Prevalence and Antimicrobial Susceptibility of Bacteria Isolated from Septic Wounds among Patients Attending Uganda Martyrs' Hospital Lubaga.

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Abstract

Background:[a]
Wound infections are associated with increased morbidity and mortality. Etiologic agents of wound infections vary with geographical locations. Pathogens that infect wounds can be part of normal flora or acquired from the hospital environment. This study aimed to determine the prevalence of wound infections, investigate the profile of pathogens cultured from an infected wound and determine their antimicrobial resistance pattern to commonly prescribed antibiotics.

Methodology:
A prospective cross-sectional study was conducted at Uganda Martyrs' Hospital Lubaga from March 2020 to May 2020. Swabs from different types of wounds were processed to investigate etiologic agents using the standard microbiological technique. Antimicrobial susceptibility tests were done using a simple disc diffusion technique.

Results:
Out of 210 wound swab samples analyzed, 125(59.5%) were culture positive. 20(16%) of the culture had mixed infections and a total of 238 bacteria were isolated from 205 cases. *Staphylococcus aureus* was the most frequently isolated pathogen which accounted for 94 (75.2%) of isolates followed by *Proteus* 11 (8.8%). The sensitivity rates of Ciprofloxacin, ceftriaxone, and gentamicin were 80.6%, 80.6%, and 74.2% respectively.

Conclusions and recommendation:
S. aureus and *Proteus* were the predominant causes of wound infections. Ciprofloxacin, ceftriaxone, and Gentamicin were the most effective drugs. Periodic surveillance of the species of bacteria involved in wound infection and determination of their antimicrobial resistance is recommended for empirical treatment.

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1 Background

Bacterial infections of wounds are among the leading causes of morbidity and mortality throughout the world and are regarded as one of the most common nosocomial infections (Ohalete *et al.,* 2012). Wound infections have been reported to vary between 3 and 11% in developed countries and are estimated to be as high as 40% in developing countries (Truong, 2011). In Uganda, previous studies at Mulago National Referral Hospital showed that 28.7% of the SSIs are due to S. aureus, and of these 31.5% are due to MRSA (Ojulong *et al.,* 2010). Wound infections increase with

the degree of wound contamination, and it is estimated that 50% of wounds contaminated by bacteria become clinically infected (Mawalla *et al.,* 2011). Drug resistance impinges on the quality of patient care through its associated mortality, morbidity, and significant economic consequences (Moremi *et al.,* 2012). In hospital practice, 30–50% of antibiotics are prescribed for surgical prophylaxis and 30–90% of these prophylaxes are inappropriate (Moremi *et al.,* 2012). Inappropriate use of antibiotics increases selection pressure favoring the emergence of pathogenic drug-resistant bacteria which makes the choice of empirical antimicrobial agents more complicated

According to Kassam *et al.,* (2017), extended-spectrum beta-lactamase (ESBL) producing organisms are another type of common bacteria resistant to antibiotics, ESBL producing Gram-negative rods (GNRs) have spread all over the world.

The prevalence of ESBL producing gram-negative rods varies across the world from 50 to 80% (Shriyan *et al.,* 2010 and Etok *et al.,* 2012). About 33% of infections by ESBL producers are deadly (Kassam *et al.,* 2017).

