PREVALENCE OF BACTERIAL OSTEOMYLYELITIS AND ANTI BIOTIC SUSCEPTIBILITY OF ISOLATE AT JIMMA MEDICAL CENTER, JIMMA TOWN, SOUTH-WEST ETHIOPIA.

By:

SARA JEMAL (BSc)

A THESIS SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MSC) IN MEDICAL MICROBIOLOGY

MAY, 2022

JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF BACTERIAL OSTEOMYLYELITIS AND ANTI BIOTIC SUSCEPTIBILITY OF ISOLATE AT JIMMA MEDICAL CENTER, JIMMA TOWN, SOUTH-WEST ETHIOPIA

BY

SARA JEMAL

ADVISORS:

1. Mr. ZEWDINEH S/MARIAM (Ass. Professor)
2. Mr. MEKIDIM MEKONNEN (MSc)

MAY, 2022

JIMMA, ETHIOPIA

ABSTRACT

***Background**: Osteomyelitis is an infection of the bone caused by microorganisms, most commonly bacteria. The infection can affect people of all ages and any bone. The three routes of acquisition of infection are through blood stream, direct invasion and adjacent soft tissue infection. Without treatment or in the events of treatment failure it becomes a chronic disease and causes disability and morbidity. Updated knowledge on prevalence and antibiotic susceptibility pattern of osteomyelitis is essential for optimal management of patient.*

***Objectives**: The aim of this study was to identify prevalence of bacterial osteomyelitis and antibiotic susceptibility of isolates From December 2019 to November 2020 among patients at J M C*

***Materials and Methods:** A cross sectional study was conducted at* JMC *from December 1, 2019 to November 30, 2020.Culture were performed to isolate bacteria and antibiotic susceptibility was assessed as standard procedure (CLSI).Demographic and related clinical data were collected by using a structured questionnaire and analyzed by SPSS version 26 for statistical analysis. Findings were interpreted in the light of study objectives and conclusions and recommendations were drawn. A p-value of < 0.05 was considered as statically significant.*

***Result;** from a total of 119 participant the overall prevalence of bacterial osteomyelitis as evidenced by culture was 58.8% (70/119). Disease prevalence in male 82.9 %(58/70) While the highest infected age group was 21-40 years (41.4%) femur was a high percentage of type of affected bone 29(41.4%).Seven different bacteria were identified. Staphylococcus aurous was the most frequently isolated pathogen which accounted for 27 (38.6%).E. coli were the second accounts 23(34.3%) followed by Streptococcus. spp6(8.6%),Acinitobacter5(7.1%),Pseudomonas aurogenosa 4(5.7%), provedentia 3(4.3%),kellebsiela 2(2.9%). 92.6% of S.aures are resistance for penicillin and 100% sensitive for gentamicin. All gram negative bacteria are resistance for ampicillin except providences spp and pseudomonas aurgenosa. . In our study MDR was observed in 46/70 (65.7%) of isolates. Of this 16/46(48.48%) Gram positive and 30/16 (81.1%) Gram negative bacteria respectively.*

***Conclusion**; S. aureus and E.coli were the predominant causes of osteomyelitis in our study. Ciprofloxacin was the most effective compared with other drugs tested against the Gram positive. Multi-drug resistance was detected in 65.7%% of the isolates. The bacteria are developing resistance to most of routinely used antibiotic. Appropriate selection of antibiotic based on culture result will help to treat the disease success fully and prevent spread of multi drug resistance.*

***Key words**; Osteomyelitis, Bacterial profile, Antibiotic susceptibility*

Acknowledgment

First of all I would like to thank the almighty God, Allah (S.W), for giving me strength and full health to achieve my educational success.

Next my deepest appreciation is goes to my advisors Mr. Zewdineh Sahlemariam and Mr. Mekdim Mekonnen for their valuable and consistent advices, suggestion, remarks and guidance throughout my work.

I would like to acknowledge Jimma University for its financial support.

I greatly acknowledge JMC Bacteriology laboratory staffs, especially Mr.Bikila Alemu and Mr. Dawit Abera for their continuous support and encouragement during the laboratory work.

Also, I would like to thank Mr. Kalid Abdela for helping me with the data entry and proof-reading. The last not the least my grateful appreciation is goes to my family specially my mother Ms. Lemlem A/gisa and my husband Mr.Leta Ashebir for their endless support till now.

TABLE OF CONTENTS

List of tables

Table **Page**

List of figures

Figures **Page**

ABBREVIATIONS AND ACRONYMS

AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
COM	Chronic Osteomyelitis
ICU	Intensive Care Unit
IAFF	Infection after Fracture Fixation
JMC	Jimma Medical Center
MRSA	Methicillin Resistant *S.aureus*
OM	Osteomyelitis
SPSS	Statistical Package for the Social Sciences Software
EOPD	Emergency Outpatient Diagnosis
AOM	Acute Osteomyelitis
GNB	Gram-Negative Bacilli

CHAPTER 1: INTRODUCTION

1.1 Background

Osteomyelitis (Osteo – bones, - myelo – marrow, - itis – inflammation) is defined as a clinical state in which bone is infected with microorganisms. Osteomyelitis is an infection of the bone caused by bacteria. It is one of the most severe complications that can arise following trauma or after operative treatment of bone. Bones, which usually are well protected from infection, can become infected through three routes blood stream, direct invasion and infections in adjacent bone or soft tissues (1).

Osteomyelitis occurs by direct inoculation of bacteria into bone or from contiguous soft tissue infection or chronic overlying open wound (2).

Chronic osteomyelitis (COM) is a severe, persistent and sometimes incapacitating infection of bone and bone marrow (3). The infection specifically affects nutritional status by increasing protein and energy requirement. Evidence shows that nutritional status or malnutrition remains undiagnosed in up to 70% of hospital patients (4). It is a persistent disease, difficult to treat or eradicate completely. In the absence of early diagnosis and prompt treatment or due to development of drug resistance, it is still an important cause of high morbidity((5). *Staphylococcus aureus* is the most common causative agent (6). Despite the growing interest in the study of Gram-negative bacilli (GNB) infections, very little information on osteomyelitis caused by GNB is available in the medical literature (7).

Treatment of osteomyelitis is challenging particularly when complex multi-resistant bacterial biofilm has already been established. Bacteria in biofilm persist in a low metabolic phase, causing persistent infection due to increased resistance to antibiotics. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common causative organism responsible for more than 50% of osteomyelitis cases. The treatment implies the administration of high doses of antibiotics (AB) by means of endo venous and oral routes and should take a period of at least 6 weeks.(8)

1.2 **Statement of the problem**

Osteomyelitis is an inflammatory bone disorder caused by infection, leading to necrosis and destruction of bone. It can affect all ages, involve any bone, become a chronic disease and cause persistent morbidity (8).Infection still represents one of the most common challenges in the treatment of open fractures. It is well known that most infections in open fractures of nosocomial origin as causative microorganisms of infection are different to those found in initial smears (9).

A Population-Based Study, on Minnesota (1969 to 2009) the overall age and sex-adjusted annual incidence of osteomyelitis was 21.8 cases per 100,000 person-years.11.4 cases per 100,000 person-years in the period from 1969 to 1979 to 24.4 per 100,000 person-years in the period from 2000 to 2009.(10)

In Germen Overall osteomyelitis prevalence increased by 10.44% from 15.5 to 16.7 cases per 100,000 inhabitants between 2008 through 2018. Out of 11,340 cases in 2018, 47.6% were diagnosed as chronic, 33.2% as acute and 19.2% as unspecified osteomyelitis. (11).

Another study in germen on pediatrics osteomyelitis Pediatric osteomyelitis remains challenging to treat from medical institutions for the time period 2009 to 2019. Incidence rates of osteomyelitis increased by 11.7% from 8.2 cases per 100,000 children in 2009 to 9.2 cases per 100,000 children in 2019.(12)

Osteomyelitis in children is a serious disease in it requiring early diagnosis and treatment to minimize the risk of squeal. Therefore, it is of primary importance to recognize the signs and symptoms at the onset and to properly use the available diagnostic tools. It is important to maintain a high index of suspicion and be aware of the evolving epidemiology and of the emergence of antibiotic resistant and aggressive strains requiring careful monitoring and targeted therapy(13).

Osteomyelitis is a common cause of morbidity in developing countries. Treatment is becoming increasingly troublesome due to rise in drug resistant isolates(14). In diabetic patient, Osteomyelitis secondary to foot ulcer is a common occurrence(15). Diabetic patients is about 15% life time risk to develop pedal ulcer (16).

Bone infections after trauma, i.e. posttraumatic osteomyelitis, pose one of the biggest problems of orthopedic surgery. Even after sufficient clinical therapy including vast debridement of infected bone and antibiotic treatment, regeneration of post infectious bone seems to be restricted (17). Post-traumatic osteomyelitis is bone infection secondary to a trauma (open fracture or bone surgery). It is a painful and frustrating disease characterized by high rates of therapeutic failures and a costly management. However, treatment is increasingly becoming difficult because of the phenomenon of antibiotic resistance(18).

One of the most challenging complications in trauma surgery is infection after fracture fixation (IAFF). IAFF may result in permanent functional loss or even amputation of the affected limb in patients who may otherwise be expected to achieve complete, uneventful healing. Over the past decades, the problem of implant related bone infections has garnered increasing attention both in the clinical as well as preclinical area (19).

Osteomyelitis is a serious issue; even in a developed and industrialized country such as Germany diagnosis of infections in osteomyelitis patients poses a challenge to clinicians due to various factors. Based on this consideration, a prospective cross-sectional study was carried out to investigate the Bacterial profile of osteomyelitis and document the antibiotic susceptibility of isolates among patients at Jimma Medical Center, Jimma.

1.3 **Significance of the study**

Osteomyelitis has remained an orthopedic clinical challenge for many decades. In resent time Accidents and trauma are a leading global cause of mortality in young adults. An infection of the bone (osteomyelitis) is one of the most predominant causes of death in traumatized patients. This is because of factors related to host and those due to trauma itself. Severely traumatized patients admitted to the ICUs are prone to get nosocomial infections due to open wounds and indwelling life saving devices.

Empiric treatment to this infection may develop drug resistance; therefore treatment should be based on the result of culture and sensitivity.

Review of the literature on the prevalence of bacterial osteomyelitis and surveillance of antibiotic susceptibility in the study area revealed no study done so far. Therefore, the present study was undertaken to contribute to the knowledge gap in this problem area.

Results from this study are anticipated to provide baseline data as well as valuable information that would aid in the decision-making and improvement in the care of patients especially in the selection of antibiotic regimen for appropriate treatment

CHAPTER 2: LITERATURE REVIEW

Osteomyelitis has been continuing as the most important cause of morbidity among patients with bone infections. This infection represents one of the most common challenges in the treatment of open fractures. Even though early detection of cases and advanced treatments, osteomyelitis is still continued as a major problem due to treatment failures and multidrug resistance (9).

