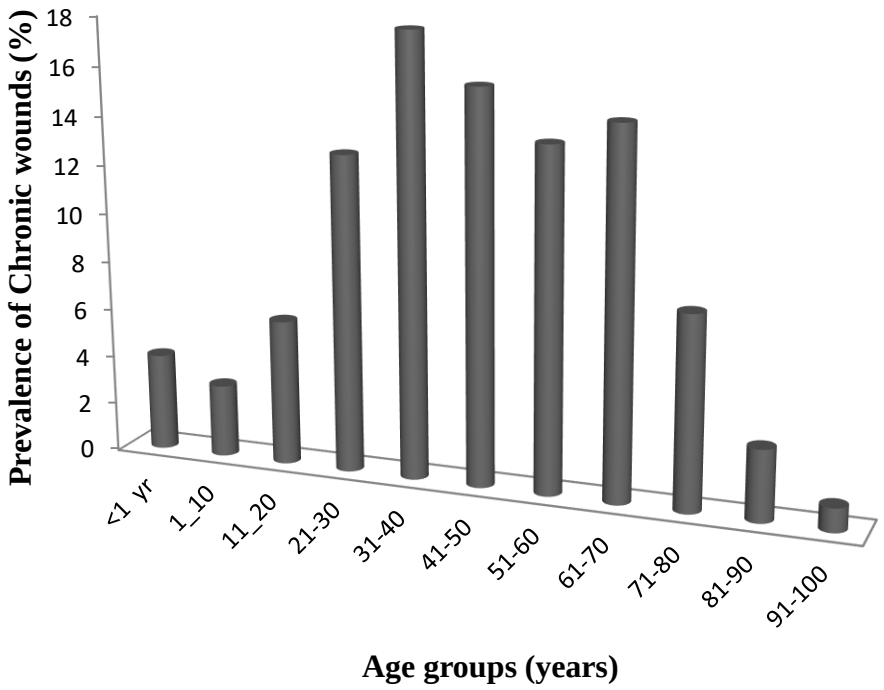

Figure 4.2: The prevalence of chronic wounds per age group

The characteristics descriptions of these study subjects were as follows: informally employed were 114 (38%), farmers were 110 (37%), children were 27 (9 %), elderly were 27 (9%), disabled were 03 (1%), students were 16 (5 %) and employed were 03 (1%).

One hundred and sixty four 164 (55%) study participants did not have any other pathological disorder either primary or secondary to chronic wound infection. The other one hundred and thirty six 136 (45%) had another primary or secondary pathological disorder (comorbidities) to the chronic wound

infection. These pathological disorders included: HIV 28 subjects, diabetes 56 subjects, hypertension 68 subjects, cancer 3 subjects, alcoholism 2 subjects, TB 1subject and osteoarthritis 12 subjects.

4.2 Quality controls of the study

To monitor quality, efficacy and potency of the culture media, sensitivity drugs and other utilities like normal saline, the principal investigator analysed quality controls every two weeks throughout the data collection and analyse period. While sterilising, autoclaving tape and *Bacillus stearothermophillus* were included to monitor the autoclave condition. In every bunch of media plates prepared, two plates were tested for any contaminant by incubating them at 37^0c for 48 hours. To ensure high quality research output, routine bacteriological techniques were practiced to produce accurate and precise findings. American Type Culture Collection (ATCC) standard reference strains (*E. coli* ATCC-25922, *E. coli* ATCC 35218 for β-lactams, *S. aureus* ATCC-25923 and *Pseudomonas aeruginosa* ATCC 27853) were employed to confirm culture media's efficacy and antibiotic disc potency. Quality control results were within the acceptable ranges.

4.3 Bacteria species that colonizes the chronic wounds.

Wound swabs specimens were received in the microbiology laboratory with a request for culture and sensitivity analysis. Those Samples taken from wounds were cultured on SBA and MAC culture plates after collection from the study participants based on the laid down standard operating procedure. Out of the 300 wound swabs culture specimens received, 244 (81.3%) after being cultured had different bacterial growth obtained. 38 from those 244 wound swabs had 2 bacteria species. 56 (18.7%) specimens after being cultured had either no growth or no significant growth obtained. All culture plates of wound swabs which grew 3 and above bacteria species were considered to be contaminated. The gender composition among those whose wounds had bacterial infection were 132 (54.1%) males and 112 (45.9%) females.

Amount of 15 distinct bacteria species were identified from cultures of 244 study participants. These bacteria isolated were as follows**:** *Staphylococcus aureus* was isolated in 84 (29.7 %) out of the 244

wound swabs emerging the most prevalence bacteria in chronic wounds of patients attending Murang'a level 5 Hospital. It was followed by *Pseudomonas aeruginosa* at 46 (16.3%) which is also the highest among gram negative bacteria. *Streptococcus agalactiae* was the least isolated, just 1 (0.3%) isolate as demonstrated below in the table 4.1. The prevalence of the isolated bacteria was not significantly different from each other df = 14, p > 0.05.

Table 4.1: The distribution of the isolated bacteria colonizing chronic wounds and their Gram stain.

NO	BACTERIA	GRAM STAIN	FREQUENCY	PREVALENCE (%)	P VALUE
1.	*Staphylococcus aureus*	POS	84	29.70%	
2.	*Pseudomonas aeruginosa*	NEG	46	16.30%	> 0.05
3.	*E. coli*	NEG	43	15.20%	
4.	*Proteus mirabilis*	NEG	38	13.40%	
5.	*Klebsiella pneumoniae*	NEG	17	6.00%	
6.	*Acinetobacter baumannii*	NEG	16	5.60%	df = 14
7.	*Proteus vulgaris*	NEG	13	4.60%	
8.	*Klebsiella oxytoca*	NEG	7	2.40%	
9.	*Streptococcus pyogenes*	POS	5	1.70%	
10.	*Coagulase Neg Staph*	POS	3	1.00%	

11.	*Citrobacter koseri*	NEG	3	1.00%
12.	*Citrobacter freundii*	NEG	2	0.70%
13.	*Enterobacter cloacae*	NEG	2	0.70%
14.	*Serratia marcescens*	NEG	2	0.70%
15.	*Streptococcus agalactiae*	POS	1	0.30%
	TOTAL BACTERIA ISOLATED		282	100.00%

The profile of bacteria species that colonizes the chronic wounds was determined as shown in the figure 4.3 below. However, the prevalence of the isolated bacteria was not significantly different from each other df = 14, $p > 0.05$.