In Tanzania, the death rate due to ESBL producing gram-negative rods is as high as 13.9% (ReAct, 2012). Comparing to Gram-negative, Gram-positive bacteria have been reported to be less prevalent causing wound infections (Osariemen *et al.,* 2013). According to Kassam *et al.*, (2017), *Staphylococcus aureus* has been reported to be the most common isolated bacteria from different wound types. *Pseudomonas aeruginosa* is commonly isolated in infected wounds following surgeries and burns whereas *Enterococcus species* (Kassam *et al.*, 2017), and *Enterobacteriaceae* are commonly isolated from wounds in immune-compromised patients and abdominal surgeries (Muhammed *et al.*, 2013). The majority of the isolates from infected wounds are known to be resistant to ampicillin and amoxicillin (Kassam *et al.,* 2017). Large numbers of *S. aureus* are methicillin-resistant *S. aureus* (MRSA) and most bacteria isolated are sensitive to quinolones, aminoglycosides, and monobactam (Etok *et al.,* 2012). Infection in a wound delays healing prolongs hospital stay, increases trauma, poses risk for disarticulation and amputation, increases the need for medical care, and increases treatment costs (ReAct, 2012 and Osariemen *et al.,* 2013). This makes infection of wounds a matter of concern and makes it necessary to study the causative agents of these infections and their antibiogram (Kassam *et al.,* 2017). Therefore, this study focused on bacterial isolates and their antimicrobial susceptibility profiles of wound infections among patients in Uganda Martyrs' Hospital Lubaga.

In resource-limited countries with inadequate diagnostic facilities, the spectrum of microorganisms causing wound infections and their antibiotic resistance patterns are only superficially understood (Lia *et al.,* 2018). Pathogen-specific treatment for severe wounds is difficult if the disease-causing agents remain unknown. The widespread and prolonged use of antibiotics leads to the emergence of resistant bacterial pathogens in wound infections contributing to high morbidity and mortality rates (Amare *et al.,* 2011).

Information on bacterial isolates and their antimicrobial susceptibility patterns from the patient at Uganda Martyrs' Hospital Lubaga is limited. Thus, this study aimed to determine the prevalence and antimicrobial susceptibility of bacterial isolates at Uganda Martyrs' Hospital Lubaga in Kampala District.

2 MATERIALS AND METHODS

Study area

The study was carried out in Lubaga hospital which is a private not-for-profit hospital under the framework of the Uganda Catholic Medical Bureau. It is located on Lubaga Hill about 3km from the city center and is bordered by 8 parishes which include; Ndeeba, Kabowa, Nateete, Mutundwe, Lubaga, Busega, and Najjanankumbi I and II. It has a catchment population of 191,107 people (Financial year Report 2019-2020). As of 2015 to date, about 340 new patients have been registered.

It is a fully-fledged hospital rendering both outpatient and inpatient services. It renders surgical, internal medicine, maternity, antenatal and diagnostic services like laboratory and radiology. It has children's wards, medical, surgical, maternity wards, and also three operating theatres.

In October 2012 the Board decided that the name of the hospital should be changed: from previously Rubaga Hospital to Lubaga Hospital. The Board also clarified that the full name of the hospital is now: Uganda Martyrs' Hospital Lubaga.

Study design and duration

A hospital-based cross-sectional study was conducted from March 2020 to May 2020 at Uganda Martyrs' Hospital Lubaga.

Study population

The study population included both outpatients and patients admitted at Uganda Martyrs' Hospital Lubaga with wound infections during the study period.

Sample size calculation

This was calculated using Kish and Leslie formula (1965)

Sample size; $N = \frac{Z^2 \, p \, q}{d^2}$

Where N- Required sample size.

Z- Standard deviation at 95% confidence interval which is 1.96.

p- Estimated proportion of the target population having the characteristics of interest.

d- Acceptable error margin which in this case is set at 0.05

q- (1-p)

$N = \frac{1.96^2 \times 0.164 \times 0.836}{0.0025}$

= 210

Therefore, the sample size was 210 study participants.

Study variables

The dependent variable was bacterial isolates associated with wound infection and drug resistance pattern whereas the independent variable was age and sex.

Inclusion and exclusion criteria

Inclusion criteria

Patients with wound infection and patients who agreed to participate by giving informed consent

Exclusion criteria

Patients who did not have wound infection based on clinical examination during the study period were excluded from the study.

Sampling procedure

Consecutive sampling technique was employed to include study participants who met the inclusion criteria

Data collection

A predesigned and structured questionnaire was developed and used for the collection of data on socio-demographic characteristics (age and sex) of the patient.