The study was carried out on changing trends in the epidemiology of vertebral osteomyelitis in Marseille, France in 2015. Among the 50 cases of vertebral osteomyelitis which represented 2.6% of 3778 patients with bone and joint infection managed in a centers overs 6-year study period , 84% of the cases were in men. The mean age was 55 years. A single organism was isolated in 92% of cases. Gram-positive bacteria were identified in 76% of cases, while Gram-negative bacilli (GNB) were found in 18% of case.(20).

Another study was conducted in 2018 on Bacterial osteomyelitis: microbiological, clinical, therapeutic, and evaluative characteristics of 344 episodes. Mean age was 52.5 ± 18.3 years and 233 (67.7%) were male. post-traumatic OM (26.2%). Tibia (24.1%) and femur (21.8%), and methicillin-susceptible *Staphylococcus aureus* (29.6%) were the most commonly involved bone and bacteria, respectively. *Staphylococcus aurous* was the most frequently isolated monomicrobial infections (29.6% of patients). The second and third most frequently isolated microorganisms were *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (4.1% each, respectively). The rate of polymicrobial infection was 35.4%. Many others pathogens were found with a lower frequency, *Staphylococcus epider-midis* (3.2%), *Escherichia coli* (2.3%), and *Enterococcus faecium*, *Proteus mirabilis*, and anaerobes <2%(21).

The research done on Southwest China (2017) A Hospital-Based Study retrospective chart review conducted on 5,368 patients diagnosed with extremity traumatic fractures 84 (1.56%) patients were diagnosed with osteomyelitis based on a positive culture result. The most commonly involved infected site was the tibia-fibula (47.62%). 66 (78.57%) of these cases were monomicrobial, and 18 cases (21.43%) were polymicrobial. The infections were predominantly caused by Gram-positive bacteria (56, 53.85%). The most common Gram-positive bacteria were *Staphylococcus aureus* (39 cases, 37.50%) and *Staphylococcus. epidermidis* (6 cases, 5.77%), which were sensitive to ampicillin, synercid/ dalfopristin, linezolid, tigecycline, macrodantin, and vancomycin. *Staphylococcus aureus* was the most

common pathogen in both monomicrobial and polymicrobial cases. All 17 cases of MRSA infection were sensitive to Imezolid, ampicillin, synercid/ dalfopristin, linezolid, tigecycline, Afuradantin, piperacillin/yaz, rifampicin, and vancomycin, respectively. The most common Gram-negative bacteria were *E. coli* (16 cases, 15.38%) and *Enterobacter cloacae* (11 cases, 10.58%), which were sensitive to thienamycin (22).

On the other research done on Brazil (2018). During the study period 193 patients with PTO were analyzed 110 (57%) presenting monomicrobial PTO and 73 (37.8%) polymicrobial PTO. Negative- culture osteomyelitis was diagnosed in 10 (5.2%) patients and they were included in the clinical and epidemiological description. In general, the PTO rate during the study period was 2.5%. Mean age was 50 (± 16-88) years, 68.9% were male. High-energy trauma due to road traffic accidents occurred in the majority (57.0%) of our study population, and consequently, femur (30.5%) and tibia (29.0%) fractures were the most frequently diagnosed. (23)

On the other study done on 2016 Bacteriological profile of chronic osteomyelitis in a tertiary care hospital in South India out 184 patients .There was a male preponderance (163 / 184, 88.5%) with majority, in the age group of10-20 years. Trauma was the major risk factor (95/ 184, 51.6%). The lower limb bones were more commonly affected of which femur (166/184, 90.2%) the predominant bone was involved. Culture was positive in 104/184 (56.6%), with the Gram positive organisms most predominant. MRSA was in 28/104 (27%) cases. Among Gram negative bacilli, *Escherichia coli* was most common organism isolated in 11/104 (10.4%). Other organisms isolated *included Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumanii, Proteus mirabilis, Enterobacter cloacae, Morganella morgagnii* and showed a high level of antibiotic resistance.(24)

The distribution of pathogens causing post traumatic osteomayilitis and pattern of their antibiotic susceptibility were analyzed India in 2016.From total of 78 samples, 53 were positive by culture. Out of the 55 positive samples 7 were polymicrobial and 48 had only single isolate, *Staphylococcus aureus* was the most commonly isolated pathogen. 33(65.4%) and39.4% of *Staphylococcus aureus* were MRSA. Other important organisms isolated included *Pseudomonas aeruginosa, E.coli, Klebsiellapneumonia* and *Proteus mirabilis*. All the isolates of *Staphylococcus aureus* were resistant to Penicillin. However, Vancomycin resistance was not detected in any of the patient with MRSA.

All Gram negative bacilli were sensitive to Piperacillin-Tazobactam and Imipenem. *Staphylococcus aureus* was the most commonly isolated organism.(1)

The study done on Tertiary Care Hospital, Jamnagar, Gujarat, India 2017 0n chronic osteomayelitis the Study group comprised 108 males and 42 females. Majority of the patients were in the age group of 20 – 70 years 117 (78%) with trauma being the most common 76 (50.67%) predisposing factor. The commonest organisms isolated were *Staphylococcus aureus60* (60.60%) and *Pseudomonas aeruginosa*13 (13.13%). Majority of Gram positive organisms were sensitive to Linezolid and Vancomycin and Gram negative organisms to piperacillin tazobactam combination and ceftazidime.(25)

The aerobic bacteriological study of chronic osteomyelitis showed *Staphylococcus aureus* is being continued to be major etiological agent followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Gram-positive isolates were sensitive to linezolid, teicoplanin while gram-negative isolates were sensitive to colistin, ciprofloxacin in the majority. The disease occurs mostly due to traumatic injuries commonly affecting the middle age group. In present study prevalence of methicillin-resistant *Staphylococci aureus* side.(26)
War-wounded civilians in Middle East countries are at risk of post-traumatic osteomyelitis (PTO). first-line antibiotics resistant bacteria (FLAR) among PTO cases in civilians from Syria, Iraq and Yemen admitted to the reconstructive surgical program of Médecins Sans Frontières (MSF) in Amman, Jordan, and to identify risk factors for developing PTO with FLAR bacteria 558 (76.7%) among 727 patients included had ≥1 positive culture results. 318 were from Iraq, 140 from Syria and 100 from Yemen. Among the 732 different bacterial isolates, we identified 228 *Enterobacteriaceae (*31.5%), 193 *Staphylococcus aureus* (26.3%), 99 *Pseudomonas aeruginosa* (13.5%), and 21 *Acinetobacter baumanii* (2.8%). Three hundred and sixty four isolates were FLAR: 86.2% of *Enterobacteriaceae*, 53.4% of *Pseudomonas aeruginosa*, 60.5% of *S. aureus* and 45% of *Acinetobacter baumannii*.(27)

Chronic osteomyelitis in Sub-Saharan Africa review (2019). The incidence of COM in SSA is far greater than high income countries. Tibia is the commonly affected bone with more than 30% of cases, while the peak age is between the intervals 10-21 years of age. 50- 93% of patients present with sinus drainage whereas *Staphylococcus Aureus* is the causative agent in 60-80% of the cases.(28)

The research done in Romania (2020) Male predominance was observed, with boys from rural areas more prone to a poor outcome. *Staphylococcus aureus* was the most common etiologic agent, with 84 patients testing positive. Disease evolution was toward chronicity in patients diagnosed late. The most frequent complications were sepsis and pathological fractures.(29)

Another study in Uganda on Chronic Osteomyelitis among Children Attending Orthopedic Services at Mbarara Regional Referral Hospital (2019) .The prevalence of children with COM was 9.7%. The female: Male ratio was 1:1.2 with a mean age of 11 years. The most infected bone was the tibia followed by the femur. The common clinical presentations were chronic bone pain and discharging sinus tracts. The concordance rate of the microorganisms between the superficial and the deep swabs was 62.5%. *Staphylococcus aureus* was the most predominant microorganism isolated (85%). All the microorganism isolates were sensitive to gentamycin. However, all *Staphylococcus aureus* isolated were resistant to penicillin (30) . *Staphylococcus aureus* is a highly successful Gram-positive pathogen capable of causing both superficial and invasive, life-threatening diseases.(31)

In a study done in Yaoundé Cameroon (2017), a total of 31 patients were recruited; the modal age range was 21-50 years (67.8%) and the sex ratio 1.8:4. The commonest bones affected were the tibia (48.5%) and femur (32.3%). Out of 31 samples, 29 yielded positive culture giving rise to 48 bacteria isolates. Fourteen samples (48%) were polybacterial. The most predominant species was *Escherichia coli* (29%), followed by *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (all 22.6%). The Gram positive organisms showed good sensitivity to Imipenem, Rifampicin, Fucidine, Lincomycin, and to Vancomycin whereas the Gram negative bacilli were mostly sensitive to Imipenem (96.7%), Amikacin (82.1%) and to a lesser extend Quinolones (54%) and Piperacillin/Tazobactam and Ceftazidime (48%)(32).

Prospective cohort study done on 30 consecutive patients(2018) On New Guinea Microbiologic results showed that Gram-negative and mixed flora accounts for more than half of chronic osteomyelitis cases while *Staphylococcus aureus* was a dominating single pathogen (39%). 83% of *S. aureus* isolates were resistant to oxacillin (MRSA). In 73% of the cases of the chronic osteomyelitis was caused by inadequate treatment of open fractures. (33)

Another study on chronic osteomyelitis (COM) on Tikur Anbessa Hospital two years study period, from Jan. 2005 to Jan. 2007A total of 442 consecutive patients .There were 336 (76%) males accounting for 76%. The mean age at the initial presentation was 18 years the youngest patient was aged one month and the oldest was 84 years. The majority (68%) of patients came from rural areas. The mean age at the initial presentation was 18 years the youngest patient was aged one month and the oldest was 84 years (68%) of patients came from rural areas. Discharging sinus was the commonest clinical presentation observed (411, 93%) followed fracture accounted for 93 (79%) of the posttraumatic onset .The main isolate was *S. aurous* and most of the organisms were resistant to the common antibiotic. Swab culture was done in half of the patients(34).