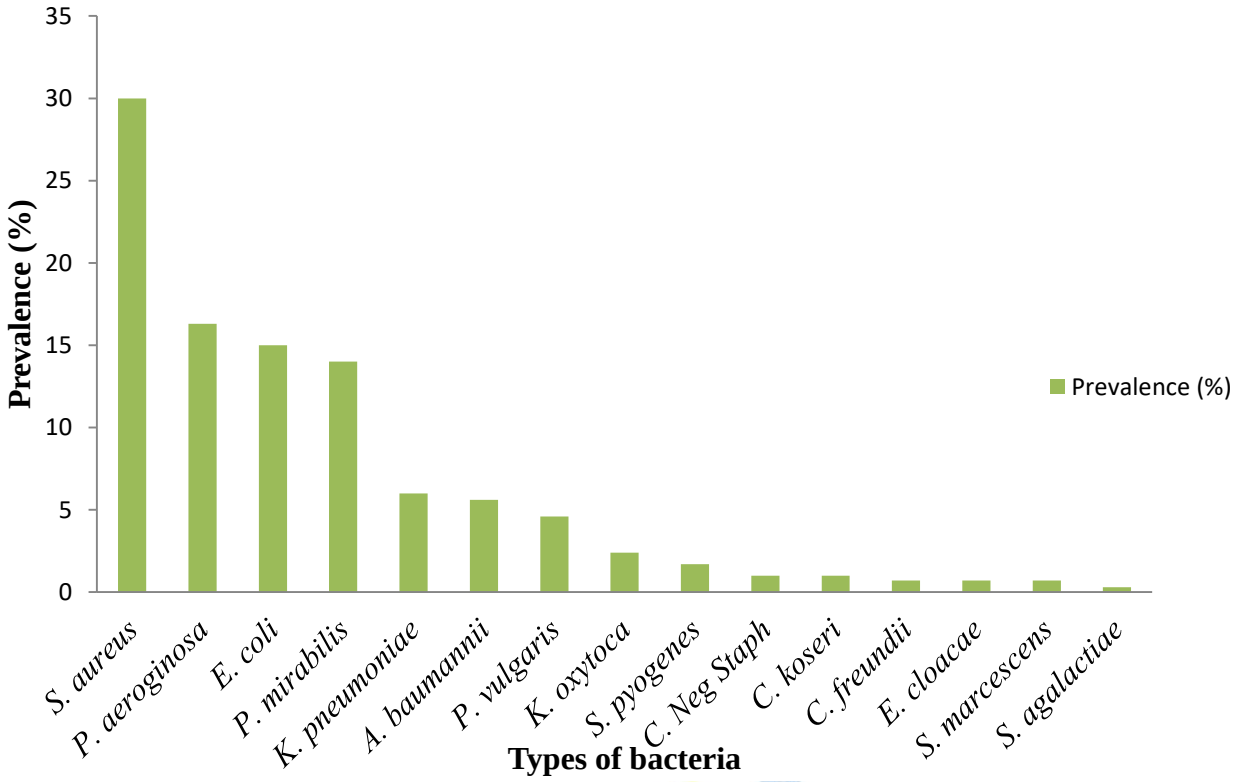

Figure 4.3: Evaluation of bacteria species that colonizes the chronic wounds.

Prevalence on Gram-negative and Gram-positive bacteria species from chronic wounds in patients attending Murang'a Level 5 Hospital was determined.

Most of the isolated bacteria were Gram negative at 188 (67%). Gram positive bacteria were 94 (33%) as shown in figure 4.4 below. However, the prevalence of Gram negative bacteria was not significantly different from that of Gram positive bacteria. CI = 95%, P > 0.05.

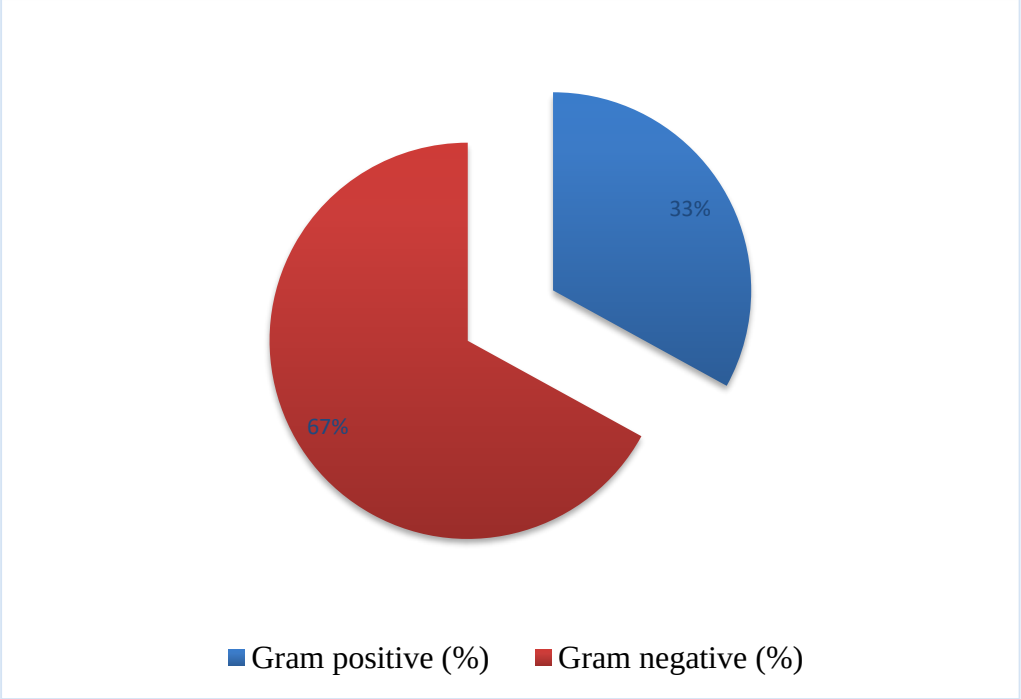

Figure 4.4: Prevalence of Gram positive and Gram negative bacteria isolate.

4.4. Antimicrobial susceptibility patterns of bacteria isolates from chronic wounds.

All identified isolates were subjected to a galaxy of antibiotics drug disks to evaluate their antimicrobial susceptibility and resistant patterns. The outcome revealed that some antibiotics were sensitive and others were resistance to the subjected microbial organisms. The antimicrobial drug susceptibility testing outcome of the current study is as follows: Out of 84 *Staphylococcus aureus* isolated, 89.5% were sensitive to gentamycin, 38.0% were sensitive to co-trimoxazole, 64.9% were sensitive to tetracycline, 77.3% were sensitive to cefoxitin, 65.4% were sensitive to clindamycin, 47.5% were sensitive to azithromycin, 96.2% were sensitive to doxycycline, 50.0% were sensitive to Augmentin. Penicillin emerged the poorest in sensitivity at 15.2% while levofloxacin was the best at 100% as summarised in the table 4.2 below. However, the sensitivity of *S. aureus* to an array of antibiotic drugs was similar. CI = 95%, P>0.05.

Table 4.2: Evaluation of *S. aureus* susceptibility pattern

ANTIBIOTIC	**SENSITIVE (%)**	**INTERMIDIATE (%)**	**RESISTANT** (%)
Levofloxacin	100	0	0
Gentamicin	89.5	3	7.5
Co-trimoxazole	38	7	55
Tetracycline	64.9	8	27.1
Cefoxitin	81	_	19
Clindamycin	65.4	4	30.6
Azithromycin	47.5	6	46.5
Doxycycline	96.2	1	2.8
Augmentin	50	5	45
Penicillin	15.2	2	82.8

NOTE: All QC results were within the acceptable range

Out of 46 *Pseudomonas aeruginosa* isolates, 44% were sensitive to ceftriaxone, 73.1% were susceptible to ceftazidime, 50% were susceptible to cefotaxime, 63.4% were susceptible to ciprofloxacin, 81.8% were susceptible to cefepime, 97.2% were susceptible to meropenem, 96.4% were sensitive to amikacin, 62.5% were susceptible to aztreonam, 81.0% were sensitive to piperacillin/tazobactam, 71.4% were sensitive to gentamycin as shown in figure 4.5 below.