Specimens collection

Samples (pus swabs) were collected from patients with wound infections by clinicians and they were sent to the laboratory immediately for culture (See appendix I).

Sample processing

On arrival in the laboratory, the swabs were inoculated onto the prepared culture media which

Consisted of blood agar plates and MacConkey plates. These plates were then incubated at 37°C for 24 – 48 hours and inspected for bacterial growth.

Biochemical test

Colonies from primary cultures were extracted and used for carrying out biochemical tests for the final isolation and identification of the organisms. Gram-negative rods were identified by performing a range of tests that included; motility tests, TSI, urease, indole, oxidase, and Simon's citrate agar.

Susceptibility testing

The drug diffusion disc technique developed by Bauer *et al.,* (1966) was used to assess the susceptibility of the bacterial isolates to the common antibiotics used for treatment at Uganda Martyrs' hospital Lubaga. The test organism was picked using a sterile wire loop, emulsified in sterile broth, and incubated for two hours to let the organisms attain their log growth phase. The density of the suspension was matched with the standard opacity of 0.5 McFarland barium sulphate solution, after which a sterile swab containing the isolate was uniformly seeded onto the Mueller Hinton agar (Oxoid) plate. Antibiotic discs impregnated with different drugs were placed at prescribed regions on the plate and incubated for 18 – 24 hours at 37°C . Zones of inhibition were indicated by the levels of clearing around the antibiotic discs and interpreted using CLSI 2014 guidelines where sensitivity was shown by clearance around the antibiotic disc and resistance indicated by no clearance measured in millimeters.

The Gram-positive isolates obtained were tested against trimethoprim-sulfamethoxazole 25/23 5μg , penicillin (10IU , erythromycin (15μ g , vancomycin (30μ g , cefoxtin (30μ g , and clindamycin (2μ g). Gram-negative bacterial isolates were tested with ciprofloxacin (5μ g), gentamicin (10μ g), trimethoprim-sulfamethoxazole (1.25/23.5μg), chloramphenicol (30μ g), nalidixic acid (15μ g) and ceftriaxone (30μ g).

Quality control

The authenticity and quality of the results to be obtained were dependent on the integrity of the

procedures and protocols that were involved from the time of collection to the analysis of the samples collected. As such, SOPs as per CLSI 2014 were strongly adhered to during the pre-analytical, analytical, and post-analytical processing of the samples.

Also, all the materials and reagents used in the study were adequately controlled to ensure sterility throughout the entire process. In addition to that, all prepared culture media batches were tested for sterility and performance.

As stated by Bauer *et al.,* (1966), standard inoculums of bacterial suspension for susceptibility testing were prepared using reference from 0.5 McFarland barium sulphate solution.

Internationally recognized control strains including *Staphylococcus aureus* (ATCC 25923),

Escherichia coli (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were incorporated into the study.

Ethical considerations

The research proposal together with an introductory letter from COVAB was submitted to the Lubaga Hospital research committee and ethical approval was obtained. Ethical issues conformed to international regulations of research, written consent was obtained from every study participant and the results from laboratory analysis were communicated to the responsible physician who then passed them on to study participants for treatment. The information from the patients was treated with confidentiality and because of this, study numbers and not names were used during data collection and presentation.

Dissemination of results

Copies of the dissertation will be made and disseminated to Makerere University, to the management of Lubaga Hospital and the final copy will be retained by the researcher

3 RESULTS

Demography

A total of 210 patients with wound infection were included in this study, out of which, 172(81.9%) were male and 38(18.1%) were female. resulting in an overall male-to-female ratio of 1:4.5. The age of the patients ranged from 1year to 68 years, with a mean age of 35 (SD=11.6) years. The infection rate was relatively high (41%) in the age group of 21-30 years old followed by the 31-40 years of age group (20%). Most of the respondents with wound infections were students (52.8%) followed by businessmen/ladies (24%). The demographic characteristics of respondents involved in this study are presented in Table (Table1)

3.1 Prevalence of wound infection

Out of a total of 210 swab samples collected and tested, 125 (59.5%) of them presented with bacterial growth whereas the remaining 85 (40.5%) of the swabs had no growth after 48 hours of incubation. So, this gave a prevalence of 59.5%.