CHAPTER 3: OBJECTIVE OF THE STUDY

3.1 **General objective**

- To determine the prevalence and antibiotic susceptibility of bacteria causing osteomyelitis at Jimma Medical Center. From November 1, 2019 to December 30 , 2020

3.2 **Specific objective**

- To describe the bacterial pathogens responsible for osteomyelitis at Jimma Medical Center
- To determine the antibiotic susceptibility pattern of the bacterial isolates at Jimma Medical Center
- To identify predisposing factors associated with osteomyelitis at Jimma Medical Center

CHAPTER 4: METHODS AND MATERIAL

4.1 **Study area and study period**

The study was conducted at Jimma University Medical Center, in Jimma town, Jimma zone in Oromia regional state south west Ethiopia. Jimma university medical center is found in Jimma town the capital town of the Jimma zone, located 352kms southwest of Addis Ababa. It was established in 1938 during the Italian occupation for the service of their soldiers. Jimma University Medical Center is one of the biggest teaching and referral hospital in the Southwestern part of the country. It provides specialized health services through its nine medical and other clinical and diagnostic departments for approximately 15,000 inpatient, 160,000 outpatient attendants, 11,000 emergency cases and 4500 deliveries per year from a catchment population of about 15 million people. It provides specialized health services through its different clinical and diagnostic departments for about 15 million people from the South West part of the country. It has a bed capacity of 800 and more than 1448 staff, including both supportive and professional staff.

4.2 **Study design**

A prospective cross-sectional study was conducted at Jimma Medical Center. From November1, 2019 -December 30, 2020

4.3 **Population**

4.3.1 Source population

All osteomyelitis suspected Patients attending at JMC during the study period.

4.3.2 Study population

Patients in all age groups and both sexes who were visit JMC for the diagnosis of osteomyelitis during the study period included in the study.

4.4 **Eligibility criteria**

4.4.1 Inclusion criteria

- All patients irrespective of age and sex suffering from osteomyelitis who was attending JMC during the study period from November 1, 2019 – December 30, 2020 are included for study.
- Patient who give consent to participate in the study

4.4.2 Exclusion criteria

- Patient not willing to give sample
- **4.5 Sample size and sampling technique**

4.5.1 **Sample size**

The sample size was determined by using single population proportion formula as stated below. Taking 95% confidence interval and ±5% marginal error, sample size (n) is determined using the following statistical formula.

$$n = \frac{(Z\ 1-\alpha/2)^2\ p\ (1-p)}{d^2}$$

Where, P= Prevalence rate of 50 %(according to the knowledge of the researcher in our country there is no similar study found in this area).

n = Sample size,

Z = Z = 95% confident interval

d= Bond on sampling error tolerated between the sample and population: ±5%

α = Critical value at 95% confidence interval of certainty (1.96)

$$n = \frac{(Z\ 1-\alpha/2)^2\ p\ (1-p) =}{d^2} \qquad \frac{(1.96)^2 \times 0.5\ (1-0.5)}{(0.05)^2} = 384$$

Since the study patient flow is low, I use correction formula by taking N the maximum population flow for study period equal to 150(1 year)

By using error correction formula since N (150) < 10,000

$$Nf = \frac{n}{1+\frac{n}{N}} = 384/\ 1+384/150 = 107.86 = 108$$

In addition, by considering 10% non-respondent rate the total sample size was 119 individuals.

4.5.2 Sampling technique

Non-probability convenient sampling technique was employed to select consecutive study subjects during the study period.

4.6 Measurement

4.6.1 Study variable

4.6.1.1 Dependent variables

- Type of bacteria
- Antibiotics susceptibility

4.6.1.2 Independent variables

Socio-demographic characteristics and clinical presentation including

- Age
- Sex
- Residence
- duration of disease
- Duration of hospital stay
- Chief complaint at presentation
- presence of foreign body
- type of affected bone
- Co-existing disease
- Duration of hospital stay

4.7 **Data collection**

4.7.1 **Data collection tools and procedures**

The participants were interviewed using pre-designed and pre-tested questionnaire to collect data on socio-demographic characteristics and other associated risk factors such as, duration of disease presence of foreign body, coexisting disease, type of affected bone.

4.7.2 Sample Collection, Handling and Transport.

The specimens were collect under aseptic precautions by physician or nurse from patient either during routine visit to the hospital or at operating table with sterile cotton tipped applicator or sterile needles and syringes. Swabs, aspirate and bone curetting were collected aseptically using standard procedures. All sample collected with care to prevent contamination with commensal.

4.7.3 Identification of organism

4.7.3.1 Culture and gram staining

Two swab or aspirate or bone curetting taken from patient .One of the two swabs or aspirate was used for gram stain to make presumptive diagnosis and the second was used for culture. For culture the swab, Bone curetting and aspirate were inoculated directly on blood agar (for gram-positive bacteria), and MacConkey agar (for gram-negative bacteria) (Oxoid, Ltd., England). The plates were incubated in aerobic and microaerophilic atmosphere at 37°C for 24 - 48 h. Candle jar was used for microaerophilic atmosphere. Positive cultures were identified by their characteristic appearance on their respective media, gram staining reaction and confirmed by the pattern of biochemical testes using the standard method.

4.7.3.2 Biochemical test

Biochemical test were performed on colonies from pure cultures for final identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests. (Oxoid, LTD). Namely, carbohydrate utilization tests, indole production, urease test, citrate utilization, lysine iron agar, oxidase test, Kligler iron agar (KIA), H2S production, and motility test. Gram-positive cocci were identified based on their gram reaction, coagulase and catalase test result and also PYR, optochin and bacitracin disk test.(34)

4.7.3.3 Antimicrobial Susceptibility Tests

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique according to Criteria set by Clinical Laboratory Standard Institute (CLSI 2020). The inoculums were prepare by picking parts of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension was determined by comparison with opacity standard on McFarland 0.5 Barium Sulphate solution. A sterile swab was dipped into the suspension of the isolate, squeezed free from excess fluid against the side of bottle and then spread over the agar plate. The test organism will uniformly seed over the Mueller-Hinton agar surface and Expose to a concentration gradient of antibiotic diffusing from antibiotic impregnated paper disk into the agar medium, and then incubate at 37°C for 16–18 hours. Diameters of the zone of inhibition around the discs was measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI. The drugs was tested for gram negative and gram positive bacteria, ampicillin (10µg),amikacin(30µg),Augmentin(30µg) ,ceftriaxone(30µg) ,cefoxtin(30µg),chloramphenicol(30µg), ciprofloxacin (5µg), cephalothin (30µg), gentamicin (10µg), tetracycline (30µg), cotrimoxazole (25µg), and. Penicillin G (10IU), erythromycin (5µg) clindamycin(2µg),vancomycin (30µg) were used.(35)

Mueller-Hinton agar was used for all gram negative and gram positive bacteria, except for *Streptococci spp*. The sensitivity test of *Streptococci* was performed on Mueller-Hinton agar with 5% sheep's blood. Only the conventional antibiotics regularly available for frequent use in the study area were considered for this study and all the disks used for the test were from (Oxoid Ltd. England). (36)

Flow chart

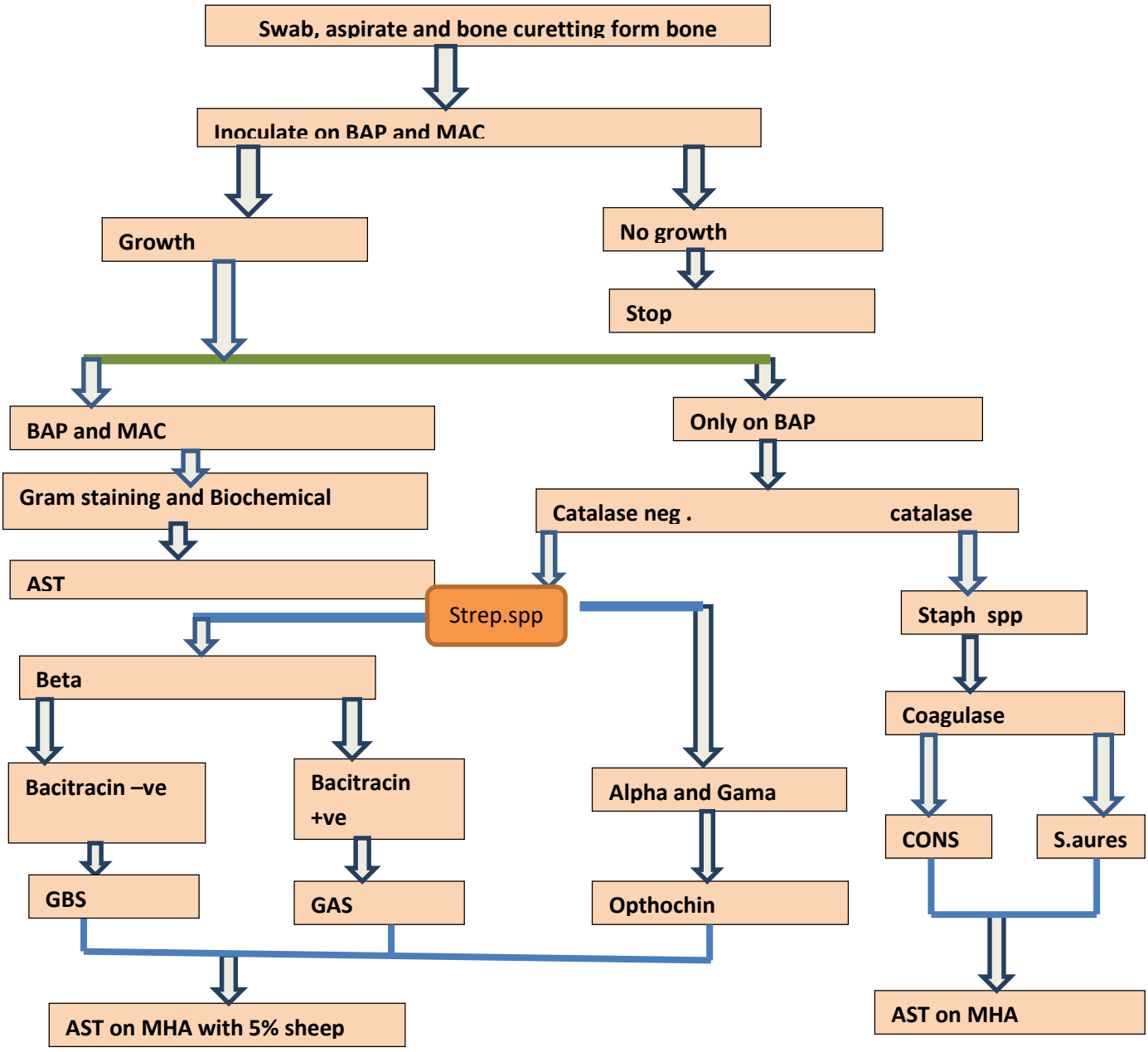

Figure 1: Simplified flow chart for isolation of bacteria from osteomyelitis specimen

4.8 **Data quality assurance**

The following measures was undertaken to control the quality of the data and laboratory investigation. Properly designed and pre-tested data collection instrument was used. Every day the collected data was cross checked for completeness, consistency and onsite correction action was made. Standard operation procedural tools were strictly followed for sample collection, storage and processing. Special emphasis was given during coding the data sheet as well as collecting samples. All reagents were checked for being stored at appropriate temperature and used within specified shelf life. At regular intervals and whenever a new batch of gram stain is prepared, control smears of appropriate organisms were stained to ensure correct staining reactions. Gram-stained smears were prepared. For identifying the gram reaction of organism and also the presence of PMN cell. To avoid measurement bias, quality control was run along with the test sample according to the manufacturer's instruction and test procedures**.**

Laboratory data

All materials, equipment and Procedures were adequately controlled. All stains and reagents were clearly labeled and stored correctly. The preparation, fixation, staining and reporting of smears as detailed in the SOPs of the microbiology laboratory of JMC was strictly followed. For each item of equipment there was clear operating and cleaning instructions, and service sheets. The operating temperature of a refrigerator, incubator, and water-bath was monitored and documented .Culture media was tested for Performance and sterility. To standardize the inoculums density of bacterial suspension for the susceptibility test, a 0.5 McFarland standard was used and standard reference strain *S. aureus* (ATCC-25923), *E. coli* (ATCC-25922) and *P.aeruginosa* (ATCC-27853) was used as Control bacteria strains. All the strains were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI)

4.9 Data analysis

Data entry and analysis procedure

Data entry, transforming and analysis was done using SPSS version 26. Frequency of variables was determined. Odds ratio with 95% confidence interval and P- value was calculated to identify statistical significance. P-value < 0.05 was considered as significant. Association between the variables was checked by first performing bivariate analysis therefore the effect of each predictor on the outcome of interest was checked.