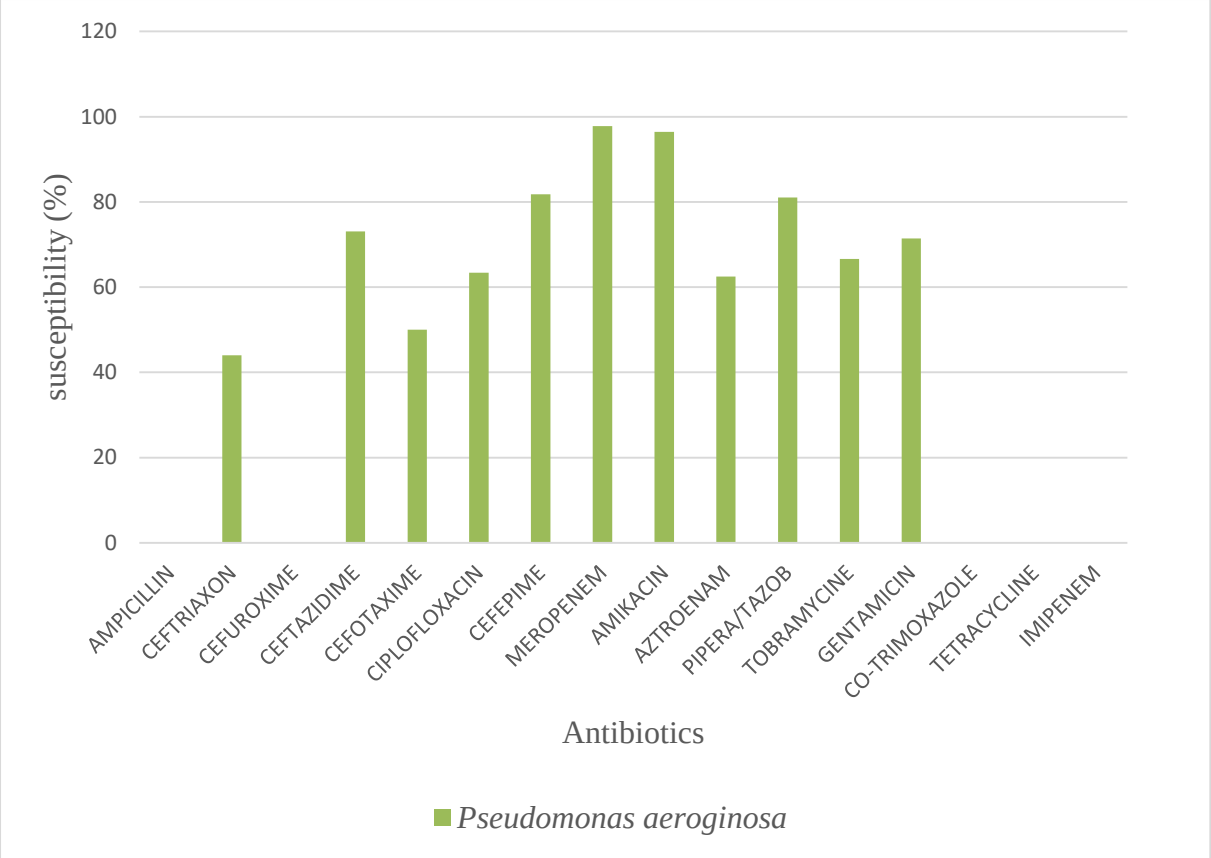

Figure 4.5: Sensitivity pattern for *Pseudomonas aeruginosa*

Out of 43 *E. coli* isolates obtained from the study, 22.2% were sensitive to ampicillin, 39.2% were sensitive to ceftriaxone, 33.3% were sensitive to cefuroxime, 46.8% were sensitive to ceftazidime, 46.1% were susceptible to cefotaxime, 58.0% were sensitive to ciprofloxacin, 63.8% were sensitive to cefepime, 100% sensitive to meropenem, 90.3% were sensitive to amikacin, 84.6% sensitive to piperacillin/tazobactam, 30.0% were sensitive to tobramycin, 78.5% were sensitive to gentamycin, 6.2% were sensitive to cotrimoxazole, 45.8% were sensitive to tetracycline, 33.3% were sensitive to doxycycline, 71.4% were sensitive to imipenem.

Out of 38 *Proteus mirabilis* isolates, only 9.0% were sensitive to ampicillin emerging the most resistant drug. 44.4% were sensitive to ceftriaxone tying the sensitivity with cefotaxime, 50.0% were sensitive to cefuroxime, 51.7% were sensitive to ceftazidime, 69.2% were sensitive to ciprofloxacin, 47.0% were sensitive to cefepime. Meropenem was 100% sensitive followed by amikacin at 94.1%.

Piperacillin/tazobactam and gentamycin came third at 87.5%. Tobramycin was sensitive to 14.2% while co-trimoxazole had 40.0%. Tetracycline balanced both sensitivity and resistant at 50.0%. Imipenem was able to clear 73.3% of the isolates as demonstrated in table 4.3 below. The susceptibility prevalence was significantly different from each other $F_{15}=6.16$, $P < 0.05$.

Table 4.3: Evaluation of Gram-negative bacteria's sensitivity pattern

	Pseudomonas aeruginosa	*E. coli*	*Proteus Mirabilis*	P VALUE
No. of isolates	46	43	38	
Meropenem	97.2%	100%	100%	
Ampicillin	-	22.2%	9.0%	
Ceftriaxone	44.0%	39.2%	44.4%	< 0.05
Cefuroxime	-	33.3%	50.0%	
Ceftazidime	73.1%	46.8%	51.7%	
Cefotaxime	50.0%	46.1%	44.4%	
Ciprofloxacin	63.4%	58.0%	69.2%	
Cefepime	81.8%	63.8%	47.0%	$F_{15} = 6.16$
Amikacin	96.4%	90.3%	94.1%	
Aztreonam	62.5%	-	-	
Piperacillin/Tazobactam	81.0%	84.6%	87.5%	
Tobramycin	66.6%	30.0%	14.2%	
Gentamycin	71.4%	78.5%	87.5%	
Co-trimoxazole	-	6.2%	40.0%	
Tetracycline	-	45.8%	50.0%	
Imipenem	-	71.4%	73.3%	

4.5. Evaluation of antimicrobial resistance strains

Out of 84 *Staphylococcus aureus* obtained from chronic wounds, 19 (22.6%) were methicillin resistance *S. aureus* (MRSA) strains. They were considered resistant after showing a clearance zone of < or = 21mm in cefoxitin. 5 (5.95%) were induced clindamycin resistance (ICR) strains. 2 (2.38%) had both MRSA and ICR strains. In all Enterobacteriaceae isolated, 19 (16.23%) were ESBL (Extended spectrum beta-lactamase) producing strains. *Escherichia coli* accounted for 10 ESBL strains while the rest 9 ESBLs were from *Klebsiella pneumoniae* as shown in the figure 4.6 below. All ESBLs producing Enterobacteriaceae were revealed to be highly resistance to third generation cephalosporins (ceftazidime, ceftriaxone and cefotaxime), Augmentin and ampicillin. Meropenem which is a carbapenem type of drug was the only drug disk that showed 100% sensitive to all ESBLs in other words, it had zero resistance to all ESBLs.

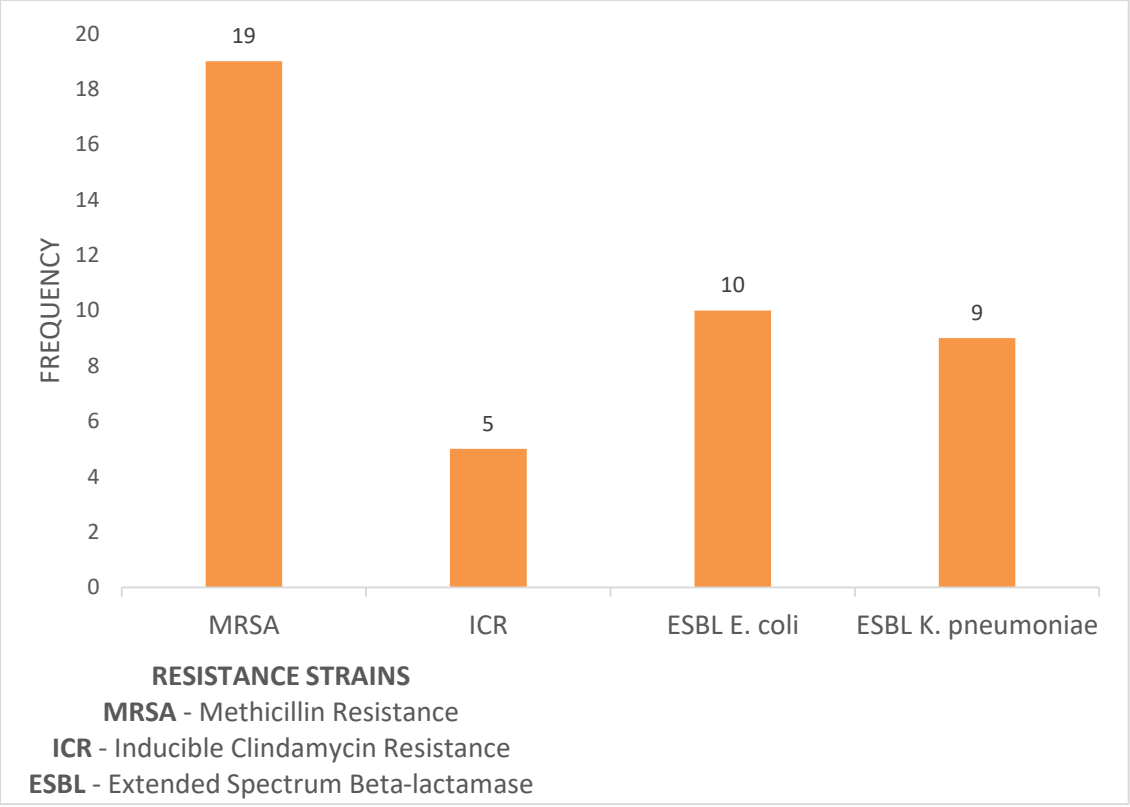

Figure 4.6: Evaluation of resistant strains

4.6 The risk factors associated with chronic wound infections

Most participants had one or more comorbidities. Hypertension was the most common comorbidities at 66 (40%) among the participants, diabetes followed at 56 (33%). Human immunodeficiency virus (HIV) came third at 28 (16%). Participants who had osteoarthritis were 12 (7%) while those who had malignancy were 3 (2%). Those who were addicted to alcohol (alcoholism) were 2 (1%) and the comorbidity which had affected the least was mycobacterium tuberculosis (TB) 1(1%). The distribution of these risk factors was determined as illustrated in figure 4.7 below. There was a significant difference among the comorbidities associated with chronic wounds infection $F_{13} = 31$, $p = 0.0001$.

