Microbial Profile and Antibiotic Usage of Patients with Chronic Wounds Attending Two Hospital facilities in Ghana

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ABSTRACT

Background: Chronic wounds continue to be a challenging problem and causing considerable healthcare burden globally especially to the developing countries. This study examines how patients with chronic wounds acquired them, site of the body commonly located, various bacterial isolates present, type of antibiotic usage, and their susceptibility pattern to commonly prescribed antibiotics. Methods: Two hundred and sixty (260) wound swab samples were collected from chronic wound patients attending the St. Patrick Hospital and the Nkenkenso Government Hospitals from December 2016 to June 2017. Bacterial isolates were tested against six commonly prescribed antibiotics identified in this study. Antimicrobial susceptibility testing of the clinical isolates was done according to CLSI guidelines. Results: A total of 211 (81.2%) isolates were recovered from the 260 samples. Staphylococcus aureus (30.8%) was the most predominate, followed by Pseudomonas spp. (24.9%). Majority of the patients (87.31%) with chronic wounds received antibiotics metronidazole and cefuroxime which was the most common (21.74%) antibiotics prescribed. More than 50% of the isolate were resistant to flucloxacillin and amoxicillin, whilst most of the isolates were susceptible to ciprofloxacin and clindamycin. Conclusion: The study observed that inflammation was the major cause of chronic wounds and that, most wounds were located on the lower extremity part of the body. The frequency of drug administration in the management of chronic wounds was 87.31%. The commonly prescribed monotherapy antibiotics were flucloxacillin and ciprofloxacin whilst metronidazole and cefuroxime were mostly prescribed together with most isolates resistant to flucloxacillin and amoxicillin.

Keywords: *Antimicrobials, Susceptibility, Monotherapy, Antibiotics.*

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1. INTRODUCTION

Even though studies on microbial profile over time and their antibiotic susceptibility in chronic wounds are lacking, reports suggest that, in developed countries, an estimated 1 to 2% of the population will experience a chronic wound during their lifetime. The cause of the wound, location on the body, infection and improper use of antibiotics can make wound difficult to treat. Hence, an appropriate antibacterial therapy using broad-spectrum antibiotics is often used. However, indiscriminate use of common antibiotics to treat some bacterial causing infection in chronic wounds may eventually lead to the emergence of strains resistant to many antibiotics [1].

One bacterium that has become notorious for its resistance to antibiotic in chronic wounds is *Staphylococcus aureus*. It is estimated that the incidence of Staphylococci with antibiotic resistance has increased about tenfold over the past decade. Antibiotics are losing their effectiveness at a rate that is alarming and the more antibiotics we use during an infection the more likely it is that our next infection will be resistant to that antibiotics [2]. It is reported that in Ghana, 42.3% of all *S. aureus*, which is one of the most common bacteria found in chronic wounds are methicillin resistant [3].

In considering the type of antibiotics to be used in the treatment of chronic wounds, sensitivity to the pathogen should be the main concern. Unfortunately, in Ghana, a direct, simple method of identification and antimicrobial susceptibility pattern is most often not done due to time constraint, lack of a qualified microbiologist or the cost involved [3]. As a result, most hospitals and herbal clinics usually administer synthetic antibiotics and herbal remedies to treat chronic wound infections which end up not being effective. In view of the diverse availability of different classes of antibiotics for therapy, it becomes important that the microbial profile and antibiotic usage of patients with chronic wound be defined by specific characterization of microbes associated with the wound and rationally implemented to facilitate effective treatment of wound infection. The present study seeks to determine how patients with chronic wounds attending two health facilities (Nkenkaasu Government Hospital (NGH) and the St. Patrick's Hospital (SPH) acquired them, the site of the body they are commonly located, antibiotic usage, the various bacterial isolates present and their susceptibility pattern to some commonly prescribed antibiotics.

