Evaluation of Antimicrobial Resistance Patterns of Bacteria Isolates From Chronic Wounds of Patients Attending Murang'a Level 5 Hospital, Kenya

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Abstract

Background: 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. 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.

Methods: It was a hospital-based cross-sectional study. Swabs were aseptically picked from chronic wounds and transported in Amies transport media in a cooler box to Microbiology laboratory. Inoculation was done on SBA and MAC and incubated at 37^0c for 24 to 48 hours. Gram stain followed for the provisional isolate's identification. The isolated microorganisms were evaluated for drug sensitivity and resistance using Kirby Bauer disk diffusion method on Mueller Hinton Agar.

Results: The positivity rate was 81.3%. *Staphylococcus aureus* lead at 29.7%, *Pseudomonas aeruginosa* 16.3% and *E. coli* 15.2%. Levofloxacin was the most sensitive antibiotic to *S. aureus*, doxycycline and gentamicin followed closely. 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. 22.6% of all *Staphylococcus aureus* isolated were methicillin resistance (MRSA) strains. 5.95% were induced clindamycin resistance (ICR) strains. 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.*

Conclusion: Frequent monitoring of antimicrobial susceptibility pattern is necessary to curb the spread of antibacterial resistance.

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

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1. Introduction

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

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). 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). *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. 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). 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.

2. Materials and methods

2.1 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 report, 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. Below is the map of the study area (figure 1)

MURANG'A COUNTY

Figure 1: Map of Murang'a county

2.2 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.

2.3 Inclusion and exclusion criteria

Clients with visible wounds, 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. 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.

2.4 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.

2.5 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.

2.6 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).

2.7 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) 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.

2.8 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.

2.9 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 (# 1776), The National Commission of Science, Technology and Innovation (# NACOSTI/P/23/26471), 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.

3. Results

3.1 Bacteria species that colonizes the chronic wounds.

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. Amounting to 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 3.1.

3.2 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 3.2 below. However, the sensitivity of *S. aureus* to an array of antibiotic drugs was similar. CI = 95%, P>0.05

Table 3.1: 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%	

Table 3.2: Evaluation of *S. aureus* susceptibility pattern

ANTIBIOTIC	SENSITIVE (%)	INTERMIDIATE (%)	RESISTANT (%)
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
Levofloxacin	100	0	0

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 3.1 below.

