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THE MICROBIOLOGICAL CAUSES OF CHRONIC OSTEOMYELITIS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA

By

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A Dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the Award of Master of Medicine in Orthopaedics and Trauma

2017

DECLARATION

I hereby declare that this dissertation entitled *The Microbiological Causes of Chronic Osteomyelitis at the University Teaching Hospital Lusaka*, represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other University elsewhere.

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DEDICATION

To my sons James and Jonathan, for making me smile and laugh even when times are hard, to my dear wife Chipo, for your unconditional support and always believing in me to achieve greater things.

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ABSTRACT

BACKGROUND: Chronic osteomyelitis is a debilitating infection of bone and its bone marrow, presenting with bone pain and an active sinus that discharges pus. It is a persistent and recurrent condition that results from infection of bone and its marrow by various microorganisms. Worldwide, staphylococcus aureus is the commonest causative organism. This study was done because the microbiological profile of causative organisms at the University Teaching Hospital in Lusaka was unknown.

MATERIALS AND METHODS: This was a cross-sectional study involving the collection of deep tissue cultures following sequestrectomy and subjecting them to microscopy, culture and sensitivity examination so as to determine the causative organisms and their antimicrobial sensitivity pattern.

RESULTS: Staphylococcus aureus was the most common (35%) isolated microorganism and the cultured organisms showed highest sensitivity to ciprofloxacin and imipenem. Chronic osteomyelitis was common in children (median age 7.5 years) and in patients of low socioeconomic status.

CONCLUSION: Staphylococcus aureus is the commonest cause of chronic osteomyelitis at the University Teaching Hospital.

KEY WORDS: Osteomyelitis, Sequestrectomy, Staphylococcus

ABBREVIATIONS AND ACRONYMS

COM	-	Chronic Osteomyelitis
UTH	-	University Teaching Hospital
Staph aureus	-	Staphylococcus aureus
M/C/S	-	Microscopy, Culture and Sensitivity
Fig	-	Figure
UNZABREC	-	University of Zambia Biomedical Research Ethics Committee

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CHAPTER ONE

1.1 Introduction

Osteomyelitis is an infection of bone and bone marrow caused by several microbiological organisms (Miller, 2008). It presents with bone pain and an active sinus that discharges pus (figure 1). The condition is subdivided into acute, subacute and chronic stages (Miller, 2008). Chronic osteomyelitis (COM) may appear denovo; not all the patients show progression through the three phases (Khan, 2013). COM is a severe, persistent and sometimes incapacitating infection of bone and bone marrow (Khan, 2013). It is often a recurring condition because it is difficult to treat definitively owing partly to the wide variety of infecting microbiological organisms (Khan, 2013). This disease follows infection with various microorganisms as may result from inadequate treatment of acute osteomyelitis, haematogenous osteomyelitis and trauma (Miller, 2008). Infection may also result following surgery (joint replacements and internal fixation of fractures) and compound fractures (Miller, 2008). COM affects both adults and children (Agaja, 2008). In adults it mostly affects the spine and pelvis, while in children it tends to affect the long bones: Humerus, radius, ulna, femur and tibia (Agaja, 2008).

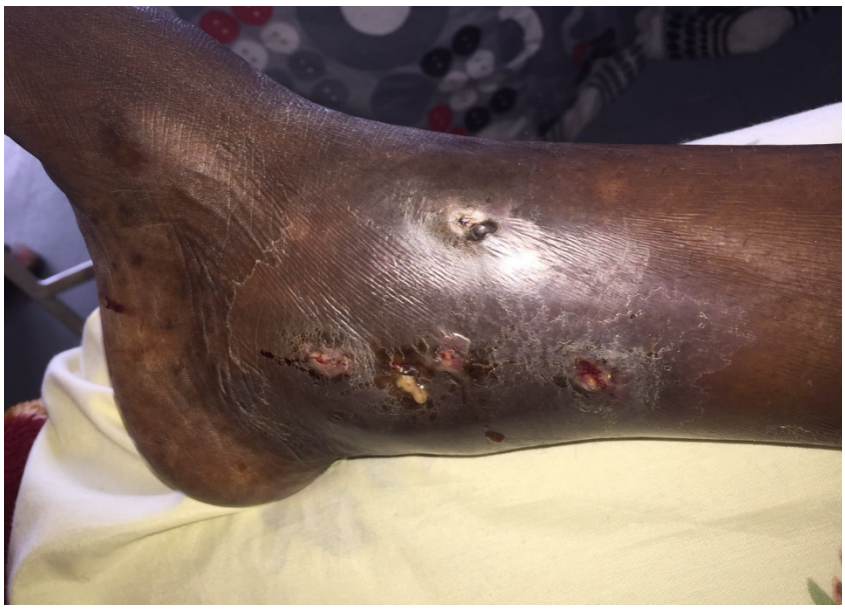


Figure 1 Photograph of COM in the distal tibia demonstrating multiple active discharging sinuses