3.2 Common bacteria associated with wound infections Gram stain reaction of the organisms cultured

From a total of 125 samples that showed bacterial growth, only 31 (24.8%) were Gram negative while the rest 94 (75.2%) were Gram positive (Table 2).

Staphylococcus aureus was the most predominant isolate with 94(75.2%) followed by *Proteus spp* with 11(8.8%), *E. coli*, *K. pneumonia, P. aeruginosa* and *Enterobacter* presenting 9(7.2%), 5(4%), 4(3.2%) and 2(1.6%) respectively.

All mixed infections in this study involved Gram-positive and Gram-negative bacteria with *S. aureus* and *E. coli* show the most common association in 9 (45%) cases. Infections with *S. aureus* and *Proteus spp,* 7(35%) *S. aureus* and *Pseudomonas aeroginesa* 4(20%) were among the mixed infections, there was no case with more than two organisms in our study.

Antimicrobial susceptibility of the bacterial isolates from the septic wound among patients seeking medical care at Uganda Martyrs' Hospital Lubaga

Gram-positive antimicrobial susceptibility pattern

Staphylococcus aureus was the only Gram-positive bacteria isolated. Their susceptibility patterns were determined against a profile set of 12 antibiotics. Vancomycin and teicoplanin showed sensitivity in all 125 (100%) of the Gram-positive isolates followed by clindamycin with 104(83.2%) sensitivity and 21(16.8%) resistance, gentamycin with 102(81.9%) sensitivity, and 23(18.1%) resistance while the least effective antibiotic was tetracycline with 37(30%) sensitivity and 88(70%) resistance, penicillin G with 32 (25.1%) sensitivity and 93(74.9%)

Table 1. Demographic characteristics of study respondents with wound infections at Uganda Martyrs' Hospital Lubagas.

Age group (years)	No. of Culture positive (N=125)	No. of culture negative (N=85)
<10	12(10)	12(14)
11-20	10(8)	16(19.2)
21-30	51(41)	26(30.6)
31-40	25(20)	18(21)
41-50	18(14)	11(12.8)
< 51	9(7)	2(2.4)
Education level		
Preschool	7(5.6)	4(4.7)
Primary restore	11(8.8)	15(17.6)
Secondary	55(44)	9(10.6)
Tertiary	52(41.6)	57(67.1)
Occupation		
Preschool	7(5.6)	4(5)
Students	66(52.8)	24(28)
Peasant	22(17.6)	19(22.3)
Business men/ Lady	30(24)	38(44.7)