2. MATERIALS AND METHODS

2.1. Study Area and Design

Material from chronic wounds (n = 260) was collected from patients visiting the Nkenkaasu Government Hospital (63 females and 65 males) and the St. Patrick's Hospital (67 females and 65 males) located in the Offinso Municipality in Ghana from December 2016 to June 2017. Prior written informed consent was obtained from patients with chronic wounds older than one month and seeking wound dressing services at the facilities as approved by the Committee of Human Research and Publication Ethics. The sociodemographic data, gender, age, wound-related information, therapeutic type, wound infection status and antibiotic history were obtained using a structured questionnaire and analysed. Children under 10 years and Adults above 75 years were excluded as well as very ill patients and those undergoing antibiotic therapy two weeks prior to the study.

2.2. Isolation of Microbes

Wound swab samples were collected aseptically using Levine's technique. The surface of the wound and the surrounding skin was cleaned with sterile water. Swab specimen was taken from fluid expressed within the wound tissue by rotating the swab over a 1cm square area and applying sufficient pressure and immersed into brain heart infusion media. On arrival to the microbiology laboratory, the swabs were promptly inoculated on Blood agar, MacConkey agar, and Chocolate agar [4] by sterile inoculation loop and incubated aerobically at 37°C for 24 to 48

hours. The swabs were immediately processed for culturing of aerobic bacteria. A series of standard biochemical and microbiological techniques were followed to isolate and identify bacterial strains for each sample obtained in the study.

2.3. Antimicrobial Sensitivity Patterns

Assessment of the antimicrobial sensitivity of the bacterial isolates was performed using the disc diffusion method. Antimicrobial discs (ciprofloxacin (25 mg), flucloxacillin (5 mg) clindamycin (2 mg), [amoxicillin (30 mg), metronidazole (25 mg), and cefuroxime (30 mg), were obtained from HiMedia Laboratories and results were interpreted as recommended by the Clinical and Laboratory Standards Institute [5]. The following reference strains were used in the study for quality control – *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

2.4. Statistical Analysis

The quantitative variables were expressed as means ± SD while qualitative variables were presented as frequencies. Statistical significance was accepted at $P < 0.05$. The statistical analyses were performed using SPSS statistical software (version 16.0).

2.5. Ethics

Ethical clearance was obtained from the committee on Human Research, Publication and Ethics of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Written informed consent was obtained from all study participants.

3. RESULTS

3.1. Socio-Demographic Characteristics of Patients

A total of 260 patients aged between 10 to 75 years were screened for the presence of microbes associated with chronic wound. 132 from SPH made up of 67 females and 65 males and 128 from NGH consisting of 63 females and 65 males (Table 1). The largest age group of the patient with chronic wounds from the two facilities was between 21-30 years (26.92%), whilst patients between the ages of 61-75 were the least (8.46%).

Table-1. Socio-demographic characteristics of Patients

Characteristics of Patients	SPH (%)	NGH (%)	Total (%)
No. of Patients	132 (50.76)	128 (49.24)	260 (100)
Males	65 (50)	65 (50)	130 (100)
Females	67 (51.53)	63 (48.47)	130 (100)
Age 10 – 20	19 (14.39)	30 (23.44)	49 (18.85)
Age 21 – 30	28 (21.21)	42 (32.81)	70 (26.92)
Age 31 – 41	25 (18.39)	23 (17.97)	48 (18.46)
Age 41 – 50	32 (24.24)	15 (11.72)	47 (18.08)
Age 51 – 60	16 (12.12)	8 (6.25)	24 (9.23)
Age 61 – 75	12 (9.09)	10 (7.81)	22 (8.46)

Abbreviations: SPH, St. Patrick's hospital; NGH, Nkenkaasu Government hospital.

3.2. Cause and Site of Body Wound Is Located on Patients Visiting the SPH and NGH

The results of wound etiology of the 128 chronic wounds investigated at NGH showed that 41.41% were acquired through cut, inflammation 27.34%, road traffic accident (RTA) 21.09%, burns 5.47% and surgery 4.69% (Figure 1). On the other hand, out of the 132 patients investigated at the SPH, inflammation was 50%, road traffic

accident 12.12%, cut 24.24%, surgery 8.33%, burns 2.27% and other sources 3.03%. Comparing the two facilities, there were significant differences in the cause of wounds ($p < 0.004$) in all cases. In the investigation into the site of chronic wounds, majority of the wounds 57.57% and 38.28% from SPH and NGH respectively were located on the legs whilst only 1.51% and 4.69 from SPH and NGH respectively were located on the arm (Figure 2).