The causative microorganisms for COM are different in various age groups and other co-morbidities like sickle cell disease, diabetes mellitus, HIV infection and other immunosuppressive conditions (Hatzenbuehlar 2012). Staphylococcus aureus though is the commonest microorganism causing COM. However, infection with microorganisms, such as Mycobacterium tuberculosis and Treponema species (syphilis) is known to occur (Miller, 2008). Furthermore contiguous spread from soft tissues as may occur with diabetic ulcers or ulcers associated with peripheral vascular disease is not uncommon (Miller, 2008). However, the various causative microorganisms of COM in Zambia have not been elucidated.

The patients with COM seen at the University Teaching Hospital (UTH) represent a fair picture of the situation in the country. During an orthopaedic clinic at UTH, a doctor attends to at least 3 patients with COM and an average of 18 patients with COM were operated on every month according to 2013 monthly audits at UTH. Additionally, UTH receives referrals from right across the country. This study looked at the microbiological profile of causative organisms of COM at UTH.

1.2 Literature Review

COM is common in the first and second decades of life (Agaja, 2008). Among individuals who have been treated for an episode of acute osteomyelitis, the prevalence of COM is about 5 to 25% in the United States (Khan, 2009). Prevalence can be as high as 30 to 40% in individuals with diabetes and 16% in foot puncture (King, 2008). In developing countries, the overall prevalence is higher (King, 2008). In Zambia, no statistics on the prevalence of COM have been published but studies indicate that patients with the condition occupy as much as one third of the bed capacity in the major hospitals of the country with an incidence rate of sixty-nine children per year (Jellis, 1981, Klenerman, 2007). In a retrospective review of COM in Malawi, the prevalence for COM was estimated at 6.7% (Beckles, 2009). According to Pelser (2009) when dealing with COM, the classification by Cierny and Mader has proven to be useful as a guide for surgical management. The

infection is classified according to its anatomical description as well as patient (host) factors as shown in the tables below.

Table 1. Anatomical classification

Type	Anatomical description
I	Medullary, with endosteal disease
II	Superficial where the cortical surface is infected and a coverage defect exists
III	Localized cortical sequestrum without instability after debridement
IV	Diffuse type with mechanical instability associated with type I,II or III

Table 2. Physiological classification

Physiological Class	Description
A	Normal immunocompetency with good local vascularity
B	Local or systemic factors that compromise immunity or healing
C	Prohibitive morbidity anticipated and/or poor prognosis for cure

Additionally, the Beit CURE classification system has also proved to be a good tool in grading severity and as a guide in the management of chronic haematogenous osteomyelitis in children. It is based on a review of the radiological features of a series of children with the condition, as a system that has prognostic value and aid surgical management. There are three main groups (A, B and C), the most common of which is the sequestrum/involucrum type (B), which has four subdivisions (Table 3). This classification system also allows description of physeal damage to long bones (Stevenson et al 2015, Jones et al 2011).

Table 3. Beit CURE classification of Childhood Chronic Haematogenous Osteomyelitis

Classification type	Radiological appearance of bone segment
A	Abscess type, osteolytic area(s), no sequestrum, no involucrum
B1	Peripheral, localized cortical sequestrum, minimal/no involucrum
B2	Sequestrum present; stable, normal-looking cortical involucrum
B3	Sequestrum present; stable, sclerotic involucrum
B4	Sequestrum present; unstable, inadequate involucrum
C	No sequestrum visible on plain X-ray, diffusely sclerotic bone segment; abscess may be present
Unclassifiable	Inadequate X-ray/disease onset >months/previous surgery

In the work-up of a patient with COM, raised inflammatory markers such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and a raised white cell count usually confirm the presence of infection, but have poor specificity (Pelser, 2009). Confirmation of the diagnosis of COM is obtained by culture and sensitivity on biopsy (Pelser, 2009). Specimens for biopsy must be comprehensive and taken from tissue that is representative of the diseased area (Miller, 2008). A minimum of five biopsies need to be taken and samples can consist of pus, soft tissue and bone (Pelser, 2009). The diagnosis of the type of infection causing COM is confirmed if two or more specimens yield a positive culture (Pelser, 2009). A sample is sent for histopathological examination, which may show changes of COM (Pelser, 2009).