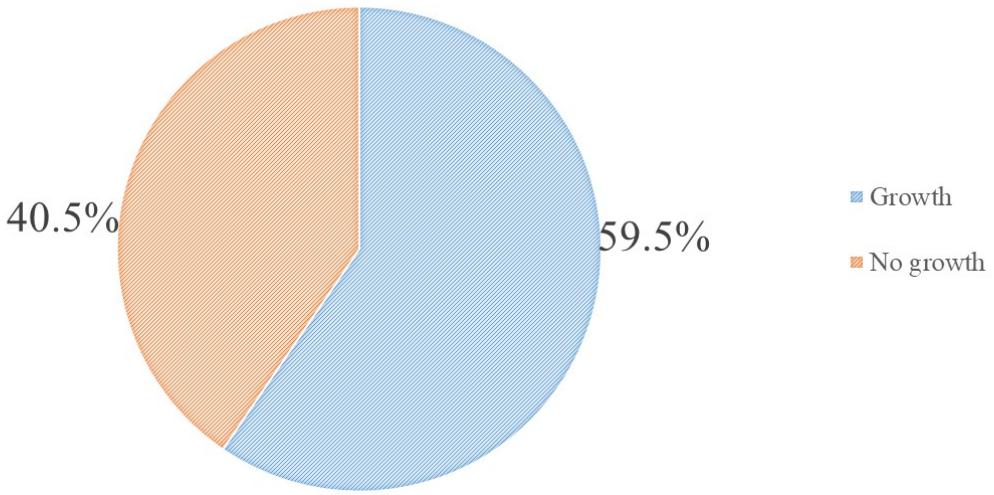

Chart 1. Prevalence of wound infections caused by bacteria among patients seeking medical care at Uganda Martyrs' Hospital Lubaga

Table 2. Gram stain reaction of the isolates obtained wound infection in respondents at Uganda Martyrs' Hospital Lubaga

Gram stain reaction	**Frequency**	**Percentage**
Positive	94	75.2
Negative	31	24.8
Total	125	100.0

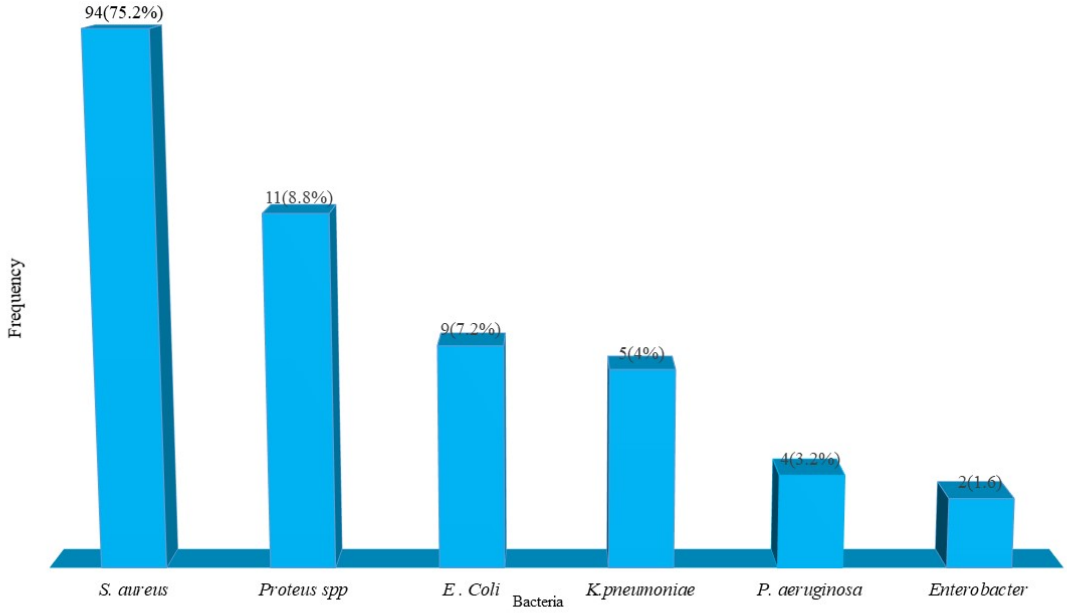

Chart 2. Frequency of the bacteria isolated from the swabs with bacterial growth in respondents seeking medical care at Uganda Martyrs' Hospital Lubaga

Table 3. Type and frequency of pathogens with mixed infection from wound infections in respondents seeking medical care at Uganda Martyrs' Hospital Lubaga

Pathogen	Frequency	Percentage (%)
S. aureus and E. coli	9	45
S. aureus and Proteus	7	35
S. aureus and P. aeruginosa	4	20
Total	20	100

resistance, ampicillin with 31(25%) sensitivity and 94(75%) resistance cotrimoxazol with 13(10%) sensitivity and 112(90%)resistance. However, *S. aureus* showed 100% resistance to ciprofloxacin, cefotaxime, and oxacillin each. Vancomycin together with teicoplanin were the most effective antibiotics from the results.