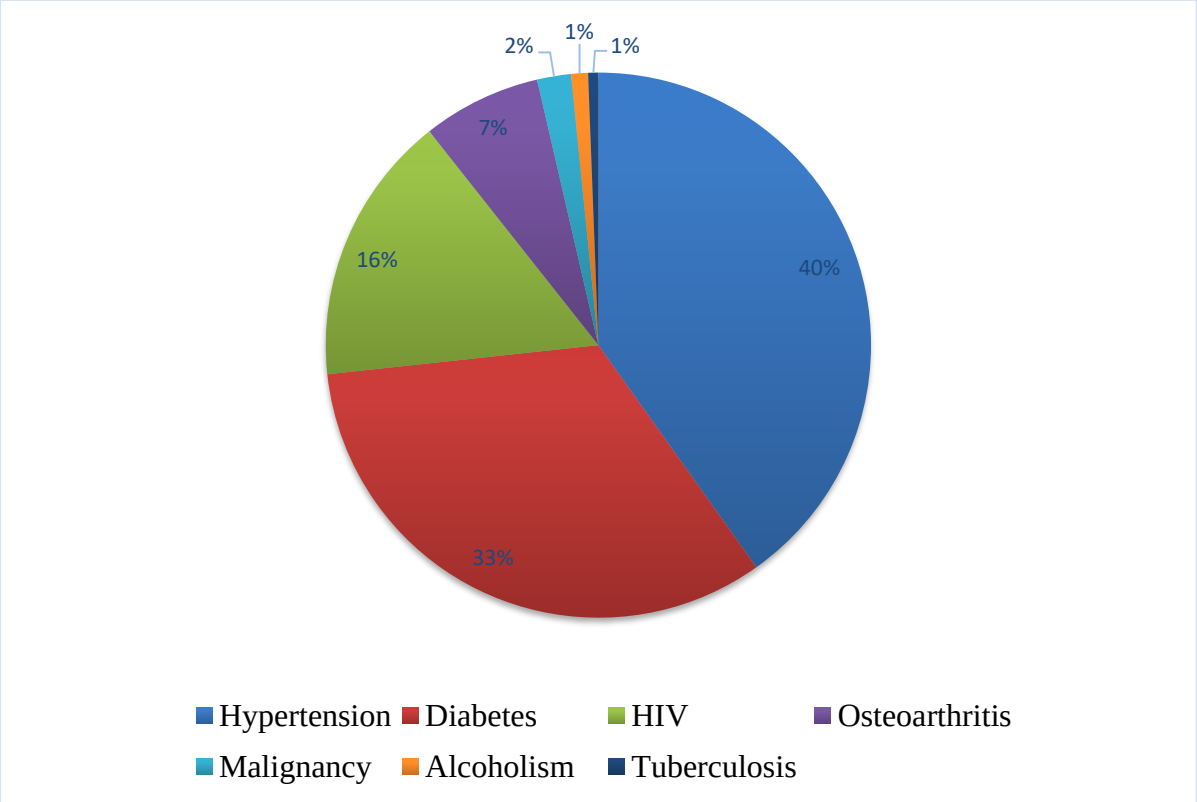

Figure 4.7: The prevalence of comorbidities in chronic wounds

4.7 The origin/causes of chronic wounds

The study established 16 different causes/origin of chronic wounds among the study subjects. Most of the participants 66 (22%) their wounds originated from cellulitis/diabetic foot. 54 (18%) had accident whose bruises progressed to be chronic wounds. 45 (15%) their wounds originated from an abscess followed closely by those who had a cut 43 (14%). Surgical site infections which failed to heal after 1 month were 25 (8%) and those who had different types of traumas were 19 (6%). Bed sores from bed ridden patients were 10 (3%) while those who had different type of incisions were 9 (3%). Bites inclusive of snakes, humans, dogs and rats were 8(2%) and those whose burns failed to heal within 1 month were 6 (2%). Those whose pimples progressed to chronic wounds were 4 (1.5%). Necrotising fasciitis and gangrenes tied at 3 (1.5%) each while ulcers and fractures also tied at 2 (1%). There was only 1 (0.5%) participant whose wound originated from a gunshot as shown in the table 4.4 below. However, the frequency of sources of chronic wounds had no significant difference among the patients, df=15, $p > 0.05$.

Table 4.4: Evaluation of origin of chronic wounds

S. NO	ORIGIN OF THE CHRONIC WOUND	FREQUENCE N=300	PREVALENCE (%)	P VALUE
1.	Cellulitis	66	22	
2	Accidents	54	18	
3	Abscesses	45	15	
4	Cut	43	14	
5	SSI	25	8	
6	Trauma	19	6	> 0.05
7	Bed sores	10	3	
8	Incisions	9	3	

9	Bites	8	2	
10	Burns	6	2	df = 15
11	Pimples	4	1.5	
12	Necrotising fasciitis	3	1.5	
13	Gangrenes	3	1.5	
14	Ulcers	2	1.0	
15	Fractures	2	1.0	

4.8: DISCUSSION

A chronic wound occurs when subcutaneous tissue is exposed for an extended period of time after skin integrity is lost. This prolonged exposure creates a warm, highly nutritive environment which favors the settlement and growth of microbes. The solemn purpose of this study was to investigate the bacteria isolates colonizing chronic wounds, the antimicrobial susceptibility patterns and the risk factors associated with chronic wounds in patients attending Murang'a Level 5 Hospital.

Microbiological analysis of chronic wounds in this study revealed a prevalence of 81.3% infection rate which is slightly less than 92% prevalence of the research study conducted in Uganda by (Wangoye et al., 2022). Conversely, however, the prevalence in this research is higher than in a Chinese study, where the prevalence was 63.9% (Guan et al., 2021). In developed nations such as Germany, the prevalence of chronic wounds is typically as low as 7.8% (Raeder et al., 2020). This is due to availability of affordable improved medical facilities and patients seek medical attention at early stages of wound infections. Nonetheless, the results of this investigation concur with a review study carried out in the United Kingdom by (Howell-Jones RS et al., 2019) where they had a chronic wound prevalence of 82%. The high overall prevalence observed through standard culture methods suggested that the samples collected contained more aerobic than anaerobic organisms, which were not cultured

because of funding and equipment shortages. The distinction between this study and others may result from differences in study design, geographical location and social economic status of the population.

The social demographic in this research revealed that the most affected gender was male with a prevalence of 51% as compared to their female counterpart who had a prevalence of 49%. This is in agreement with several studies which showed that men are most prone to chronic wounds probably due to heavy manual job they do exposing them to injuries. Even though this study is in keeping with others, the prevalence of male was lower in comparison to a research conducted in China by (Zhou et al., 2022), which had a prevalence of 60.8%. This study is in contrary to a study done in Brazil where female had high prevalence of 57% as compared to male (da Rosa Silva et al., 2017).

The average age group in the study was 31 – 40 years which had 53 participants while the mean age was 45 years. Similarly, a study of chronic wound infections done in Ethiopia revealed a high prevalence of chronic wounds at the age of 40 years (Mohammed et al., 2017). However, studies from developed nations show that the aged population has a higher prevalence of chronic wounds contrary to this study (Gupta et al., 2021). This difference is highly contributed by the socioeconomic status of middle aged in our country where majority are manual labourers. In fact, out of 300 participants in this study, only 03 (1%) were formally employed.