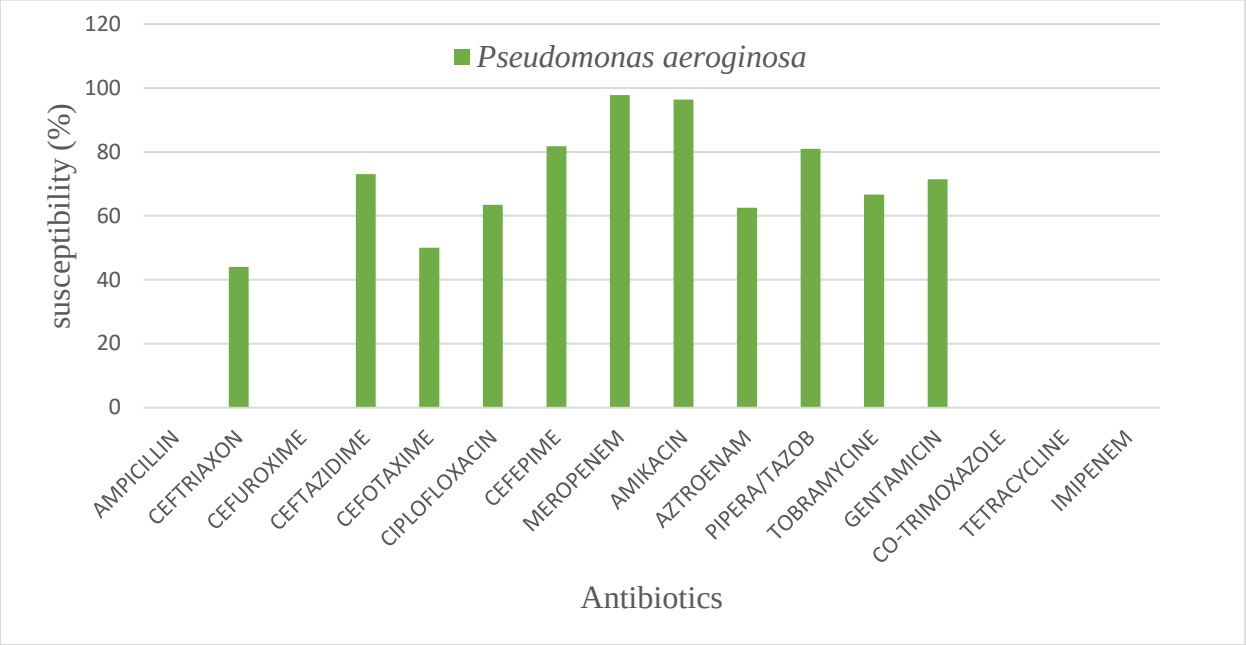

Figure 3.1: Sensitivity pattern for pseudomonas aeruginosa

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 3.3 below. The susceptibility prevalence was significantly different from each other F_{15}=6.16, P< 0.05.

Table 3.3: Evaluation of Gram-negative bacteria's sensitivity pattern

	Pseudomonas aeruginosa	*E. coli*	*Proteus mirabilis*	P VALUE
No. of isolates	46	43	38	
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%	$F_{15} = 6.16$
Meropenem	97.2%	100%	100%	
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%	

3.3 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 3.2 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.

3.4 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 3.3 below. There was a significant difference among the comorbidities associated with chronic wounds infection $F_{13} = 31$, $p = 0.0001$.