3.3 Gram negative antimicrobial susceptibility pattern

Proteus vulgaris 11(8.8%), *E. coli* 9(7.2%), *Klebsiella pneumoniae* 5(4%) *Pseudomonas aeroginesa* 4(3.2%), and *Enterobacter species* 2(1.6%) were the gram-negative isolates obtained in the study, representing a total of 31 isolates. The drug susceptibility patterns for the isolates were tested against a panel of 6 antibiotics and the results revealed that ciprofloxacin and ceftriaxone had the highest sensitivity of 25(80.6%). Gentamycin had sensitivity of 23(74.2%), Ampicillin with 21(67.7%), nalidixic with 19(61%) while chloramphenicol had the highest resistance of 21(67.7%). This information showed that ceftriaxone, ciprofloxacin and gentamycin are the best drugs of choice for treating infections caused by Gram negative bacteria.

4 DISCUSSION

A prevalence of 59.5% of wound infections was observed in this study. This was higher than that previously reported in Mbarara Uganda with a prevalence of 16.4% (Lubega *et al.,* 2017), Nepal with a prevalence of 23% (Giri *et al.,* 2013), Tanzania 26% (Mawalla *et al.,* 2011) and Ethiopia 10.6% (Mulu *et al.,* 2012). However, the prevalence observed in this study is lower than that previously observed in North East Ethiopia 70.5% (Mulugeta *et al.,* 2011),

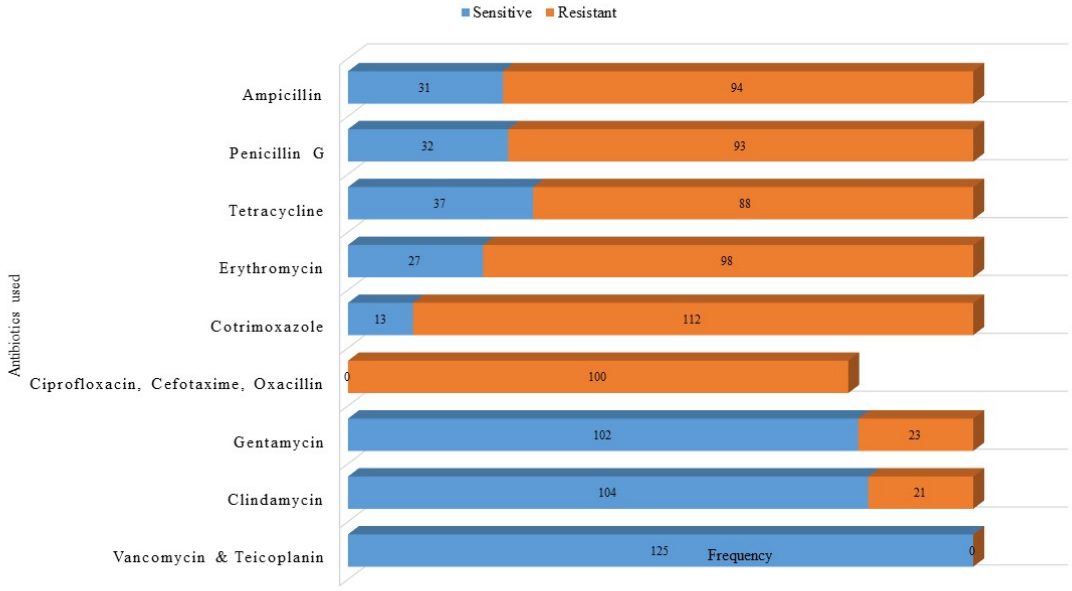

Chart 3. Gram positive antimicrobial susceptibility pattern in respondents seeking medical care at Uganda Martyrs' Hospital Lubaga