Objective I: Bacteria species that colonizes the chronic wounds

Many wounds had monomicrobial growth while 38 chronic wounds had polymicrobial growth. There were 15 different types of bacteria which were isolated. Most of the isolated bacteria 188 (67%) were Gram negative. Gram positive bacteria were 94 (33%). This study outcome is in agreement with other studies that chronic wounds are mainly colonized by Gram negative bacteria (Rahim et al., 2017), (Wu et al., 2019). *Staphylococcus aureus* was the most colonizing bacteria at 29.7%, It's especially high because skin serves as *Staphylococcus* species' natural habitat which increases the risk of chronic

wound infections. This is consistent with a Chinese study that was conducted by (Guan & Haonan Dong, et al., 2021), they had a *S. aureus* prevalence of 29.2%. Another study done in Tertiary Care Hospital by Ravichandran, (2017) also had similar findings of 29.26%. However, this prevalence is less than that of research conducted at Mogadishu's Shaafi Hospital, Somalia with a *S. aureus* prevalence of 39.47% but still emerging the most colonizing bacteria in that study (Najma Mohamud et al., 2021). The main reasons for the increased attention in *S. aureus* chronic wound infections are its involvement in hospital cross-infections and the occurrence of virulent species of the bacteria that are resistant to antibiotics (Methicillin resistance strains).

Pseudomonas aeruginosa came second in bacteria colonizing chronic wounds and first in gram negative bacilli in this study. It had a prevalence of 16.3% followed closely by *E. coli* at 15.2%. These findings fall in line with a number of studies that found that these 3 bacteria (*S. aureus, P. aeruginosa and E. coli*) are the predominant bacteria that mostly cause chronic wound infections (Guan & Haonan Dong, et al., 2021), (Sisay et al., 2019). Even though, this study findings were comparatively different from findings in a study done in Kenyatta National Hospital which found *Proteus mirabilis* to be the predominant bacteria in chronic wounds with a prevalence of 17.6% (Kisoi, 2021).

In our study, *Proteus mirabilis* was fourth in line of bacteria colonizing chronic wounds at 13.4% while *Klebsiella pneumoniae* was fifth.at 6.0%. Although *Acinetobacter* has recently emerged as a nosocomial pathogen and a significant contributor to immobilization and mortality, mainly in chronic wounds victims, in this investigation we got only 5.6%. *Proteus vulgaris* was 4.6% while *Klebsiella oxytoca* was 2.4%. Other pathogens isolated were *Streptococcus pyogenes* 1.7%, Coagulase Negative *Staphylococcus* 1.0%, *Citrobacter koseri* 1.0%, *Citrobacter freundii* 0.7%, *Enterobacter cloacae* 0.7% and *Serratia marcescens* 0.7%. *Streptococcus agalactiae* had the least prevalence at 0.3%.

Due to a lack of laboratory space at the time the study was done, obligatory anaerobes were not examined. These are the kinds of bacteria that thrive without oxygen, examples are; *Bacteroides*

fragilis, Clostridium tetani and *Peptostreptococcus*. These pathogens rarely result in chronic wound infections, instead they usually cause deep-seated acute infections.

Objective II: Antimicrobial susceptibility patterns

All identified isolates were subjected to a galaxy of antibiotics drug disks to test their antimicrobial susceptibility and resistant patterns. The outcome demonstrated that some antibiotics were effective and others were not effective to the subjected microbial organisms. All *Staphylococcus aureus* in this study were 100% sensitive to levofloxacin which is a fluoroquinolone with great bioavailability and a wide range of antibacterial activity that targets common infections including Gram positive bacteria and Enterobacteriaceae found in chronic wound infections. This discovery aligns with research conducted by (Oberdorfer et al., 2017) where levofloxacin demonstrated superior penetration into the tissue around the wounds, resulting in a microbiological cure. Doxycycline also showed a good sensitivity of 96.2%, this is consistent with a collective result done by (Bidell et al., 2021). Their review showed that, Adults with wound infections who are known or strongly suspected to have been colonized by *S. aureus*, particularly MRSA, may benefit from therapy with doxycycline if they take it for the prescribed length of time (e.g., 5 – 10 days). Gentamicin was third in sensitivity to *S. aureus* at 89.5%, this is similar though slightly higher to a study in Uganda with 87.5% (Anguzu & Olila, 2017). According to this investigation, most *S. aureus* isolates have high levels of Penicillin resistance. According to (Schito, 2016) Penicillinase or beta-lactamase synthesis is the cause of this resistance which inactivates beta-lactam antibiotics by hydrolysis of their beta-lactam ring. Co-trimoxazole also showed a high threshold of resistance followed by Augmentin which was 50% resistance. These results might be the consequence of the research population's reckless consumption of these antibiotics since these drugs are widely used because they are more affordable leading to resistant bacteria.

Pseudomonas aeruginosa showed highest sensitivity to meropenem (97.8%) which is parenterally administered carbapenem antibiotic. It exhibits outstanding bactericidal efficacy against nearly all

aerobes and anaerobes that are clinically important. This study agrees with (Edwards, 2015), who revealed that meropenem is highly active to *Pseudomonas aeruginosa* and all Enterobacteriaceae. Other antibiotics that showed high sensitivity were amikacin, cefepime, piperacillin/tazobactam, ceftazidime, gentamicin and aztreonam respectively. Even though, *Pseudomonas. aeruginosa* showed resistant to cefotaxime and ceftriaxone. The sensitivity and resistant pattern of this *P. aeruginosa* confirms research findings done by (Pokharel et al., 2019), in the Government of Nepal. They found that, meropenem, amikacin, cefepime and piperacillin/tazobactam to be the most suitable option for treating an illness brought on by this bacterium. Also, they found this organism to have resistance to cefotaxime and ceftriaxone. The only difference is that their *P. aeruginosa* had resistant to ceftazidime. This drug has in-vitro activities which are effective to all enzymes of *P. aeruginosa.*

All of the Gram negative bacteria in this investigation were very sensitive to meropenem, amikacin, piperacillin/tazobactam, gentamicin, imipenem, cefepime and ciprofloxacin in that order. They were intermediate to tetracycline, tobramycin, cefotaxime and cefuroxime. They exhibited resistance to ampicillin, co-trimoxazole, Augmentin, and ceftriaxone. This study is comparable to one conducted at Salem's Tertiary Care Hospital. They discovered that the best efficient antibacterial drugs for Gram negative bacterial strains are meropenem, amikacin, and piperacillin/tazobactam (Sugandhi et al., 2017). Other investigations done in Pakistan

also revealed similar results on these most effective antibiotics (Rahim et al., 2016).

Of all *Staphylococcus aureus* isolated, 22.6% were methicillin resistance (MRSA) strains. Induced clindamycin resistance (ICR) strains were 5.95%. A few had both MRSA and ICR strains 2.38%. Compared to the 60% of MRSA that was isolated from intensive care units in the US, 22.6% in this research is a very low percentage (Sakoulas et al., 2018). Additionally, these results are at disagreement with a Tanzanian study which had 44.4% MRSA still higher than our findings (Manyahi, 2017). Even though, this study findings are slightly higher than 18.8% MRSA isolates found in a study by (Mawalla et al., 2021). The findings of this data confirm the rising trend of MRSA infections both nationally and

internationally. Moreover, it was shown that a few of these isolates exhibited multiple resistance to routinely recommended antibiotics including clindamycin hence there are rising incidences of inducible clindamycin resistant. These findings demonstrate evidence that it's a necessity to test susceptibility in guiding the treatment of Staphylococcal and other infections.

In all Enterobacteriaceae isolated, 19 (15.07%) were Extended spectrum beta-lactamase (ESBL) producing species. *E. coli* accounted for 10 ESBL strains while the rest 9 ESBLs were from *Klebsiella pneumoniae*. This finding partially agrees with a recent study done in Tertiary Care Hospital, Saudi Arabia which revealed a prevalence of 17.73% (Binsuwaidan et al., 2023) This ESBL prevalence is low as compared to 55.74% prevalence found by (Oli et al., 2017). It was discovered that all ESBL-producing Enterobacteriaceae were extremely resistant to Augmentin, ampicillin, and third-generation cephalosporins (ceftriaxone, cefotaxime, and ceftazidime). Meropenem which is a carbapenem type of drug was the only drug disk that showed 100% sensitive to all ESBLs in other words, it had zero resistance to all ESBLs.