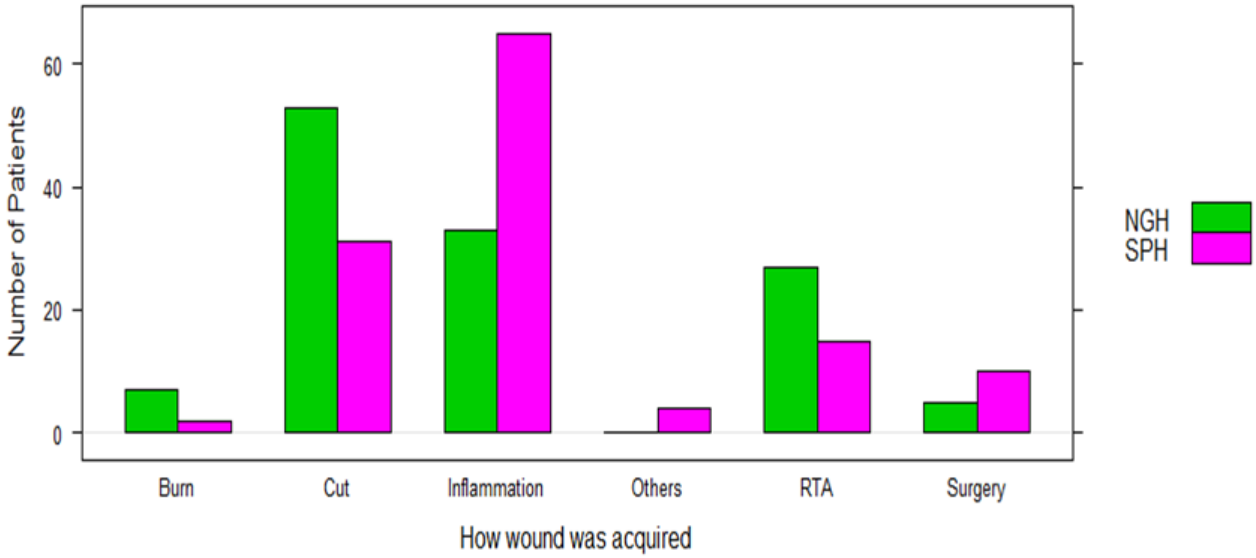

Figure-1. Comparison of cause of wounds of patients attending the SPH and NGH facilities