Those variables having significant association ($p < 0.25$) was a candidate for multivariate analysis hence, inserted it into multivariate logistic regression where effect of confounding factors was controlled and $p < 0.05$ was considered as statistically significance in this study. The results was presented in the form of tables, figures and texts as using frequencies and summary statistics such as mean, standard deviation and percentage to describe the study population in relation to relevant variables.

4.10 **Ethical Consideration**

Ethical clearance was obtained from Jimma University Ethical Review Committee. Following the endorsement by the research review committee, JMC was informed about the objective of the study through a support letter from department of medical laboratory sciences and pathology. In relation to the study, the individual participant was not subjected to any harm. As far as the confidentiality is concerned, the collected data was never accessed by a third person except the principal investigator, and was kept with a firm confidentiality. To keep the confidentiality no personal identifiers was be used on data collection form. Informed consent was obtained on willingness to participate in the study from each participant who was involved in the study.

4.11 Operational definition

Presence of foreign body	the material inter in to the bone for fixation purpose

CHAPTER 5: RESULT

5.1 **Socio-demographic and Clinical Characteristics of Study subjects.**

A total of 119 patient was included in the study to identify bacterial etiologic agents of osteomyelitis. We noted a predominance of the male gender with a male to female ratio of 2.6:1. The mean age of the respondents was 27.69 years (± 16.56). The majority of the respondents were in the age group of 21-40 years which accounts 49 (41.2%) and least was 60 years and above which accounts 3(2. 5%). Most of the respondents 82(68.9%) were rural dwellers.

More than half of the patients, i.e., 80/119(67.2%) were admitted to the Orthopedics ward while 37/119(31.1%) received medical care at EOPD (emergency outpatient diagnosis) each one of the following remaining two patients was at the time of the study managed in surgical and intensive care units.

The main cause of the primary bone lesion was road traffic accident of all type accounting for 58/119(48.7%) of which 37.8% and 10.9% were due to car and motor bicycle accidents, respectively. Trauma due to sharp material was the second most frequent cause of bone lesion accounting for 35.3% (42/119) while gunshot wound was responsible for a small minority of cases (3.4%). The commonest site affected was the femur (38.7%) followed by the tibia/fibula (24.4. %), foot (23.5%) and humerus /radius/ulna (13.4%).

Regarding clinical and physical symptoms at presentation, discharge was the most frequent presenting symptom (55.5%) followed by swelling (36.1%) and pain (8.4%). from suspected osteomyelitis patient 25(21.1%) has foreign material (material used for bone fixation like metal) Sixteen one patient (51%) had chronic osteomyelitis (symptoms had been evolving for greater than two weeks) while the remaining 48.7% had acute bone disease.

Regarding to duration of hospital stay 76/119(63.9%) of study participant were less than two week.

Table 1.Sociodemographic and Clinical Features versus Culture Results among Patients with Osteomyelitis at JMC, December 2019 - November 2020

Variables	Categories	Frequency (n=119)	**Culture positive(n=70)**	**culture negative(n=49)**
Sex	Male	86 (72.3%)	58(82.9%)	28(57.1%)
	Female	33(27.7%)	12(17.1%)	21(42.9%)
Age group (Yrs)	≤ 20	45(37.8%)	26(37.1%)	19(38.8%)
	21-40	49(41.2%)	29(41.4%)	20(40.8%)
	41-60	22(18.5%)	13(18.6%)	9(18.4%)
	≥ 61	3(2.5%)	2(2.9%)	1(2.0%)
Residence	Rural	82(68.9%)	47(67.4%)	35(71.4%)
	Urban	37(31.1%)	23(32.9%)	14(28.6%)
Place of admission	Orthopedics	80(67.2%)	48(68.6%)	32(65.3%)
	EOPD	37(31.1%)	21(30.0%)	16(32.7%)
	Surgical	1(0.8%)	0(0.0%)	1(2.0%)
	ICU	1(0.8%)	1(1.4%)	0(0.0%)
Type of accident	Car accident	45(37.8%)	25(35.7%)	20(40.8%)
	Motor bicycle accident	13(10.9%)	8(11.4%)	5(10.2%)
	Gunshot wound	4(3.4%)	2(2.9%)	2(4.1%)
	Sharp Item Trauma	42(35.3%)	23(32.9%)	19(38.8%)
	Others(cutting of clothed	15(12.6%)	12(17.1%)	3(6.1%)
Type of infected bone	Femur	46(38.7%)	29(41.4%)	17(34.7%)
	Tibia/Fibula	29(24.4%)	15(21.4%)	14(28.6%)
	Humerus/radius/ulna	16(13.4%)	10(14.3%)	6(12.2%)
	Foot	28(23.5%)	16(22.9%)	12(24.5%)
Type of Trauma	Acute	58(48.7%)	24(34.3%)	34(69.4%)
	Chronic	61(51.3%)	46(65.7%)	15(30.6%)
Co-existing disease	Diabetic Mellitus	8(39.9%)	5(7.1%)	3(6.1%)
	Skin infection	9(45.2%)	7(10.0%)	2(4.1%)
	Others(epilepsy, tonsillitis)	3(14.9%)	2(2.9%0	1(2.0%)
Presence of foreign body	Yes	25(21%)	18(25.7%)	7(14.3%)
	No	94(79%)	52(74.3%)	42(85.7%)
Chief complaint	Pain	10(8.4%)	3(4.3%)	7(14.3%)
	Swelling	43(36.1%)	23(32.9%)	20(40.8%)
	Discharge	66(55.5%)	44(62.9%)	22(44.9%)
Duration of hospital stay	< 2 weeks	76(63.9%)	36(51.4%)	40(81.6%)
	>2 weeks	43(36.1%)	34(48.6%)	9(18.4%)
Type of specimen	Bone curetting	16(13.4%)	11(15.7%)	5(10.2%)
	Aspirated abscess	94(79%)	51(72.9%	43(87.8%)
	Sinus tract	9(7.6%)	8(11.4%)	1(2.0%)

Etiology of osteomyelitis

Out of 119 sample analyzed, 70(58.8%) were culture positive, from which 33(47.1 %) were gram- positive whereas the remaining 37(52.9%) were gram-negative. Seven different

bacteria were identified. *Staphylococcus auras* 27(38.6%), were the predominant one followed *by E.coli 23(32.9%), Streptococcus spp. 6(8.6%), Acenetobacter.spp 5(7.1%).psedomonas aureginosa 4(5.7%), Provedencia 3(4.3), Kellebsiela 2(2.9%).* (Shown below in figure 2)

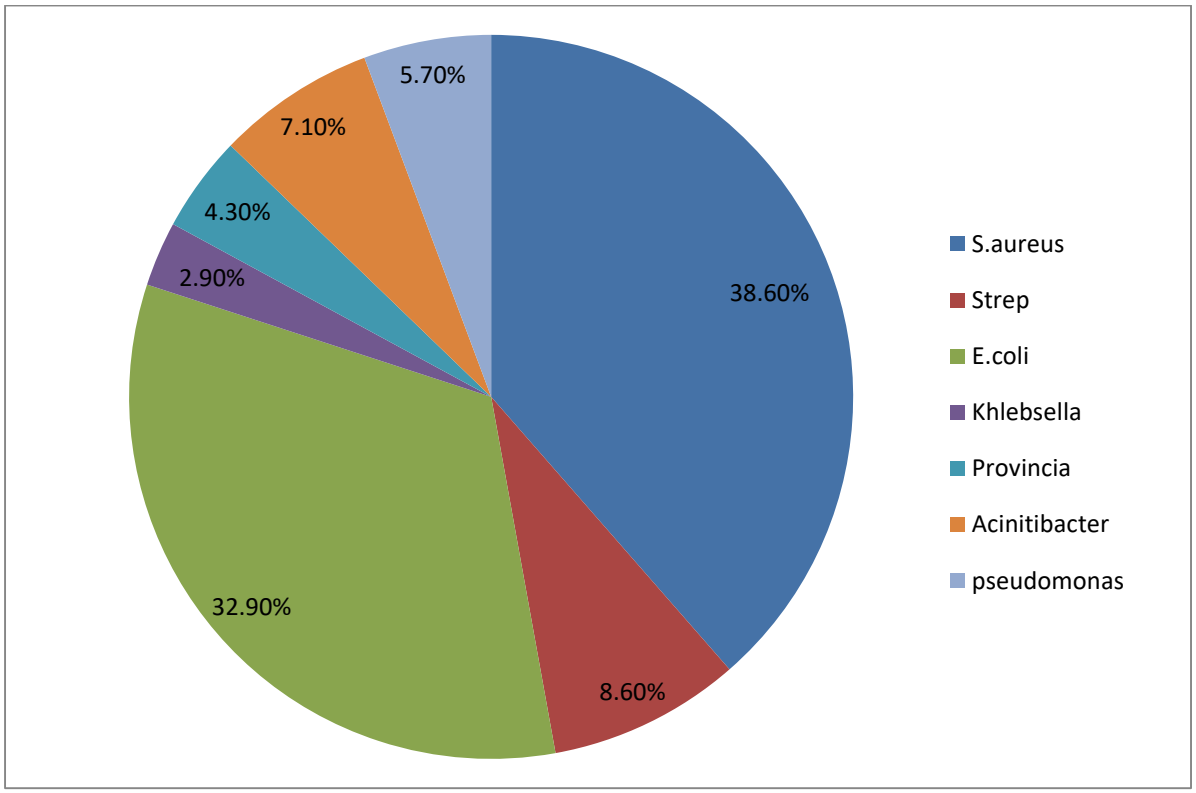

Figure 2 Type and frequency of bacterial species isolated from patients with osteomyelitis at Jimma medical center, December 2019-November 2020

5.2 Association between osteomyelitis infection and socio-demographic characteristics

Binary logistic regressions analysis was conducted to see the effect of independent variable on outcome variables while controlling cofounders. Among 119 osteomyelitis sample 70(58.8%) were confirmed to have osteomyelitis infection. Of the 70 positive cases, 58(82.9%) were males and 12(17.1%) were females. There is statistically significant association with sex (p=0.003) and the odds of having osteomyelitis infection (AOR = 4.26, (95% CI, 1.504, 12.072). Twenty nine (41.4%) of the patients who were confirmed to

have osteomyelitis infection were in the age group of 21-40 years even though not statistically significant (p>0.05).from seven candidate (p<0.25) sex, type of accident, type of trauma, presence of foreign body, chief complaint at presentation, duration of hospital stay .from this candidate three Of them are statistically significant association with culture positivity(p<0.05) sex, type of accident, and type of trauma .