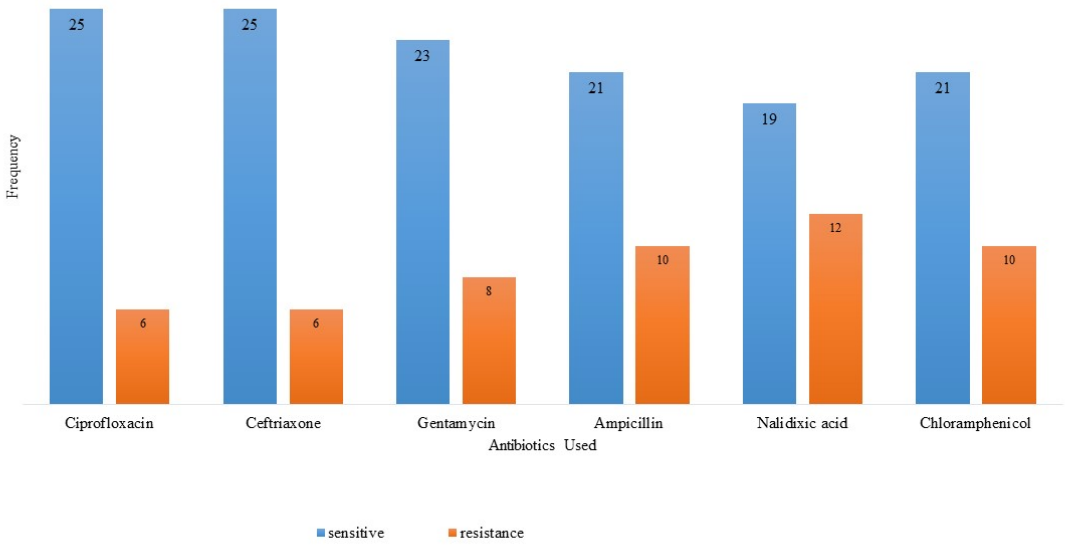

Chart 4. Gram negative isolate antimicrobial susceptibility pattern in respondents seeking medical care at Uganda Martyrs' Hospital Lubaga

Addisa Ababa Ethiopia 83.3% (Tigist *et al.,* 2012). The higher prevalence of wound infection in this study could be attributed to poor sterility during surgical procedures, poor hygiene due to poor standards of living in communities, and lack of reliable surveillance methods for discharged patients.

The Gram-positive bacteria isolated from the pus swab collected from wound infections was *Staphylococcus aureus* 94(75.2%) as the most prevalent bacterial agent isolated in this study. This observation agreed with several previous studies conducted at different places (Mulugeta *et al.,* 2011; Tigist *et al.,* 2012). However, Girma *et al.,* (2013) reported *Proteus species* as the most prevalent agent for wound infections. This deviation might be due to variation in the distribution of microbial agents between different geographical locations and regions within the same country. The possible reason for the high frequency of *Staphylococcus aureus* is that these bacteria are commonly found in the hospital environment (Girma *et al.,* 2013) which might increase wound infection rate and cross-contamination among admitted patients. Also, these bacteria are normal flora in a healthy person (especially *S. aureus* on the skin) when they get the skin damaged and soft tissue they can easily spread as explained by Khana *et al.,* (2010).

The prevalence of mixed infections 20(16 %) observed in this study was slightly lower than that reported in Jimma University Specialize Hospital Ethiopia 22.9% (Girma *et al.,* 2013) and Nigeria 33.2% (Agbe *et al.,* 2011) reported in previous studies.

Amongst the Gram-negatives, *Proteus vulgaris* 8.8% (11/31) was the most prevalent isolate. Others including, *E. coli* 9(7.2%), *Klebsiella pneumonia* 5(4%), *Pseudomonas aeroginesa* 4(3.2%), and *Enterobacter* 2(1.6%) were amongst the isolates obtained. The findings are in agreement with a similar study done by Ekrami *et al.*, (2014) in which *proteus* was the most predominant Gram-negative isolate identified.