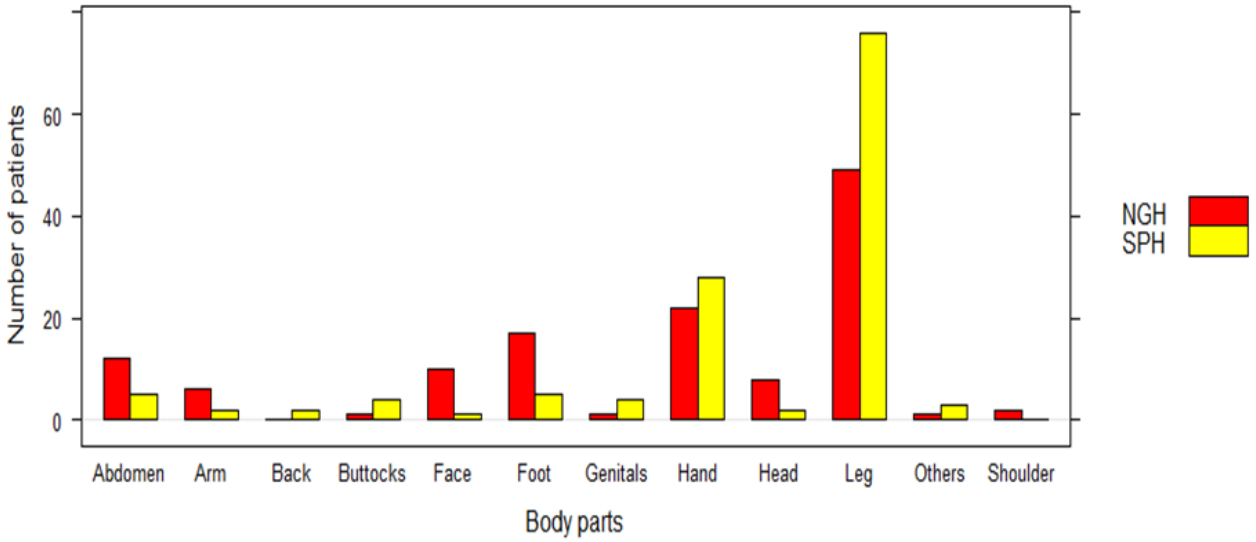

Figure-2. Distribution sites of the chronic wounds investigated at the SPH and NGH (n = 260)

In all, 32% of the chronic wounds acquired through cut were located on the legs and only 1.9% on the arm. 57% of inflammation wounds were located on the legs and 17% on foot. All surgery wounds were located on the abdomen, whilst 42% and 28% of burn wounds were located on the leg and foot respectively. 30% of road traffic accident wounds were located on the legs and only 2% were on the head (data not shown). At both facilities, most of the wounds investigated were between 1-3 months old (78.08%) and very few of the wounds were older than 12 months (5%) (Table 2). Out of the 13 chronic wounds older than 12 months investigated, 77% was located on the leg. Also, of the 44 chronic wounds between the months of 3-12 investigated 63% was located on the legs, 14% on the foot and 9% on the hand (data not shown).

Table-2. Duration of wound investigated from SPH and NGH

Wound Duration (Months)	NGH (%)	SPH (%)	Total (%)
1-3	112(87.50)	91(68.94)	203(78.08)
3-12	12(9.38)	32(24.24)	44(16.92)
>12	4(3.12)	9(6.82)	13(5.00)
Total	**128**	**132**	**260**

3.3. Occurrence of Bacteria Isolates Present in the Investigated Wounds at the SPH and NGH

Out of a total of 260 chronic wound swabs taken, 211 (81.2%) produced bacterial growth. A total of 237 bacteria were isolated, out of which 85 (35.9%) were Gram Positive and 152 (64.1%) Gram negative. Of the 85 Gram positive isolates, 73 were *Staphylococcus aureus*, 11 were *coagulase negative Staphylococci* and the rest (2), were *Streptococcus* spp (Table 3). *Staphylococcus aureus* (30.8%) was the predominate organism isolated, followed by *Pseudomonas* spp. (24.9%). The occurrence of Gram negative isolates was as follows: *Pseudomonas* spp. 24.9%, *Escherichia coli* 10.5%, *Klebsiella* spp. 8.0%, *Enterobacter spp.* 9.7% and *Proteus* spp. 10.5% (Table 3). There was a significant difference in the frequency of occurrence in samples from Gram-positive versus Gram-negative isolates for *Pseudomonas* spp ($p = 0.002$), *Escherichia coli* ($p = 0.01$), *Enterobacter* spp ($p = 0.01$), *Klebsiella spp* ($p = 0.002$) and *Proteus* spp ($p = 0.001$).

Table-3. Occurrence of bacteria isolates present in the investigated wounds at the SPH and NGH

Bacteria isolated	Gram Strains	NGH (%)	SPH (%)	Total (%)
Staphylococcus aureus	+ve	31(29.2)	42(32.1)	73(30.8)
Pseudomonas spp.	-ve	25(23.6)	34(26.0)	59(24.9)
Escherichia coli	-ve	11(10.4)	14(10.7)	25(10.5)
Klebsiella spp.	-ve	9(8.5)	10(7.6)	19(8.0)
Enterobacter spp.	-ve	11(10.4)	12(9.2)	23(9.7)
Proteus spp.	-ve	11(10.3)	14(10.7)	25(10.5)
CoN Staphylococci spp.	+ve	7(6.6)	4(3.1)	11(4.6)
Streptococci spp.	+ve	1(0.9)	1(0.8)	2(0.8)
Total		**106**	**131**	**237**