Twelve (17.1%) and 23 (32.9%) of the respondents to have osteomyelitis infection were from other category (clothed shoes trauma, falling from height, mat chin injury) and Sharp Item Trauma, respectively there was statistically significant association (p=0.007) and (p=0.034). The odd of culture positivity for patients who come with sharp item trauma was 77% less likely than those who come with car accident [AOR: 0.23, 95CI: 0.059, 0.895] Whereas Out of sixty one chronic osteomyelitis (the patient diagnosis more than two week after onset of infection) suspected cases 46(65.7%) positive for osteomyelitis infection (p=0.020) The likelihood of culture positivity for patients with chronic trauma was 83.2% less likely as compared to those with acute trauma [AOR: 0.168, 95CI: 0.037, 0.751].

Table 2: Bivariate analysis of factors associated with culture positivity among suspected with osteomyelitis infection at JMC, southwest, Ethiopia, 2020

Variables	Categories	Culture		P-value	COR(95% CI)	p-value	AOR(95% CI)
		Positive	Negative				
Sex	Male(86)	58(82.9%)	28(57.1%)	1			
	Female 33	12(17.1%)	21(42.9%)	0.003	3.625(1.564, 8.401)	0.006	4.261(1.504, 12.072)
Age group	≤ 20 (45)	26(37.1%)	19(38.8%)	1			
	21-40 (49)	29(41.4%)	20(40.8%)	0.89	0.944(0.415, 2.145)		
	41-60 (22)	13(18.6%)	9(18.4%)	0.918	0.947(0.336,		

					2.668)		
	≥ 61 (3)	2(2.9%)	1(2.0%)	0.764	0.684(0.058, 8.107)		
Resident	Rural (82)	47(67.4%)	35(71.4%)	1			
	Urban (37)	23(32.9%)	14(28.6%)	0.619	1.223(0.552, 2.711)		
Place of admission	Orthopedics (80)	48(68.6%)	32(65.3 %)	1			
	EOPD(37)	21(30.0%)		0.74	1.143(0.519,2.57)		
	Surgical(1)	0(0.0%)	1(2.0%)	1	>>(000)		
	ICU(1)	1(1.4%)	0(0.0%)				
Type of Accident	Car accident(45)	25(35.7%)	20(40.8%)	1			
	Motor vehicles accident(13)	8(11.4%)	5(10.2%)	0.102	3.2(0.793,12.913)	0.585	0.595(0.092,3.8390)
	Gun shoot accident(4)	2(2.9%)	2(4.1%)	0.287	2.5(0.462,13.521)	0.915	1.174(0.062,22.138)
	Sharp accident(42)	23(32.9%)	19(38.8 %)	0.244	4.00(0.388,41.228)	0.034	0.230(0.059, 0895)
	Others(clothed shoes,	12(17.1%)	3(6.1%)	0.095	3.304(0.812, 13.447)	0.007	0.088(0.015, 0.511)

	felling, machine injury)(15)						
Type of infected bone	Femur(46)	29(41.4%)	17(34.7%)	1			
	Tibia/Fibula(29)	15(21.4%)	14(28.6%)	0.334	1.592(0.620,4.087)		
	Humorous/ radius/ulna (16)	10(14.3%)	6(12.2%)	0.969	1.024(0.316,3.317)		
	Foot(28)	16(22.9%)	12(24.5%)	0.614	1.279(0.491,3.336)		
Type of Trauma	Acute(58)	24(34.3%)	34(69.4%)	1			
	Chronic(61)	46(65.7%)	15(30.6%)	0.0001	0.230(0.105,0.503)	0.020	0.168(0.037, 0.751)
Co-existing disease	Diabetic Milletus(8)	5(7.1%)	3(6.1%)	1			
	Skin infection(9)	7(10.0%)	2(4.1%)	.494	.476(0.057,3.990)		
	Others(epilepsis,tonsilitis)(3)	2(2.9%0	1(2.0%)	.898	.833(0.051,13.633)		.
Presence of foreign body	Yes(25)	18(25.7%)	7(14.3%)	1			
	No(94)	52(74.3%)	42(85.7%)	0.137	2.077(0.793,5.441)		
Chief	Pain(10)	52(74.3%)	42(85.7%)	1			

compl aint	Swelling(43)	23(32.9%)	20(40.8%)	0.191	0.373(0.085,1.6360)	0.062	0.124(0.014, 1.111)
	Discharge(66)	44(62.9%)	22(44.9%)	0.037	0.214(0.050,0.910)	0.161	0.227(0.029, 1.801)
Duration of hospital stay	< 2 weeks(76)	36(51.4%)	40(81.6%)	1			
	>2 weeks(43)	34(48.6%)	9(18.4%)	.001	4.198(1.773,9.936)	0.134	0.283(0.054, 1.475)
Type of specimen	Bone cutting(16)	11(15.7%)	5(10.2%)	1			
	Aspirate from abscess(94)	51(72.9%	43(87.8%)	.278	3.636(0.353,37.457)	0.928	1.088(0.173, 6.835)
	Sinus tract(9)	8(11.4%)	1(2.0%)	0.077	6.745(0.811,56.087)	0.620	0.510(0.036, 7.276)

N.B: Variable candidate for multivariate analysis at P-value<0.25, and shows reference group

Antibiotic Susceptibility of Isolates

Antibiotic susceptibility of isolated was performed based on the 2020 CLSI guideline. Out of the 70 culture positive samples, 27 (38.6%) were *staphylococcus aureus.* Antibiotic susceptibility testing of these isolates revealed high rates of resistance particularly to penicillin (92.6%) and trimethoprim-sulfamethoxazole (59.3%). S*taphylococcus aureus* isolates showed good sensitivity to ciprofloxacin (92.6%), chloramphenicol (81.5%), tetracycline (74.1%), gentamycin (63%) and clindamycin (51.9%). Twenty-six percent of the Staphylococcus *aureus* isolates were resistant to oxacillin (MRSA). No strain of S. *aureus* showed vancomycin resistance. Isolates of S*treptococcus species* exhibited susceptibility in excess of 50% to ampicillin, erythromycin, clindamycin, penicillin and tetracycline**.**

Table 3 Antibiotic Susceptibility Patterns of Gram positive Bacterial isolate from osteomyelitis infections at JMC, from December 2019-November 2020

Micro-organism(n)		**Antibiotic resistance in percentage(resistant/total)**										
		AMP	**OX**	**CAF**	**CIP**	**CLD**	**SXT**	**ERY**	**P**	**T**	**CN**	**V**
s.aureus(27)	R	NT	25.9	18.5	7.4	40.7	59.3	48.1	92.6	25.9	37	0
	S	NT	74.1	81.5	92.6	59.3	40.7	51.9	8.4	74.1	63	100
Streptococcus(6)	R	33.3	NT	NT	NT	50	NT	33.3	33.3	50	NT	NT
	S	66.7	NT	NT	NT	50	NT	66.7	66.7	50	NT	NT

R-resistance, S-sensitive AMP- Ampinicillin, OX –Oxacillin, CAF Chloramphenicol, CIP - Ciprofloxacin, CLD-Clindamycin SXT-Trimethoprim-Sulfamethoxazole , ERY-Erythromycin P-penicillin, T-Tetr cycline ,CN - Gentamycin, , FOX - Cefoxitin, and NT- not tested

Generally, gram-negative bacterial isolates exhibited a high degree of antibiotic resistance. *E. coli* showed significant degrees of resistance to ampicillin (100%), trimethoprim sulphamethoxazole (82.6%), ceftriaxone and cefuroxime (78.3%), and ceftazidime (65.2%). All clinical isolates of *Acinetobacter species* were resistant to ampicillin, Augmentin, ceftriaxone, cefuroxime, ciprofloxacin, and trimethoprim sulphamethoxazole.

The two strain of *kellebsiella app* 100%sensitive to amikacin and chloranpnenicol. *psedomonas aurogenosa* 75% resistance to meropenem. *provedentia* shows (66.7%-100%) sensitive to all the drug we use except ampicillin 33.3%.(See table 3)

Table 4 Antibiotic Susceptibility Patterns of Gram negative Bacterial Isolates from osteomyelitis infections at JMC, from December 2019-November 2020.

Micro-organism(n)		**Antibiotic resistance in percentage(resistant/total)**													
		AMC	**AMP**	**AMK**	**CAZ**	**CRO**	**CXM**	**CAF**	**CIP**	**SXT**	**IMP**	**MRP**	**P+t**	**T**	**CN**
E-coli(23)	R	34.8	100	21.7	65.2	78.3	78.3	26.1	47.8	82.6	NT	NT	NT	NT	0
	S	65.2	0	78.3	34.8	21.7	21.7	73.9	52.2	17.4	NT	NT	NT	NT	100
Kllebsiella.spp (2)	R	50	100	0	50	50	50	0	50	50	NT	NT	NT	NT	50
	S	50	0	100	50	50	50	100	50	50	NT	NT	NT	NT	50

	R	33.3	66.7	0	33.3	33.3	33.3	33.3	33.3	33.3	NT	NT	NT	NT	33.3
Provincia(3)	S	66.7	33.3	100	66.7	66.7	66.7	66.7	66.7	66.7	NT	NT	NT	NT	66.7
Acinetobactor.spp(5)	R	100	100	40	60	100	100	80	100	100	40	40	40	40	100
	S	0	0	60	40	0	0	20	0	0	60	60	60	60	0
Pseudomonas aeruginosa(4)	R	75	75	50	75	75	75	75	100	75	50	75	50	0	75
	S	25	25	50	25	25	25	25	0	25	50	25	50	100	25

R-Resistance, S-Sensetive, AMC- Amoxicillin-clavulanate , AMP- Ampicillin , AMK-Amikacin , CAZ-Ceftazidime, CRO - Ceftriaxone , CXM-Cefuroxime , CAF-Chloramphenicol, CIP – Ciprofloxacin, SXT-Trimethoprim-sulfamethoxazole, IMP- Imipenum , MRP – Me ropenem P+T=Peperacilin tazobactam T-tetracycline, CN - Gentamycin and, NT- not tested.