Radiological changes of COM can be varied and may include osteopaenia, scalloping and cortical thinning (Pelser, 2009). Other features may include loss of trabecular and cancellous architecture (Pelser, 2009). While radiodense appearance of sequestrum, periosteal changes and soft tissue swelling are a frequent radiological finding (Pelser, 2009). (Figure 2)

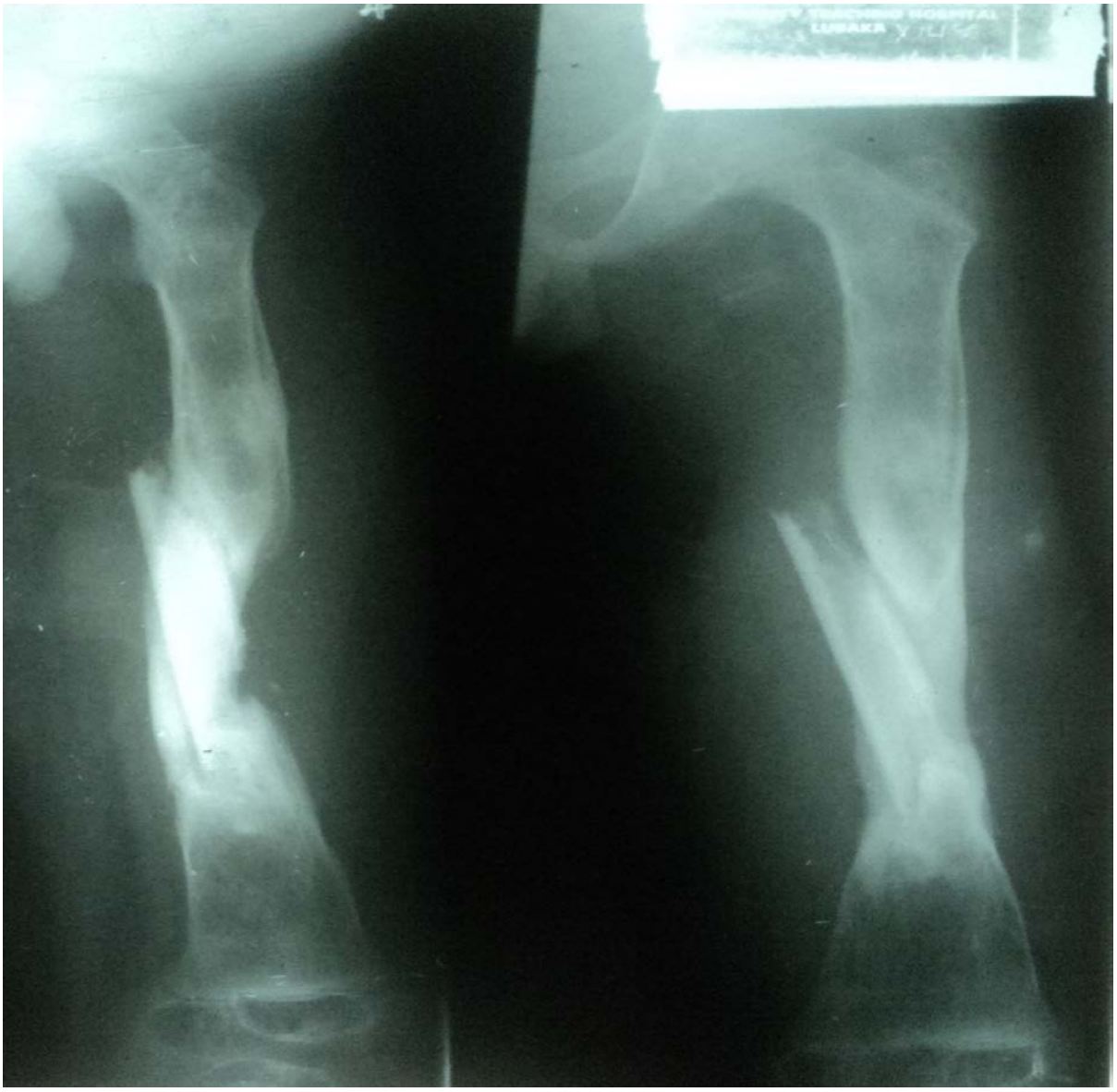


Figure 2 : Radiograph of COM in the left femur of an adult male patient

Most common pathogens of COM depend on a patient's age (Hatzenbuehler, 2011). Staphylococcus aureus however is common in both adults and children (Miller, 2008). In a study in Nigeria, staphylococcus aureus is reported to have been isolated from sinuses of more than 90% of patients with COM (Alonge et al, 2002 and Agaja2008). In another study from Oxford, United Kingdom, Staphylococcus aureus was the most commonly isolated microorganismaccounting for 32% (Sheehy, 2010). Group A streptococcus, streptococcus pneumoniae and kingella kingae are the most common in children, while group B streptococcus occurs in newborns (Hatzenbuehler, 2011). Other organisms that may be cultured include Klebsiella, coliforms, Escherichia coli,

proteus and pseudomonas (Agaja, 2008). These microorganisms frequently cause COM secondary to infection at another anatomical site (Miller, 2008).