Objective III: Risk factors associated with chronic wound infections

The risk factors associated with chronic wound infections were found to contribute in the virulence of the organisms. Most participants had one or more comorbidities. Hypertension was the most common comorbidities at 66 (40%) among the participants, diabetes followed at 56 (33%). Human immunodeficiency virus (HIV) came third at 28 (16%). Participants who had osteoarthritis were 12 (7%) while those who had malignancy were 3 (2%). Those who were addicted to alcohol (alcoholism) were 2 (1%) and the comorbidity which had affected the least was mycobacterium tuberculosis (TB) 1(1%). The prevalence of these findings partially disagrees with a study done in Portugal where their hypertension prevalence was 63%, diabetes was 30% (Furtado et al., 2020).

Despite the fact that old age has been reported to be a significant association with chronic wound infections, this study did not confirm this relation since majority of those who had chronic wounds were from the age group of 31-40 years. A significant consequence of diabetes that can result in amputations is diabetic foot ulcers hence immobilization and lowered self-esteem. To curb this menace, clinical microbiology laboratories have role to play in early diagnosis and giving susceptibility antibiotics patterns for effective management of these infections.

Study strength

This study was done in a big hospital which is well equipped and has a high population hence the researchers were able to get diversity of bacteria out of which majority met the threshold recommended by WHO and Clinical and Laboratory Standards institute (CLSI) of 30 isolates per organism to evaluate antibiogram profile. The Laboratory in which the cultures were done is ISO 15189 accredited. Third of all isolates identified were submitted to National Microbiology Reference Laboratory (NMRL) for further clarifications and the results correlated 100%. Furthermore, the principal investigator analysed Microbiology External Quality Controls and she scored 100% meaning her work is accurate and specific.

Limitations

Obligate anaerobes bacteria were not isolated and also resistant genes were not established.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary and conclusion

Chronic wound is a serious yet often neglected health care issue that increases the burden on healthcare professionals, causes patients' distress and immobility, and may even result in their inability to work. The chronic wound bacterial growth rate was 81.3%. According to this study, chronic wounds are commonly colonised by Gram-negative bacteria even though the major predominant isolate was *Staphylococcus aureus,* followed by *Pseudomonas aeruginosa*. The study found significant resistance to beta-lactam antimicrobial agents which are in many cases the antibiotics of choice. Antibiotic resistance to beta-lactams emerges more quickly when drugs are uncontrolled and overused. Their efficacy is significantly threatened by bacterial resistance to penicillin and cephalosporins.

All Gram negative bacteria in this investigation were sensitive to meropenem, amikacin, piperacillin/tazobactam, gentamicin, cefepime and ciprofloxacin in that order. They were resistance to ceftriaxone 60.8%, Augmentin 45%, co-trimoxazole 55% and ampicillin 91%. Ceftriaxone and metronidazole were the most empirical treatment given to wound patients probably contributing to the resistant of this ceftriaxone due to its misuse. Levofloxacin, doxycycline and gentamicin were found to be the most sensitive antibiotics to gram-positive bacteria. Antibiotics with high resistance were penicillin 82.8%, co-trimoxazole and Augmentin.

Bacteria resistant to antibiotics are causing an alarming rise in infections. Resistant bacterial strains may have emerged as a result of inconsistent antibiotic policies and indiscriminate antibiotic use. To stop the spread of antibiotic resistance, clinicians must prescribe evidence-based antibiotic treatments and continuously assess the trend of antibiotic susceptibility. Microbiological results should be embraced into consideration when prescribing antibiotics whenever possible.

5.2 Recommendations

1. Fluoroquinolones antibiotics and gentamicin gave satisfactory sensitivity to both Gram-negative and Gram-positive bacteria hence they are recommended for the empirical treatment in preference to penicillin, co-trimoxazole and ceftriaxone.
2. As a tactic to reduce the spread of resistance organisms, infection prevention and control strategy is recommended.
3. Periodic monitoring of aetiology and antimicrobial susceptibility is also recommended. Patients benefit from isolate testing, which also helps doctors to choose an appropriate empirical course of treatment in places without a microbiological lab.
4. To stop resistance, surveillance and antimicrobial stewardship must be strengthened.
5. For the sake of future research to be comprehensive, it is recommended that the microbiology laboratory's capability be increased to include strict anaerobe bacteria and genotyping.

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APPENDICES

Appendix I: Culture Media Preparation

Mueller-Hinton Agar

Principle

Beef extract, starch, agar, and casein acid hydrolysate are all present in Mueller Hinton Media. Beef extract and casein acid hydrolysate offer vital nutrients such as nitrogen, sulfur, carbon, vitamins, and amino acids. Starch is used to help absorb any toxic metabolites that are produced. Starch is hydrolyzed to create dextrose, which is an energy source. Agar is the solidifying agent.

Procedure

1. As directed by the manufacturer, make MHA using a commercially supplied dehydrated base.
2. To fully dissolve the medium, boil for one minute while heating with continuous stirring.
3. At 121°C, autoclave for 15 minutes.
4. Place the agar in a 45–50°C water bath to chill it right away after autoclaving.
5. Transfer the recently made and chilled medium onto glass or plastic Petri dishes with a flat bottom that are level and horizontally oriented, ensuring that the depth is consistent at around 4 mm. For plates measuring 150 mm in diameter, this equates to 60–70 ml of medium, and 25–30 ml for plates with a diameter of 100 mm. If the agar plates aren't going to be used right away, let them come down to room temperature and then store them in the refrigerator (2 to 8°C).
6. If sufficient measures aren't done, like covering in plastic, to reduce the agar's drying, after preparation, utilize the plates within seven days.
7. To check for sterility, every set of plates should have a representative sample incubated at 35 ± 2°C for a minimum of 24 hours.

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8. Once the medium is ready, Verify each batch of MHA's pH. After gelling, the agar medium needs to be examined to ensure that its pH is within the recommended range of 7.2 to 7.4 at room temperature.

9. Avoid supplementing MHA with extra calcium or magnesium cations.

MHA quality control

Positive controls:	**Expected results**
ATCC® 25922 *Escherichia coli*	Light straw-colored colonies, moderate growth
ATCC® 27853 *Pseudomonas aeruginosa*	Moderate growth; colonies with straw color
ATCC® 25923 *Staphylococcus aureus*	Moderate growth; golden colonies
Negative control:	
Plain medium plate	No growth or color change

MacConkey medium

Principle

Gram-negative enteric bacteria are isolated and lactose-fermenting gram-negative bacteria are distinguished from non-lactose-fermenting gram-negative bacteria using MacConkey agar. The pancreatic digest of gelatin and peptones (meat and casein) provides essential minerals, vitamins, and nitrogenous components that promote the growth of microorganisms. Lactose monohydrate is a fermentable type of carbohydrates. The selective effect of this medium is caused by bile salts and crystal violet, which inhibit most gram-positive bacterial species. Sodium chloride maintains the

osmotic balance in the medium. Neutral red, a pH indicator, turns red when the pH drops below 6.8 and stays colorless when the pH rises above 6.8. Agar is the solidifying agent.

Procedure

1. One liter of filtered water contained 49.53 grams of the medium suspended in it.
2. Heating was done with frequent agitation and boiling for one minute in order to completely dissolve the medium.
3. This solution underwent a 15-minute autoclave at 121°C.
4. After adding the media to petri dishes, they were let to cool.
5. The media was then packaged and kept for later use in a refrigerator between 2 and 8 degrees Celsius.

Sheep blood agar

Principle

An enhanced-nutrient medium, blood agar can be used as a general medium when blood is not available, or blood can be added to it to help certain organisms develop. The blood injected into the base provides the medium with extra growth components that these particular organisms require, hence enhancing its flourish. The blood also aids in the visualization of the hemolytic effects of different microorganisms. The blood used as the medium, peptone, and tryptose provide the bacteria with the carbon, nitrogen, amino acids, vitamins, and minerals they require. Tryptose and peptone are soluble in water, which makes it easier for the organism to absorb nutrients. Sodium chloride is supplied to the medium in order to maintain its osmotic equilibrium and prevent the medium's pH from altering as the media expands. The distilled water dissolves the nutrients, which makes it easier for the bacteria to eat them. Agar, a hardening material, provides the organism with a stable surface on which to grow,

allowing for the counting of organisms and the examination of colony shape. Periodically, phenolphthalein phosphate is added to the medium to identify Staphylococci that produce phosphate, and salt and agar are added to evaluate surface contamination.