Multi drug resistance of the isolates

From the total of 70 isolates tested for antimicrobial susceptibility, multiple drug resistance (resistance to three or more drugs) was observed in 46 of 70 (65.71%). Of which 16 of 33 (48.48%) were from Gram positive and 30 of 37(81.08 %) were seen in Gram negative bacteria. Among gram positive isolates *staphylococcus aures 14(51.85), streptococcus spp 2(33.3%) only 2(7.4%) s.aures* are sensitive to all antibiotic (no resistance). The highest MDR shown on gram negative bacteria(100%)resistance showen on *acineto bacter spp ,Eschirichi .coli(86.95%),pseudomonas aureginosa (75%),kellepsiela.spp(50%) and provedientia spp (33.5%) respectively.*

Table 5: Multidrug resistance of gram positive bacteria identified from osteomyelitis infection at JMC from December 2019 to November 2020

Organism isolate	Ro	R1	R2	R3	R4	R5	R6-12	MDR
s.aures (27)	2(7.4%)	6(22.2%)	5(18.5%)	1(3.7%)	5(7.4%)	2(7.4%)	6(22.2%)	14(51.85%)
Streptococcus spp (6)	3(50%)	1(16.7%)	0	0	0	2(33.3%)	0	2(33.3%)
Total (33)	5(15.15%)	7(21.21%)	5(15.15%)	1(3.03%)	5(15.15%)	4(12.1%)	6	16(48.48%

Key R0 = no resistance to antibiotic, R1= resistance to 1 antibiotics. R2= resistance to 2 antibiotics R 3=resistance to 3 antibiotics, R4 =resistance to 4 antibiotics, R5 =resistance to 5 antibiotic, R 6-9=resistance to 6-9 antibiotics

Table 6.Multidrug resistance of gram negative bacteria identified from osteomyelitis infection at J MC from December 2019 to November 2020

Organism isolate	Ro	R1	R2	R3	R4	R5	>R5	MDR
E .coli(23)	0	3(13.0%)	0	0	1(4.34%)	1(4.34%)	18(78.3%)	20(86.95%)
Klepsella spp(2)	0	1(50%)	0	0	0	0	1(50%)	1(50%)
Provedentia(3)	1(33.33)	1(33.33					1(33.33)	1(33.33)
Acinetobacter(5)	0	0	0	0	0	0	5(100%)	5(100%)
Pseudomonas aurogenosa(4)	0	1(25%)	0	0	0	0	3(75%)	3(75%)
total (37)	1(2.70%)	6(16.21%)	0	0	1(2.70%)	1(2.70%)	28(75.67%)	30(81.08%)

Key R0 = no resistance to antibiotic, R1= resistance to 1 antibiotics. R2= resistance to 2 antibiotics R 3=resistance to 3 antibiotics, R4 =resistance to 4 antibiotics, R5 =resistance to 5 antibiotic, R 6-9=resistance to 6-9 antibiotics

CHAPTER 6: DISCUTION

Clinical profile

Osteomyelitis is one of the most challenging diseases among most of the developing countries. In the absence of early diagnosis and increase in antibiotic resistance strains causing failure in antibiotic therapy, osteomyelitis has become prominent disability, bone deformity finally Couse morbidity. Proper management of osteomyelitis requires careful isolation of microorganisms

Hence this present study was done to know the aerobic bacteriological etiologic agents of osteomyelitis and antibiotic susceptibility pattern of various isolates along with study of common predisposing factors

In one year prospective study of osteomyelitis at Jimma medical center a total of 119 osteomyelitis suspected patients were included the prevalence was 58% the incidence of osteomyelitis infection was more common in males (82%) than in females (18%). (With a sex ratio of 2.6:1) This is in agreement with studies done in different parts of country like India (25), Brazil (24) Austria (22) and France(21) this finding were not comparable with Uganda female are more predominant than male(30) and a greater number of patient were in the age groups between 21-40 (41.2%). These results are similar to those obtained in a study done in India in 2017 also reported a higher cases of osteomyelitis among younger age groups of 31-40 (32.8%) followed by 21-30 (25.6%) (14). A study done in yaounde-cameron, 2017 was reported similar results(18)).This study was not co relate with the study conducted in central India 2019 the highest age group was 41-60(48%) (26) This high predominance of the male gender and the youthful age group could be attributable to the greater likelihood of trauma and this population group in relation with their daily activities. This might be explained by the fact that traditionally, in our country, mainly males are participating in occupations such as construction works, transportation and industry works where they likely exposure to trauma is common .in our study on the assessment of osteomyelitis Chief complaint at presentation was discharge sinus 66(55.5%) the predominant one. This co relate with the study done sub Saharan Africa (50-93%)of patient presented with sinus drainage (28).In Uganda the common clinical presentation were chronic bone pain and discharge sinus(30).

Femur was commonest bone affected in the study done in china (30.5%) and central India 38%(19) (23) .this shows similar to my study femur was the commonest bone involved accounts (38.7%) followed by tibia/fibula (24.4%), foot(23.5%)and humerus/radius/ulna(13.4%) this differ in Austria, Uganda and Cameron the commonest bone was tibia (21)(30)(32).The high number of femur and tibia shown because of they are long bone during accident and trauma they have a greater chance to get fracture in case of open wound they have greater chance to get contamination. the most factor leading to osteomyelitis was road traffic accidents 48%% (car (37.8%), motor cycle accident (10.8%)) of the causes. the nature of roads in our setting and the non-respect of road security measures may Couse for this result but this number is lower than the study done in Cameron 65% and brazil 57%(20)(23). The next predominant cause of injury was sharp item trauma (35.3%). The p value was =0.034

With respect to duration of illness, 48.7% of our patients had an acute infection while 51.3% had a chronic infection. The number of chronic infection increase it might be in case of drug resistance or not early visiting of health facility. In the study done in north India in 2017, 52% of the patients had chronic osteomyelitis in contrast to 48% who had an acute infection.

Culture results

In our samples, 58.8 % gave positive culture results and 52.9% gram negative and 47.2% gram positive organisms. Gram negative bacteria were the most predominant organisms. the commonest single bacteria isolated were *Staphylococcus aureus27* (38.6%),followed by *E.coli* (32.9%), *streptococcus spp* (8.6%) *acenitobact*er spp(7.1%),*pseudomonas aurogenosa* (5.7%) and *provedencia spp* (4.3%).*Staphylococcus aures* is often the first causative agent in different counties this co relates with India, France, Austria, South India and Uganda (1)(20)(21)(24)(30) Another study done in Cameron and north India however reported *E.coli* was more predominant than *Staphylococcus aurous* (14)(32). *Staphylococcus aureus* is a highly successful Gram-positive pathogen capable of causing both superficial and invasive, life-threatening diseases. Of the invasive disease manifestations, osteomyelitis or infection of bone is one of the most prevalent, with *S. aureus* serving as the most common etiologic agent. Treatment of osteomyelitis is arduous, and is made more difficult by the widespread emergence of antimicrobial resistant strains, the capacity of staphylococci to exhibit tolerance to antibiotics despite originating from a genetically susceptible background, and the significant bone remodeling and destruction that accompanies infection (31)

Sensitivity testing

High level resistance to different antibiotic was seen among Gram positive and gram negative bacteria. Among gram positive bacteria 92.6% strains of *Staphylococcus aureus* were resistance to penicillin and 59.3% resistance for trimethoprim sulfamethoxazole.92.6% sencitive to ciproflocacin (floroquinolone)74.1%for tetracyclin,63% for gentamicin(amino glycoside),59.3% for clindamycin(licosanemide)and 51.9% for erythromycin(macrolid)) while 74.1% were susceptible to Oxacillin (MSSA). Their sensitivity for quinolones, aminoglycosides, and vancomycin was generally high (greater than 50%) or which reported a good (51.9-92.6%) except penicillin and trimethoprim Sulfamethoxazole. These findings are different from those obtained in the study carried out in Cameron it accounts lower than 50%(32). Good sensitivety for amino glycoside, tetracyclin, fluoro quinolones, macrolid and lincosannimide).low sensitivity 40% for sulphanomide and 8.4%for penicillin. The difference could be explained by the fact that they mostly had community acquired bacteria. Another study on Uganda all strain of *s.aures* is resistance to penicillin. This had almost similar results with ours 92.6% (30) .In guinea 92.0% sensitive to gentamycin and *S.aures* are dominant single pathogen.83% were MRSA this not correlate with to our study (26%) (33).

In our study all gram negative bacteria are 100% resistance to ampicillin except *provedencia* and *pseudomonas aurgenosa* has 66.7% and 75% resistance respectively. *Acenitobactor spp* shows 100%resistance for ampicillin, amoxacilin -clavelunic acid, Ceftra exone, ciprofloxacin Trimethoprim selfamethoxazon and gentamicin.80% for chloramphenicol and 40% for piperacilin/tazobactam and carbapenum (imipenem and meropenum). *psedomonas aurogenosa* 75% resistance for amoxa/clavulinanic acid, ampicillin ,ceftazidim, ceftraexone, chloranphnicol, trimethoprim- sulfamethoxazol, gentamycin and meropenum.100%resistance for tetracycline .this finding co relate with the study done in sauth india 2016 organism isolate like *pseudomonas aurugenosa, klebsiela spp*. showed high level of antibiotic resistance(24).

CONCLUSSION

Based on the results from present study the prevalence of osteomyelitis infection was 58.8% Gram negative and Gram positive bacteria were responsible for osteomyelitis infection. Gram negative organism shows high prevalence 37 (52.9%) gram positive were 33 (47.1%).*Staphylococcus aureus* was the most frequently isolated Gram positive bacteria (38.6%) of seven (26 %) were MRSA, whereas *E.coli* was the most frequently isolated Gram negative bacteria. The overall rate of MDR (resistant to three or more antibiotics) 46(65.71%) among gram positive isolates was 16(48.48) *staphylococcus aures 14(51.85), streptococcus spp 2(33.3%) and only 2(7.4%) s.aures are sensitive to all antibiotic (no resistance)* .the highest MDR shown on *acineto bacter spp* (100%) *,E.coli(86.95%),pseudomonas aureginosa (75%),kellepsiela.spp(50%)and provedientia.spp (33.5%)respectively.* Ampicillin, penicillin and trimethoprim sulfamethoxazol shows high level resistance. In andition to this alrming result on 3rd generation cephalosporin like ceftriaxone and ceftazidim (see table 3).