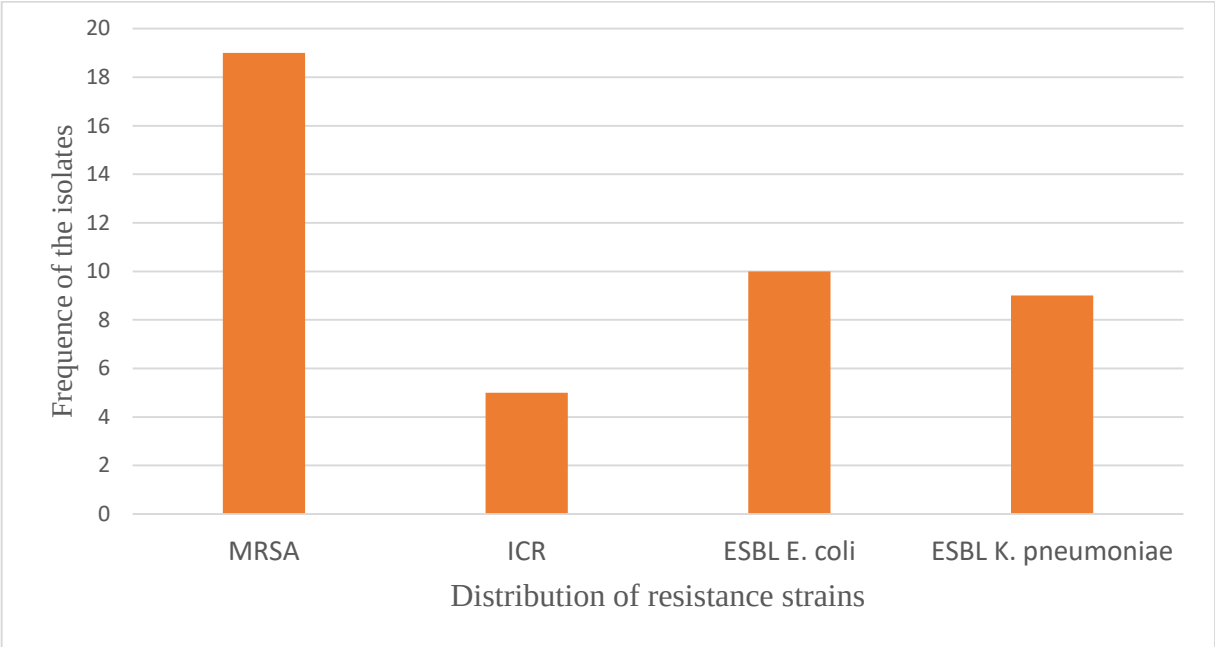

Figure 3.2: Distribution of resistant bacteria

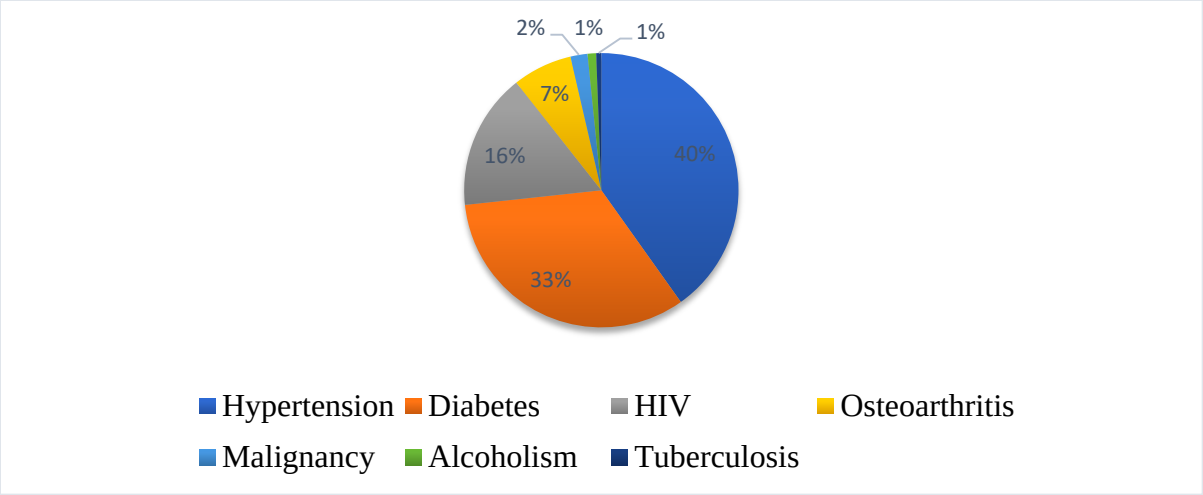

Figure 3.3: The prevalence of comorbidities in chronic wounds

3.5 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 3.4: 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	> 0.05
4	Cut	43	14	
5	SSI	25	8	
6	Trauma	19	6	
7	Bed sores	10	3	df = 15
8	Incisions	9	3	
9	Bites	8	2	
10	Burns	6	2	
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. 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 microbials. 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.

On bacteria species that colonizes 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%.

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).

22.6% of all *Staphylococcus aureus* isolated were methicillin resistance (MRSA) strains. 5.95% were induced clindamycin resistance (ICR) strains. 2.38% had both MRSA and ICR strains. 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 is 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.

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 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.

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, Augmentin, co-trimoxazole and ampicillin. 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, 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.

Recommendations

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. As a tactic to reduce the spread of resistance organisms, infection prevention and control strategy is recommended. Periodic monitoring of aetiology and antimicrobial susceptibility. Patients benefit from isolate testing, which also helps doctors to choose an appropriate empirical course of treatment in places without a microbiological lab. To stop resistance, surveillance and antimicrobial stewardship must be strengthened. 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.

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Conflicts of interest

The authors have got no competing interests.

Abbreviations

AMR: Antimicrobial resistance.
AMS: Antimicrobial sensitivity.
CLSI: Clinical and laboratory standards institute.
ESBL: Extended spectrum beta-lactamase.
ICR: Inducible clindamycin resistance.
MRSA: Methicillin resistant *Staphylococcus aureus*,
MSSA: Methicillin sensitive *Staphylococcus aureus.*

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