According to the study, the most effective antibiotic for Gram-positive isolates was vancomycin with 100% sensitivity, followed by clindamycin and trimethoprim-sulfamethoxazole with 83% and 61% sensitivities respectively. However, an increased resistance was observed to penicillin, cefoxtin, and erythromycin with 93%, 48%, and 48% resistance respectively. Similar studies revealed 100% resistance rates for penicillin G, and clindamycin (Abdallah *et al.,* 2015) and 100% resistance to penicillin G (Kajela, 2013). These trends could be as a result of the increasing poor use and abuse of antibiotics by the general population resulting in a rise in the number of resistant bacterial strains and their relatively high availability as they are cheap to obtain.

The study deduced the most effective antibiotic for Gram-negative isolates was ciprofloxacin,

ceftriaxone, and gentamycin with 80.6%, 80.6%, and 74.2% sensitivity respectively. However, the least effective antibiotic was chloramphenicol with 67.7% resistances. Other similar studies reported low susceptibilities to erythromycin at 7.1% and chloramphenicol at 6.5% (Kihla *et al.,* 2014). The high resistance is reflective of the ready availability of these drugs in the population and the increasing abuse of prescriptions.

5 CONCLUSIONS AND RECOMMENDATION

6 Conclusion

The overall general prevalence of the isolated microbes was high with 59.5%. The results from the study also showed that *Staphylococcus aureus* was the most predominant bacteria found contaminating wound infections. Other bacterial isolates included *Klebsiella pneumoniae*, Proteus vulgaris, E. coli, pseudomonas spp, and Enterobacter spp.

Recommendations

A high prevalence of potential nosocomial infection-causing pathogens observed implies insufficient sterilization and decontamination protocols and therefore, we recommend reconsiderations about the type, strength, usage, and quantity of disinfectant used by the

Hospital administration.

From the study, increasing resistance trends of bacteria against single or multiple antibiotics were observed. This is possibly due to the abuse of antibiotics described and poor decontamination and sterilization protocols.

Several organisms were resistant to multiple antibiotics from the drug susceptibility patterns from the study. We, therefore, recommend the use of vancomycin for Gram positives, ciprofloxacin, and ceftriaxone for Gram-negatives which can come in handy most especially in the empiric treatment as bacterial culture results tend to take a while before returning to the ward.

Additionally, routine infection control surveillance and monitoring systems should be put in the hospital and the whole country at large to identify emerging pathogens and their respective susceptibility patterns to antibiotics.

Vancomycin is the most recommended antibiotic of choice for the treatment of *Staphylococcal* infections followed by clindamycin. Ciprofloxacin was the recommended drug of choice for the treatment of gram-negative infections.

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8 LIST OF ABBREVIATIONS AND ACRONYMS

AHS Allied Health Sciences
API Analytical profile index
CDC Centre for Disease Control and prevention
CLSI Clinical and Laboratory Standards Institute
CoNS Coagulase Negative Staphylococcus species
ESBL Extended spectrum beta-lactamase
GNRs Gram-negative rods IHSU International Health Sciences University
MDR Multidrug resistance
MRSA Methicillin Resistant S. aureus
REC Research Ethics Committee
SSI Surgical site infections
VRE Vancomycin-Resistant Enterococus
WHO - World Health Organization

9 Definition of Terms

Septic wounds: These are also known as infected wounds. Wound care centers define infected wounds as in which bacteria and other microorganisms have colonized, causing a delay in wound healing or deterioration of the wound.

Multidrug resistance: This is an isolate that is non-susceptible to one or more agents in three antimicrobial classes.

Nosocomial infections: Are defined as hospital-acquired infections developing at least 48–72 hours after hospital admission.

Surgical site infections: These are infections that occur within 30 days after the operation and these infections involve the skin and subcutaneous tissue of the incision. The infection may be indicated by the presence of pus or abscess, fever with the tenderness of the wound, or separation of the edges of the incision exposing the deeper tissues (CDC).

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