Knowledge of the microbial organism of osteomyelitis and the resistance pattern are important tools in the management of osteomyelitis and are also useful in formulating rational antibiotic policy.

RECOMMENDATION

- In the future, the prevalence and drug susceptibility pattern of osteomyelitis infections should be done by including anaerobic bacteria, fungus and other micro-organism those can be important causes of infections.
- It is recommended that penicillin; Ampicillin, Trimethoprim Celfamethoxazol and Ceftriaxone are not good for empiric treatment.
- Empirical treatment to osteomyelitis infections may provoke drug resistance; therefore treatment should be based on the result of culture and sensitivity.

LIMITATION

It was not possible to include anaerobic bacteria due to poor laboratory facilities constraints

Political and social problem of our country limit patient flow and make dalliance in our work.

References

1. Carballini J, Viana F. Using synthetic aperture sonar as an effective tool for pipeline inspection survey projects. 2015 IEEE/OES Acoust Underw Geosci Symp RIO Acoust 2015. 2016;6(1):367–72.
2. Shettigar S, Shenoy S, Bhat S, Rao P. Microbiological Profile of Deep Tissue and Bone Tissue in Diabetic Foot Osteomyelitis. J Clin Diagnostic Res. 2018;12(May 2017):2017–9.
3. Biruk WL, Ababa A. Chronic Osteomyelitis at Tikur Anbessa Hospital, Addis Ababa University, Ethiopia. East Cent African J Surg. 2007;12(1):33–41.
4. Nigatie A. iMedPub Journals Nutritional Status and Associated Factors among Adult Chronic Osteomyelitis Patients in Felege Hiwot Referral Hospital , Bahir Dar , North West Ethiopia Study area. J Food, Nutr Popul Heal. 2017;1(3):1–5.
5. Wadekar MD. Chronic Osteomyelitis: Aetiology and Antibiotic Susceptibility Pattern. Int J Recent Trends Sci Technol. 2014;9(3):337–40.
6. Wu T, Wu C, Yu C, Tseng C, Chan H, Chen P, et al. Open Fracture Wound Infections Caused by Aeromonas species. Appl Med Res. 2015;1(2):39.
7. Carvalho VC De, Rosalba P, Oliveira D De, Dal-paz K, Paula AP De, Félix S, et al. Gram-negative osteomyelitis: clinical and microbiological profile. Brazilian J Infect Dis [Internet]. 2012;16(1):63–7. Available from: http://dx.doi.org/10.1016/S1413-8670(12)70276-3
8. Gomes D, Pereira M, Bettencourt AF. Osteomyelitis : an overview of antimicrobial therapy. Brazilian J Pharm Sci. 2013;49.
9. Kale AR, Sonawane CS, Waghmare VU, Kalambe H. Open Fractures and Incidence of Infection in Tertiary Care Government Hospital. 2017;5(403):24–8.
10. Urgery S, Ncorporated I, Kremers HM, Nwojo ME, Ransom JE, Wood-wentz CM, et al. Trends in the Epidemiology of Osteomyelitis. J BONE &JOINT Surg. 2015;837–45.
11. Walter N, Baertl S, Alt V, Rupp M. What is the burden of osteomyelitis in Germany ? An analysis of inpatient data from 2008 through 2018. Walter al BMC Infect Dis. 2021;6–13.
12. Walter N, Bärtl S, Alt V. The Epidemiology of Osteomyelitis in Children. 2021;4–9.
13. Chiappini E, Mastrangelo G, Lazzeri S. A Case of Acute Osteomyelitis : An Update on Diagnosis and Treatment. Int J Environ Res Public Heal. 2016;(Figure 2).

14. Khatoon R, Khan SA, Jahan N, Khatoon R, Med JR, Feb S. Antibiotic resistance pattern among aerobic bacterial isolates from osteomyelitis cases attending a Tertiary care hospital of North India with special reference to ESBL , AmpC , MBL and MRSA production. Int J Res Med Sci. 2017;5(2):482–90.
15. Bergman BS, Pharm D, Id B, Shah PJ, Pharm D. Diabetic Foot Infections. Infect Prim Care. 2016;2007(Cdc 2014).
16. Ravitheja A, Jyothirmayee K, Reddy PC, Dushyanth M. A Clinical Study of Surgical Complications and Management of Diabetic Foot. Int J Contemp Med Res. 2017;4(1):65–7.
17. Wagner JM, Zöllner H, Wallner C, Ismer B, Schira J, Abraham S, et al. Surgical Debridement Is Superior to Sole Antibiotic Therapy in a Novel Murine Posttraumatic Osteomyelitis Model. journal pone. 2016;1–13.
18. Handy ED, Manfo AA, Gonsu KH, Essi MJ NNB and SM. Clinical and microbiological profile of post-traumatic osteomyelitis in tertiary hospital in Yaoundé – Cameroon. Int J Orthop Sci. 2017;3(3):1180–4.
19. Metsemakers WJ, Kuehl R, Moriarty TF, Richards RG, Verhofstad M, Borens O, et al. Infection after fracture fi xation : Current surgical and microbiological concepts. Int J Care Inj. 2016;
20. Doutchi M, Seng P, Menard A, Meddeb L, Adetchessi T, Fuentes S, et al. Changing trends in the epidemiology of vertebral osteomyelitis in. New Microbes New Infect. 2015;7:1–7.
21. García E, Collazos J, Cartón JA, Camporro D. Original Bacterial osteomyelitis : microbiological , clinical , therapeutic , and evolutive characteristics of 344 episodes. Off J spanish Soc Chemother. 2018;0(3):217–25.
22. Peng J, Ren Y, He W, Li Z, Yang J, Liu Y, et al. Epidemiological , Clinical and Microbiological Characteristics of Patients with Post-Traumatic Osteomyelitis of Limb Fractures in Southwest China : A Hospital-Based Study. J Bone Jt Infect. 2017;2:149–53.
23. Jorge LS, Fucuta PS, Oliveira MGL, Nakazone MA, Matos JA De, Chueire AG, et al. Outcomes and Risk Factors for Polymicrobial Posttraumatic Osteomyelitis. J Bone Jt Infect. 2018;3.
24. Lakshmi V, Sudhaharan S, Chavali P, Mamidi N. Bacteriological profile of chronic osteomyelitis in a tertiary care hospital in South India V. Int J Infect Dis. 2016;45:141.

25. Shah R V, Sanghavi R V. Bacteriological Profile in Chronic Osteomyelitis Patientsand TheirAntibiogram in A Tertiary Care Hospital , Jamnagar ,. IOSR J Dent Med Sci. 2017;16(10):47–50.
26. Pandey A, Shaw P, Johar A. Bacteriological Profile of Chronic Osteomyelitis with Special Reference to Antibiotic Resistance Mechanisms/Patterns – A Cross-sectional Prospective Study from Tertiary Care Hospital in Central India. J Adv Med Med Res. 2020;32(3):43–52.
27. Fily F, Ronat JB, Malou N, Kanapathipillai R, Seguin C, Hussein N, et al. Post-traumatic osteomyelitis in Middle East war-wounded civilians: Resistance to first-line antibiotics in selected bacteria over the decade 2006-2016. BMC Infect Dis. 2019;19(1):1–8.
28. Olivier K, Eugene U, Lynn L, Don Eliseo III L-P. Chronic osteomyelitis in Sub-Saharan Africa—A Review. Glob Surg. 2019;5(3):1–5.
29. Popescu B, Tevanov I. Acute hematogenous osteomyelitis in pediatric patients : epidemiology and risk factors of a poor outcome. J Int Med Res. 2020;
30. Edson T, Kyengera K, Andrew B, Joel B. Chronic Osteomyelitis among Children Attending Orthopedic Services at Mbarara Regional Referral Hospital : Prevalence , Etiological Agents and Their Drug Susceptibility patterns. Microbiol Res J Int 28(5). 2019;(October).
31. Gimza BD, Cassat JE. Mechanisms of Antibiotic Failure During Staphylococcus aureus Osteomyelitis. Front Immunol 12638085. 2021;12(February):1–8.
32. Nonga NB, Ngo Nonga CB. Clinical and microbiological profile of post-traumatic osteomyelitis in tertiary hospital in Yaoundé – Cameroon. Int J Orthop Sci IJOS. 2017;3(33):1180–4.
33. Jerzy K, Francis H. Chronic Osteomyelitis - Bacterial Flora , Antibiotic Sensitivity and Treatment Challenges. Open Orthop J. 2018;12:153–63.
34. monica Cheesbrough. District laboratory practice in tropical countries volumeII:microbiology.cambridge(uk):cambrige university press; 2nd editio. 2006.
35. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. 2016;(December 2009):1–23.
36. Standards P, Testing AS. M100 Performance Standards for Antimicrobial. 30th editi. 2020.

ANNEXES

ANNEXE-I: INFORMATION SHEET AND CONSENT FORM

Explanation on procedures and conditions of agreement

I am Sara Jemal. I came from Jimma University, college of health science. I am here to study the problem of the disease called osteomyelitis. The purpose of this study is to determine the prevalence and its antimicrobial susceptibility pattern among osteomyelitis suspected patients.

The study involves osteomyelitis suspected patients so you are selected to be one of the study participants. If you are willing to participate, I am so happy and I need you to clearly understand the aim of this study. If you are agreed to participate, you are kindly requested to give your response in the interview and giving sample (osteomyelitis swab) to be tested. Your participation in this project is very important in determining the prevalence and antimicrobial susceptibility patterns among osteomyelitis suspected patients in JMC and for you the laboratory diagnosis is done without any payment and the result will be immediately send to the concerned physician this helps you to get appropriate drug for the disease you phase.

In doing so you may feel few discomfort or pain during sample collection. I also honestly tell is that; you do not provided any incentives to participate in this project and the information collected from you will be kept secret and stored in a file, without your name by assigning a code number to it. You have also the full right to refuse from participating in this study and full right to withdraw from this study at any time you wish. If you have understood the explanation well enough, I am asking you to participate in this study. If you decide to volunteer I am kindly ask you to put your signature as illustrated below.