P<0.0001 (Significant difference between Gram-positive and Gram-negative strains).
Abbreviations: +ve, positive; -ve, negative; CoN, Coagulase negative

Polymicrobial growths were detected in cultures of 26 patients. The occurrence of mixed species infections was higher in SPH (62%) than NGH. More females 57.7% had polymicrobial culture growth than the males (data not shown). However, only 5% of patients with chronic wounds between the duration of 1-3 month had mixed species infections, whilst 25% and 87.5% of patients with wounds duration between 3-12 months and greater than 12 months respectively had mixed species infections. *Pseudomonas* and *Proteus* spp. were the most common mixed infections detected 30.8%, followed by *Pseudomonas* spp. and *Staphylococcus aureus* 23% (data not shown).

3.4. Type of Antibiotic Prescription and Usage

Majority of the patients (87.31%) with chronic wounds had received antibiotics, with metronidazole and cefuroxime combination being the most common (21.74%) prescribed. This is followed by metronidazole and amoxiclav 15.22% (Figure 3). A total of 34.78% of the patients received only one antibiotic prescription where flucloxacillin (10.87%) and cefuroxime (10.87%) were the most commonly prescribed. Prescription of two combinations of drugs was 58.75%. Even though 87.31% of the patients received antibiotic treatment only 6.23% of them received it base on wound swab results. At NGH, none of the patients who received antibiotic treatment (n= 107), did so base on wound swab results.

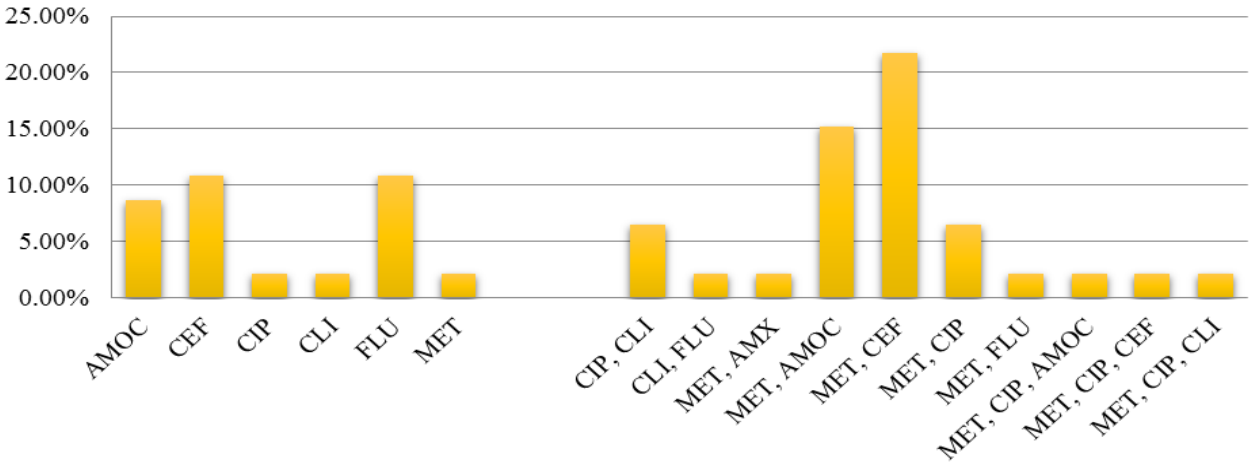

Figure-3. Type of antibiotic prescription and usage at the SPH and NGH.
Abbreviations: CIP - Ciprofloxacin, CEF - Cefuroxime, CLI - Clindamycin, FLU - Flucloxacillin, AMX – Amoxicillin, AMOC – Amoxyclav, MET - Metronidazole.