Procedure

1. Fill a 1-liter conical flask with 500ml of distilled water that has been measured with a measuring cylinder.
2. Using a weighing scale, weigh 20g of Blood Agar Base (BA).
3. Add the measured BA to the 500 milliliters of purified water.
4. Fully combine. To fully dissolve the medium, the suspension is brought to a boil.
5. After that, it is autoclaved for roughly 15 minutes at 121°C and 15 pounds of pressure to sanitize it.
6. After exiting the autoclave, the medium is chilled to a temperature of 40 to 45°C.
7. Add 5% v/v sterile defibrinated blood aseptically to this and mix thoroughly.
8. Set the petri dishes on the pristine safety hood and carefully pour the heated blood agar
9. Using a bunsen burner, carefully invert and pass the flame over the blood agar that has been added to the plate to remove any air bubbles.
10. Before storing the petri plates in a refrigerator, cover them and let the blood agar congeal.

Indole medium:

It is a biochemical process used to assess bacterial species' ability to produce indole from tryptophan when tryptophanase is present.

Procedure

1. The colony from a culture incubated for 18–24-hour is inoculated into the broth medium or agar media in the tube.

2. A tiny amount of the infected soup is taken in a different tube for the liquid medium.
3. The tubes are thereafter incubated for a full day at 37°C.
4. After adding three drops of Kovac's reagent along the tube's side, the meniscus changes color.

Simmons citrate medium:

Procedure

1. Dissolve 24.28 grams in 1000 milliliters of purified water.
2. Heat until the medium completely dissolves, without boiling.
3. Thoroughly combine and transfer into flasks or tubes. Pour 4.0 to 5.0 ml into 16 mm tubes for the tubes.
4. 4. Sterilize in an autoclave for 15 minutes at 121°C and 15 pounds of pressure.
5. Remain cool with a lengthy slant and a shallow butt.
6. To guarantee a shelf life of six to eight weeks, tubes should be kept in a refrigerator.
7. Because of the bromothymol blue and the pH of the sample, the uninoculated medium will have a rich forest green color.

Triple sugar iron agar:

Procedure

1. Add 1000 milliliters of distilled or filtered water to 64.42 grams, or the weight of the dehydrated medium per liter, and dissolve.
2. Bring the mixture to a boil to fully dissolve the medium.
3. Thoroughly combine and transfer into test tubes.
4. Sterilize for 30 minutes or as long as the validated cycle lasts, by maintaining 10 pounds of pressure (115°C).
5. Let the medium to solidify in the slanted shape with a 2.5 cm-long butt.

6. Using a straight inoculation needle, make contact with the top of a colony that has been well separated.
7. To inoculate TSI, pierce through the medium to the bottom of the tube first, and then streak the surface of the agar slant.
8. Incubate the tube at room temperature for 18 to 24 hours at 35°–37°C after leaving the top loosely attached.
9. Examine the medium's response.

Voges Proskauer

Procedure

1. Inoculate the broth with well-isolated colonies of sample bacteria that are 18 to 24 hours old using a sterile inoculating loop.
2. For 18 to 24 hours, incubate the tubes aerobically at 35±2°C.
3. After incubation, pour 2 milliliters of broth into a sterile, or as clean as feasible, test tube.
4. Include six drops of Reagent A (a 5% α-naphthol solution) and thoroughly shake to combine.
5. Include two drops of Reagent B (a 40% KOH solution) and thoroughly shake to combine.
6. After 30 minutes, look for the medium's surface to start taking on a reddish-pink hue. Shake the tube vigorously for the full thirty minutes that you have to wait.
7. If there is no color development (a negative reaction), re-incubate the leftover soup for a further day and repeat the test.

Appendix II: Consent Form

I am Magdaline Wairimu Kamande, a Mount Kenya University Medical School postgraduate student pursuing MSc. in Medical Laboratory Science, Medical Microbiology option. I am collecting wound swab samples from patients suffering from chronic wound infections at Murang'a Level 5 Hospital.

Study purpose

This study to be done at Murang'a Level 5 Hospital will look into the bacteria that colonize chronic wounds, the frequency of antimicrobial resistance to common antibiotics, and the risk factors that go along with chronic wounds. The results will help guide current practice regarding antibiotic choice in wound infections, leading to better outcomes.

Procedure to be followed

The researcher will obtain the patient's wound swab for analysis. Following your interview, as a study participant, you will have to answer some questions on a questionnaire

Risks

No risk exposure during the procedure of obtaining the wound swab. You will only feel a little sensation while swab is being obtained.

Benefits

Results that will be obtained will be given to the clinician to assist in your care for better management and quick recovery.

Compensation and cost

Participating in this study does not come with any extra costs. Participants in the study won't receive anything for their participation.

Confidentiality of records

Information gathered from the participants will be coded, and the number given to the participant will only be used for this study. Participants' names will not be taken, and details that can be used to recognize the patient will not appear in the report.

Participation basis

Each participant is free to accept or reject this study's invitation to participate. If you would want more information or have any questions, you may reach the investigator through her mobile number 0723871391.

Participant's Statement

I've been given an explanation of the purpose, benefits, and hazards of this study. I understand that taking part in the study is completely voluntary, and I can stop at any moment. Having gone through the above information and been given a chance to ask any question, I now accept participating in the research. I will answer any questions the investigator asks and allow her to collect wound swab for further analysis. I accept that my patient's file can be accessed for patient history that may be necessary for the research. By appending the signature below, I confirm that I voluntarily accept to be a participant without being coerced.

Signature …… Date ……

(Participant)

I, the investigator, have explained all the above information to the participant and have sought his/her informed consent.

Signature …….... Date …….

(Investigator)

Appendix III: Questionnaire

Study identification number……. Date…….

TITTLE OF THE STUDY: EVALUATION OF ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA ISOLATES FROM CHRONIC WOUNDS OF PATIENTS ATTENDING MURANG'A LEVEL 5 HOSPITAL

Give your answers by appropriately responding in the blank space provided. It will involve writing the responses or ticking your choice as the question requires.

1. What is your age? _____ years
2. What is your gender? Male / female
3. What is your Occupation……employed ☐ informal employment ☐
4. What is your BMI …….
5. What is the wound duration? .....
6. How often do you feel depressed because of your wound?.....
   Never ☐ Rarely ☐ Sometimes ☐ Often ☐ Always ☐
7. Do you have any comorbidity? ……
8. Hospital stays if in patient……
9. What is the cause/origin of the wound? ……..
10. Have you used any antibiotics for the last 3 weeks? ……..
11. If yes which one…….

SECTION B:

To be filled by a qualified Microbiologist personnel

culture and sensitivity report

1. Which organism isolated? ……..
2. Is it gram positive or gram negative? .....
3. Which biochemical test carried out? .....

Fill the table below

Gram positive bacteria

ANTIBIOTIC	SENSITIVE	INTERMEDIATE	RESISTANCE

Gram negative bacteria

ANTIBIOTICS	SENSITIVE	INTERMEDIATE	RESISTANCE

Appendix IV: Laboratory Request Form

Lab No: ……

Mark box √ **Pathology** ☐ **Microbiology** ☐ **Hematology** ☐ **Biochemistry** ☐	**NAME** _____ **AGE** _____ **GENDER** _____ **PHONE NO** _____ **PATIENT RESIDENCE** _____
Date sample received _____ **Time Sample received** _____	**OP NO.** _____ **IP NO.** _____**WARD** _____

Type of sample **Send Report to:**

Date of collection **Time**

Investigation required:

DIAGNOSIS: _____

HISTORY: (Therapy including antibiotics)

CLINICIAN'S NAME _____ **Signature** _____ **Date** _____

REPORT:

TEST DONE BY …… **SIGN** …… **DATE** ……. **TIME** ……

REVIEWED BY …… …… **SIGN** …… **DATE** …… **TIME** ……...