Name of the participant: _____Signature:_____ Date_____

Name of investigator: - Sara Jemal Address, sarajemal56@gmail.com

Cell number, 0913336967

For more information contact us by the address and cell number listed below:

Name of advisors: 1. Zewdineh Sahlemariam Address, zewdineh.sahlemariam@ju.edu.et

Cell number, 0913173050

2. Mekdem Mekonin Address, makface2002@gmail.com

Cell number, 0911718518

Amharic version information sheet and consent form

የመረጃ መስጫ ና የስምምነት መግለጫ ቅፅ

የስራ ቅደም ተከተል ና የስምምነት ሁኔታ

ስሜ ሣራ ጀማል እባላለሁ የመጣሁት ከጅማ ዩኒርስቲ ከጤና የትምህርት ክፍል ሲሆን አሁን እዚህ የተገኘሁት አጥንትን የሚጎዱ ተዋሲያን በሽታ ለማጥናት ነው:: የጥናቱ ዋና አላማም ስለ አጥንት በሽታ አምጪ ህዋሳት ስርጭት ና የመደሃኒት መቋቋም ብቃትን ለማወቅ ይረዳል:: ጥናቱ የሚካሄደው በ አጥነት በሽታወች ተጠረጣሪ ህሙማን ላይ ነው:: ስለሆነም እርሰዎ በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ በጣም ደስተኛ ነኝ እናም ስለጥናቱ አላማ በግልጽ አሳውቀወታለሁ:: በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ ለሚጠየቁትን ጥያቄ ተገቢውን መልስ እና ለላቦራቶሪ ምርመራ አስፈላጊውን ናሙና እንዲሰጡኝ ስል በትህትና እጠይቃለሁ:: በዚህ ጥናት ላይ መሳተፈዎ ጂማ ሆስፒታል ውስጥ ስለአለው የአጥንት በሽታ አምጪ ህዋሳት ስርጭት እና የመደሃኒት መቋቋም ብቃትን ለማወቅ ትልቅ አስታዋጽዎ አለው ለእርሰዎም የላቦራቶሪ ምርመራ ያለምንም አይነት ክፍያ ከተሰራለዎት በኋላ ውጤቱም ወዲያውኑ ወደመረመረዎት ሀኪም በመስጠት ተገቢዉን መድሃኒት እንዲያገኙ ይረዳዎታል::

ይህም ሲሆን ናሙና በሚወሰድበት ጊዜ ትንሽ ያለመመቸት ስሜት ሊሰማዎት ይችላል:: በእውነተኝነት ልነግረዎት የምፈልገው በዚህ ጥናት ላይ ሲሳተፉ ምንም አይነት ክፍያ አይከፈለዎትም ከእርሰዎ የሚወሰደውም መረጃ ስመዎት ሳይገለጽ ቁጥር በመሰየም ሚስጥሩ እንደተጠበቀ በመዝገብ ላይ ተመዝግቦ ይቀመጣል:: ከዚህ በተጨማሪ በዚህ ጥናት ላይ ያለመሳተፍ ና በፈለጉበት ሰአትም አቃርጠው የመውጣት ሙሉ መብተዎ የተጠበቀ መሆኑን ልገልጽለወት እወዳለሁ:: ስለ ሁኔታው ማብራሪያ በደምብ ከተረዱ በዚህ ጥናት ላይ እንዲሳተፉልኝ እና መስማማተዎትንም በፊርማዎት ከዚህ በታች በተቀመጠው ቦታ ላይ እነዲገለጹልኝ ስል በትህትና እጠይቃለሁ::

የተሳታፊው/ዋ ሥም._____ ፊርማ _____ ቀን_____

ጥናቱን የሚያካሂደው ሰው ሥም ሣራ ጅማል አድራሻ, sarajemal56@gmail.com

ስልክ ቁጥር 0913336967

ለበለጠ መረጃ ከታች የተጠቀሱትን አድራሻ እና ስልክ ቁጥር መጠቀም ይችላሉ::

የጥናቱ ዋና አማካሪ ሥም. 1. ዘዉድነህ ሳህለማሪያም

አድራሻ, zewdineh.sahlemariam@ju.edu.et

ስልክ ቁጥር, 0913173050

3. መቅድም መኮንን አድራሻ, makface2002@gmail.com

ስልክ ቁጥር, 0911718518

ANNEXE -II: **Data collection format**

JIMMA UNIVERSITY

DEPARTEMENT OF MEDICAL LABORATORY SCIENCE & PATHOLOGY

Socio demographic factors

- Code _____**card no_____

1. Sex 1. Male_____
   2. Female_____
2. Age in year's _____
3. Marital status_____

4. Residence 1-rural____
   2-urban------

5. Ward 1.orthopedict
   2. EOPD
   3. ICU
   4. Other----------

6. Type of accident

1. Car accident
2. Motor bicycle accident
3. Gunshot wound
4. Sharp item trauma

Other----------

7. Type of infected bone

1. Femur
2. Tibia/fibula
3. Humerus/radius/ulna
4. Foot

8. Type of trauma

1. Acute

2. Chronic

9. Presence of co-existing disease

1. Diabetic mellitus

2. Skin infection

3. Other

10. Presence of foreign body

Yes

No

11. Chief complain at presentation

1. Pain 2. Swelling

3. Discharge

12. Duration of hospital stay_____

1-<2weak

2->2weak

II. Laboratory Data

- Date of specimen collection_____

2. Type of specimen

1. Bone cutting

2. Aspirate from abscess

3. Swab from abscess

3. .Media used _____

4. Gram stains result _____

5. .Biochemical test_____

6. .Organism isolated _____

7. Drug susceptibility pattern

1. Sensitive to _____

2. Intermediate to _____

3. Resistance to _____

III. Comments_____

Name of principal investigator _____

Signature _____ Date _____

ANNEXE -III: **General laboratory procedures**

Laboratory procedure for collection, transportation and culturing of sample

1. Cleaning the wound with normal saline prior to obtaining swab specimens
2. Rotate sterile cotton-tipped applicator 1cm square area for 5 seconds with sufficient pressure to express fluid and bacteria to surface
3. Placing the swabs in to sterile test tubes having 0.5 ml of sterile normal saline solution
4. Label the sample as soon as possible with the patient code number
5. Transport the specimen to the laboratory at room temperature within 30 minutes of collection

II. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with patient code number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or chemicals (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranine stain for 2 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

- Gram positive bacteria ----------dark purple
- Gram-negative bacteria ----------pale to dark red

A. Culture media preparation

MacConkey agar

1. 52.2g of dehydrated powders was prepared to every 1000ml of distilled water and boiled.
2. It was sterilized by autoclaving at 1210C for 15 minutes.
3. The medium was cooled to 50–550C, and mixed well or homogenously.
4. It was aseptically dispensed in 20ml amount in sterile petri dishes.
5. The medium was given date and it a batch number.
6. The plates were stored at 2–80C preferably in plastic bags to prevent loss of moisture.

Blood agar

1. 40g of blood agar base was suspended into 1L of distilled water and boiled.
2. The suspension was sterilized by autoclaving at 1210C for 15 minutes and transferred to 500c water bath.
3. After cooling 5% (50ml sheep blood) was added to the suspension and mixed homogenously.
4. It was aseptically dispensed in 15ml amount in sterile peter dishes.
5. The plates were stored at 2–80 c.

Chocolate agar

1. Blood agar was prepared using above procedure.
2. The medium was heated in a 700c water bath until it becomes brown in color.
3. The medium was allowed to cool to about 45 0c, remixed
4. It was aseptically dispensed in 15ml amount in sterile peter dishes.
5. The plates were stored at 2–80 c.

B. Biochemical testing procedures

a. Catalase test

This test is used to differentiate Staphylococci (+ve) from Streptococci (-ve)

Procedure

1. 2-3 ml of 3% hydrogen peroxide was poured to a microscopic slide.
2. The test organism was taken by using a sterile wooden stick and immersed into the hydrogen peroxide solution
3. It was looked for immediate bubbling
4. Interpretation: Active bubbling--positive test and no release of bubbles negative test

b. Coagulase test:

This test is used to differentiate *Staphylococcus aureus* from other *Staphylococcus spp.*

Procedure

1. A drop of physiological saline was placed on two separate slides
2. The test organism was emulsified in each of the drop to make thick suspension
3. One drop of plasma was added to one of the suspensions and mixed gently. The organism was looked for clumping within 10 seconds
4. Interpretation Clumping within 10 seconds ----------*S. aureus*

No clumping within 10 seconds ----------other Staphylococcus species

Identification of Gram negative bacteria was based on their test result with a series of biochemical tests.

Procedure

1. A suspension of the test organism was prepared with nutrient broth.3-4 colony of test organism in 5ml nutrient broth.
2. A loop full of the bacterial suspension was inoculated in to SIM, citrate agar, KIA, oxidase, LDC and urea agar medium.
3. Media was incubated at 35-37 O c for 18-24 hours.
4. Media was looked for color change (turbidity for motility) of the medium
5. The test organism was identified by considering the result of this biochemical

C. Turbidity standard (McFarland standard)

1. A 1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mixed.
2. A 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dehydrate barium chloride (BaCl2.2H2O) in 50 ml of distilled water.
3. 0.6 ml of BaCl2.2H2O was added to 99.4 ml of the H2SO4 solution, and mixed.
4. A small volume of the turbid solution was transferred to a capped tube or screw cap bottle of the same type as used for preparing the test and control inoculum.

D. Antimicrobial sensitivity testing

Procedure

a) A suspension of the test organism was prepared by emulsifying several colony of the organism in a small volume of normal saline and the turbidity of suspension was matched with turbidity standard.

b) Sample was taken with a sterile swab from the suspension (the swab was squeezed against the side of the test tube to remove the excess fluid).

c) The inoculum was spread evenly over the Muller-Hinton agar plate with the swab.

d) The antimicrobial disc was placed using sterile forceps/needle on the inoculated plate.

e) The plate was incubated aerobically at 35-37OC for 18-24 hours.

f) The test was read after checking that the bacterial growth is neither heavy nor light. The diameter of the inhibition zone was measured.

g) The reaction of the test organism was interpreted to each antibiotics used as sensitive, intermediate, or resistance as per the standard. Sensitive – zone of diameter is wider or equal to the control. Intermediate –zone of radius is more than three mm smaller than the control. Resistance – no zone of inhibition.

E. Methicillin Resistant Staphylococcus

Test for detection of MRSA was done by cefoxitin (30µg) disk diffusion method on MullerHinton agar with 4% NaCl (MHA). After overnight incubation at 350c the inhibition zones of diameter less than or equal to 21mm of cefoxitin disc indicated MRSA. Staphylococcus aureus ATCC 25923 was used as quality control strain.

Declaration

I, the undersigned, MSc Medical Microbiology student declares that this thesis is my original work in partial fulfillment of the requirement for the degree of master science in Medical Microbiology. Where others work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

Name of principal investigator

Sara Jemal Signature _____ Date_____

Approved by advisors;

Mr. Zewedeneh S/Mariam (MSc, Ass. Professor) Signature_____ Date_____

Mr. Mekdim Mekonnin (MSc) Signature _____ Date_____

Approved by Assessors

Mr. Kedir Abdella (MSc, Assistant Professor) Signature _____ Date

_____ Signature _____Date_____