3.5. Antimicrobial Susceptibility Patterns of Isolates from Patients Attending SPH and NGH.

The bacterial isolates were tested against six commonly prescribed antibiotics identified in this study. The CLSI guidelines were followed for testing and interpretation of drug susceptibility and multiple antibiotics were used for the antimicrobial susceptibility assay and were confirmed by studying MIC values using HiComb strips. It was observed that the isolates showed different susceptibility to all antimicrobial tested. Majority of the isolates were susceptible to ciprofloxacin and clindamycin, with *Pseudomonas* spp. showing the highest resistance level of 24% and 36% to ciprofloxacin and clindamycin respectively (Table 4). However, more than 50% of all organisms tested showed resistance to flucloxacin and amoxicillin. It was observed that 30.47% and 25.76% of the patients investigated from NGH and SPH respectively, had used herbs on their chronic wounds before (data not shown). As the age of patients increases the percentage of patients that uses herbs also increased.

Table-4. Antimicrobial susceptibility profile of bacterial isolates from both SPH and NGH

Number of resistance pathogens to antimicrobial agents (%)						
Bacterial isolate (n)	**CIP**	**CLI**	**CEF**	**FLU**	**AMX**	**AMOC**
Staphylococcus aureus (73)	2(3)	11(15)	29(40)	47(64)	51(70)	49(67)
Pseudomonas spp. (59)	14(24)	21(36)	45(76)	54(92)	46(78)	24(41)
Escherichia coli (25)	4(16)	2(8)	6(24)	18(72)	22(88)	10(40)
Klebsiella spp. (19)	3(16)	1(5)	5(26)	15(79)	17(89)	9(47)
Enterobacter spp. (23)	5(22)	5(22)	8(35)	19(83)	15(65)	8(34)
Proteus spp. (25)	2(8)	1(4)	10(40)	18(72)	18(72)	7(28)
CoN Staphylococci spp. (11)	0(0)	0(0)	2(18)	9(82)	6(56)	5(45)
Streptococci spp. (2)	0(0)	0(0)	1(50)	2(100)	2(100)	1(50)

Abbreviations: CIP - Ciprofloxacin, CEF - Cefuroxime, CLI - Clindamycin, FLU - Flucloxacillin, AMX – Amoxicillin, AMOC – Amoxyclav, MET – Metronidazole

4. DISCUSSION

The number of patients developing chronic wounds is increasing with the worldwide increase in lifestyle diseases. This study was a comprehensive analysis of microbial profile and antibiotic usage of patients with chronic wounds attending two hospital facilities in Ghana. At the SPH, the major cause of chronic wounds was inflammation (50%) and cut accounted for 24.2% (Figure 1) as has been observed in earlier studies by [6]. Inflammation is one of the body's ways of dealing with infection and will lead to abscesses [7] and eventually become chronic. [8] also reports that, in a new wound, the inflammatory response starts the healing process by activating the immune system. However, interestingly, we found that, in NGH, most of the wounds were acquired through cut (41%). This could be due to the fact that about 80% of the population are farmers. The main tool

farmers in these areas use in their work is cutlass, which could easily cut them during farming related activities and may lead to chronic wounds if not treated effectively. 57% of the patients' wounds were located on the lower extremity part of the body [9]. Showed that about 70% of chronic wounds are located on the leg and may probably be due to the fact that lower extremity wounds are difficult to heal [10] because they are located on a high risk anatomical site which easily comes into contact with bacteria leading to infection [11].

More than 200 different species of bacteria normally live on the skin and an open wound provides a moist, warm and nutritious environment perfect for microbial colonisation and proliferation [8]. Among the 260 study subjects, bacterial pathogens were isolated from 211 patients with the isolation rate of 81.2%. This was higher than a previous study in Addis Ababa of 42% [12]. The high rate in the present study may be due to the criteria for selection of patient and the technique used to collect wound swab specimen. However, our results were found to be consistent with similar studies [13]. On the other hand, our results were lower than a similar study conducted in Nigeria (94%) [14]. The predominant bacteria isolates were *Staphylococcus aureus* (31.6%) and *Pseudomonas* spp. (24.9%) (Table 3). A number of similar previous studies from different part of the world also indicated that *Staphylococcus aureus* and *Pseudomonas* spp. as the most frequent isolates from wounds [15, 16]. This may be due to the fact that *Staphylococcus aureus* is a commensal and act as opportunistic pathogen when there is a disruption of natural skin barrier to cause infection. We also found marginal but insignificant variation ($p = 0.592$) in bacteria in samples from different age groups of individuals and in males and females.