Appendix V: Ethical Clearance Certificate

Mount Kenya University

REF: **MKU/ISERC/2784** Date: 19 May 2023
TO: **MAGDALINE KAMANDE**
REG: **MMLS/2022/50406**

Dear Sir/Madam,

RE: EVALUATION OF ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA ISOLATES FROM CHRONIC WOUNDS OF PATIENTS ATTENDING MURANG'A LEVEL 5 HOSPITAL, KENYA

This is to inform you that **Mount Kenya University** has reviewed and approved your above research proposal. Your application approval number is **1776.** The approval period is **19/05/2023 - 18/05/2024.**

This approval is subject to compliance with the following requirements;

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

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

Yours sincerely,

The Chairman
Mount Kenya University
Ethics Review Committee
P. O. Box 342 - 0100, Thika

Dr. Peter G. Kirira
Chairman, Mount Kenya University ISERC

Appendix VI: Letter of Introduction

Mount Kenya University

DIRECTORATE OF GRADUATE STUDIES

MMLS/2022/50406

22nd May, 2023

National Commission for Science Technology & Innovation (NACOSTI)
Off Waiyaki, Upper Kabete
P.O Box 30623- 00100
NAIROBI, KENYA

Dear Sir/Madam,

RE: MAGDALINE KAMANDE- REGISTRATION NO. MMLS/2022/50406

The purpose of this letter is to introduce the above named student who is pursuing **Master of Science in Medical Laboratory Science Degree** in the Department of **Medical Laboratory Science** in Medical School.

The title of the research is **"Evaluation of Antimicrobial Resistance Patterns of Bacteria Isolates from Chronic Wounds of Patients Attending Murang'a Level 5 Hospital, Kenya."** It has been cleared by the University's Ethics Review Committee (Certificate attached) and now has to proceed to the field to collect data between **May, 2023 and July, 2023.**

Any assistance accorded to the student will be highly appreciated.

Thank you.

Mount Kenya University
P.O. Box 342 - 01000, THIKA
Office of the Director
Graduate Studies

Dr. Samuel M. Kareru, Ph.D
Director, Graduate Studies
Enc.

Appendix VII: Research Permit from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **913093**

Date of Issue: **15/June/2023**

RESEARCH LICENSE

This is to Certify that Ms.. Magdaline Wairimu Kamande of Mount Kenya University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Muranga on the topic: EVALUATION OF ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA ISOLATES FROM CHRONIC WOUNDS OF PATIENTS ATTENDING MURANG'A LEVEL 5 HOSPITAL, KENYA for the period ending : 15/June/2024.

License No: **NACOSTI/P/23/26471**

913093

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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

See overleaf for conditions

Appendix VIII: Authorization From Ministry of Health

MAGDALINE WAIRIMU KAMANDE

PO BOX 69- 10200

MURANGA

0723871391

Magdas0729@gmail.com

16th June 2023

TO

THE DIRECTOR OF HEALTH

MURANGA COUNTY GOVERNMENT

DEAR SIR,

RE: REQUEST FOR PERMISSION TO CONDUCT A SCIENTIFIC RESEARCH

I am a student at Mount Kenya University pursuing Master's degree in Medical Laboratory Science, Microbiology option. I hereby request your office to authorize me conduct a scientific study. The title of the study is *"Evaluation of Antimicrobial Resistance Patterns of Bacteria Isolates from Chronic Wounds of Patients Attending Murang'a Level 5 Hospital"*

All ethical procedures shall be followed and the study population will participate on basis of informed consent. Attached are the NACOSTI license, ERC approval and Institutional Introduction letter.

Your assistance will be highly appreciated.

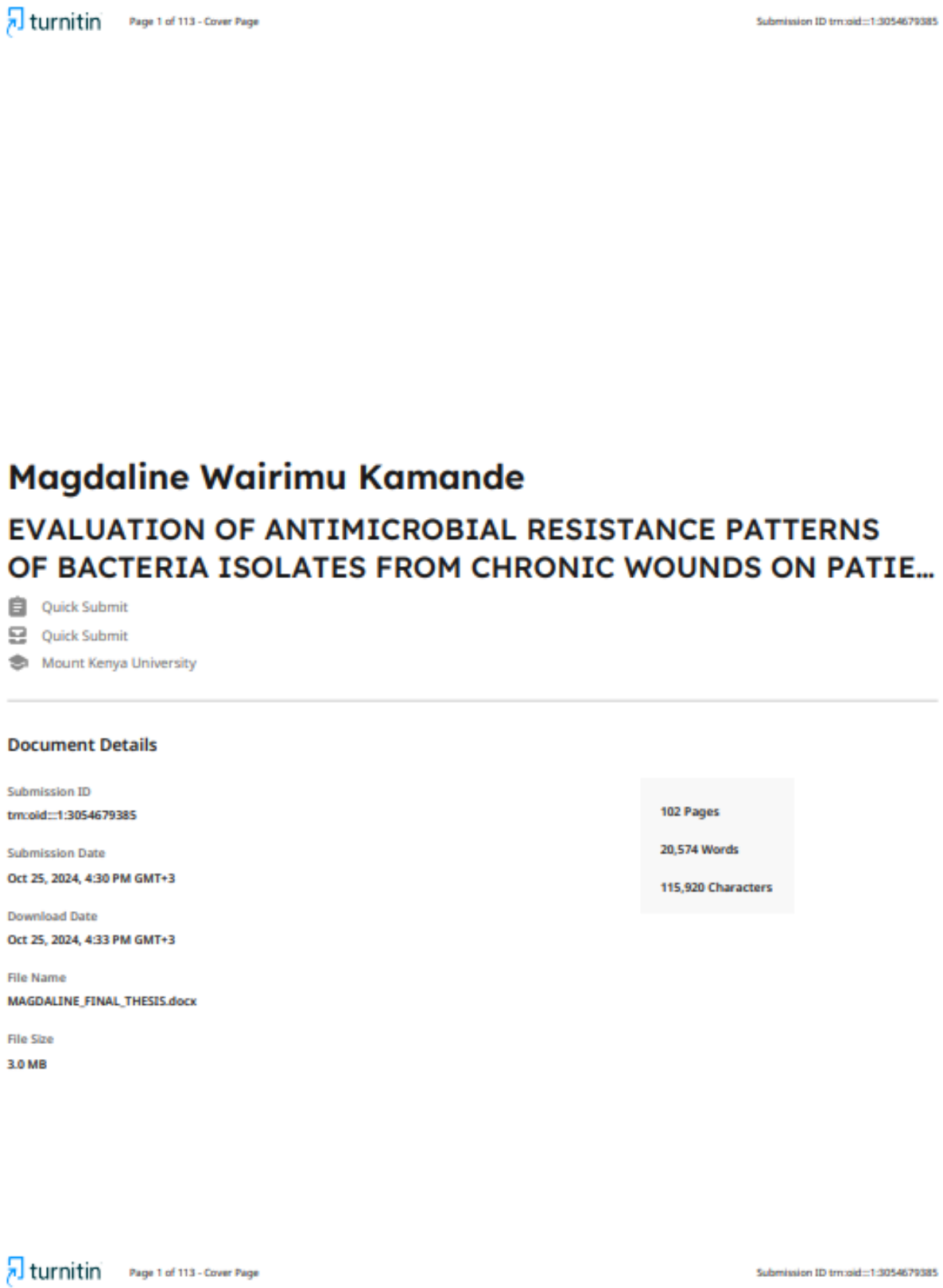
Yours faithfully

Magdaline Wairimu Kamande

CC

1. THE COUNTY COMMISSIONER
   MURANGA COUNTY
2. THE DIRECTOR OF EDUCATION
   MURANGA COUNTY

Appendix IX: Similarity Index



12% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

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- Bibliography

Match Groups

- 138 Not Cited or Quoted 10%
  Matches with neither in-text citation nor quotation marks
- 30 Missing Quotations 2%
  Matches that are still very similar to source material
- 0 Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

9% Internet sources
5% Publications
6% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

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Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

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  Matches with neither in-text citation nor quotation marks
- 30 Missing Quotations 2%
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- 0 Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

9% Internet sources
5% Publications
6% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	Student papers	
Mount Kenya University		1%
2	Internet	
erepository.uonbi.ac.ke		0%
3	Internet	
edubirdie.com		0%
4	Internet	
www.science.gov		0%
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ir.jkuat.ac.ke		0%
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9	Student papers	
Kenyatta University		0%
10	Student papers	
University of the Philippines - Manila		0%

Appendix X: Murang'a County Map

This is Murang'a County, Central Kenya region located at latitude -0.7180443636466275 and longitude 37.16071899705351 (plus code 75J6+M7 Murang'a). It occupies a total area of 2,558.8 KM^2 and has a high population of 942,581 (CHS report, 2017). It is bounded by 5 counties, Kiambu at South, Nyeri at North, Nyandarua at West and Kirinyaga and Machakos to the East.