The most frequently isolated pathogens were Gram-negative bacteria with Gram-positive bacteria which is in agreement with earlier studies [17] even though other studies indicate that Gram-positive bacteria were the ones most frequent isolates. These differences may be due to the various factors in our research studies, such as wound etiology, culture technique and different population and sample size.

Though the present study indicated that monomicrobial growth is higher than was reported in Ethiopia (81.7%), it is similar to other retrospective study of (91.6%) [18, 19]. In our study, polymicrobial infections were mainly made up of *Pseudomonas* and *Proteus* spp. (30.8%), and *Pseudomonas* spp. and *Staphylococcus aureus* (23%) which is inconsistent with other reports where *Klebsiella spp.* and *S. aureus* were identified as the common cause of polymicrobial infections.

Patients with chronic wounds who received antibiotics prescription was 87.31% and is comparable to 82.4% obtained elsewhere. However, our results contrast the average recommended by the World Health Organization of 20 -26.8%. It is also higher than previous studies from other developing countries like Tanzania (39%) and Nigeria (50.1%) [20, 21]. This over prescription and misuse of antibiotics at these facilities could possibly be that, even in hospitals with good laboratory facilities, culture and susceptibility tests are not requested, because it takes several days for results to be available or the huge cost involved.

Flucloxacillin and ciprofloxacin were the most prescribed monotherapy antibiotics followed by amoxiclav (Figure 3). Metronidazole and cefuroxime was the most prescribed combination therapy followed by metronidazole and amoxiclav even though it is reported elsewhere that Ciprofloxacin and Metronidazole was the most prescribed antibiotics at a teaching hospital facility in Ghana. The low cost of metronidazole, probably may explain its high prescription rate. Flucloxacillin on the other hand, is mainly prescribed to treat infections likely to be caused by gram-positive organisms, the most common being *staphylococcus aureus* whilst ciprofloxacin is the drug of choice in the treatment of wide variety of infections, especially those caused by *Pseudomonas aeruginosa* and other Gram-negative bacteria.

The present study has demonstrated that most isolates were resistant to flucloxacillin and amoxicillin (Table 4). In a similar study, reported that amoxicillin has the highest resistance rate of 78.9% and 83% respectively [18].

Reduced effectiveness of flucloxacillin and amoxicillin could be due to frequent prescription and use of these antibiotics without culture and sensitivity test. Ciprofloxacin and clindamycin were the least resistance to all isolates tested. Ciprofloxacin is the drug of choice in the treatment of wide variety of infections, especially those caused by *Pseudomonas aeruginosa* and other Gram-negative bacteria. Resistance of *pseudomonas spp.* to cefuroxime (76%) was very alarming (76%) and may be due to the fact that most people in developing countries with chronic wounds and admitted in hospital have infection caused by *pseudomonas* s*pp.* due to lack of proper hygienic conditions [22].

This study demonstrates that, inflammation was the major cause of chronic wounds and that, most wounds were located on the lower extremity part of the body. The isolation rates of *Staphylococcus aureus* and *Pseudomonas* spp. were the highest. The commonly prescribed monotherapy antibiotics were flucloxacillin and ciprofloxacin whilst metronidazole and cefuroxime were mostly prescribed together. High levels of bacterial resistance to flucloxacillin and amoxicillin was observed, whilst majority of the isolates were susceptible to ciprofloxacin and clindamycin. Due to this situation, we propose that health workers must be made aware of the various bacterial pathogens in chronic wounds and their antimicrobial resistance levels for proper management, whilst efforts are made to perform culture and susceptibility test before antibiotics prescription.

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