A number of studies with varying sample sizes have been done in trying to elucidate the causative organisms of COM. In two studies in the United Kingdom on the effect of surgical resection on infection free survival and showing the lack of microbiological concordance between bone and non-bone specimens in COM, 50 participants were enrolled in both (Simpson, 2001 and Zuluga, 2000). Additionally, in a prospective study on the etiologic diagnosis of COM, 100 participants were enrolled (Zuluga, 2006). Furthermore, 18 participants were enrolled in a study on treatment of COM using calcium hydroxyapatite ceramic implants impregnated with antibiotics (Yamashita, 1998). Similarly, in a study on COM in pin tracks after external fixation, 14 participants were enrolled (Green, 1984).

Atypical microorganisms may rarely cause COM and this makes its diagnosis and management challenging (Miller, 2008). Some atypical organisms that have been cultured are Staphylococcus lugdunensis and Bacteroidaceae (Massimo, 2012). Nevertheless, after years of antibiotic therapy and other treatment modalities, the culture may be altered to include strains of Enterococcus and Corynebacterium (Massimo, 2012). Another uncommon form of COM is the non-bacterial type (Miller, 2008). Chronic non-bacterial osteomyelitis is a disease of young girls and is a diagnosis of exclusion because its etiology is largely unknown (Khan, 2012). It has non-specific imaging features of osteolytic bone destructive lesions with surrounding sclerosis (Khan, 2012). Histological findings are also non-specific, showing inflammatory changes and granulocytic infiltration (Khan, 2012). The typical location of bony swelling, characteristic slow remitting and relapsing course, with negative tests for malignancy and infection are clues to the diagnosis (Khan, 2012). One example of non-bacterial COM is that due to candida (Kil, 2012). It may be diagnosed late because in many cases, it does not manifest such typical symptoms as pyrexia (Kil, 2012). Moreover, in many cases it presents with non-specific laboratory findings (Kil, 2012). Despite this wide range of causative microorganisms no growth may be cultured in some cases of COM (Alonge et al, 2002).

There is controversy over whether COM rates are increasing or decreasing (Dodwel, 2013). Changes in epidemiology may be related to improved methods of diagnosis (Dodwel, 2013). The pathogens responsible for COM in children have changed with alterations in immunization practices, emergence of resistant bacteria, and changes in patterns of immune modulating diseases and medications in children (Dodwel, 2013). Special culture techniques and PCR may help to identify pathogens that are difficult to culture (Dodwel, 2013).

In Zambia, in a study on Factors influencing the outcome of acute haematogenous osteomyelitis at the University Teaching Hospital, it was found that 69% of the patients enrolled in the study came from a low social economic group. Additionally, 26% were of poor nutrition status that predisposed them to the osteomyelitis in keeping with their low social economic status while 35% had no risk factors (spontaneous onset) (Chowa, 2003)

COM may cause lifelong morbidity and a significant reduction in the quality of life for the patient (Agaja, 2008). In the past, it was regarded as incurable, but a combination of modern surgical techniques and medical treatments have often achieved a prolonged disease-free interval and in some cases, cure (Agaja, 2008). Direct sampling of sinuses in COM for microbial identification and antimicrobial sensitivity is essential to target treatment and reduce antibiotic systemic toxicity (Hatzenbuehler, 2011). This furthermore, has reduced the time and cost of hospitalization (Hatzenbuehler, 2011). Therefore, where available, an anti-microbial profile of the causative organisms of COM can be used to guide empiric treatment with improved outcomes in management. There is no known microbiological profile of COM currently in Zambia.

1.3 Study Justification

In spite of COM being a significant problem at UTH, the microbiological profile of causative organisms still remains unknown. Such a profile can help guide the treatment of COM in the country and significantly improve the outcomes of

management. Ultimately, the debilitating effects of COM in both children and adults can thus be prevented.

1.4 Research Question

What is the microbiological profile of causative organisms of COM seen at UTH?

1.5 Null Hypothesis

The microbiological profile of causative organisms of COM does not include a wide spectrum of organisms.

CHAPTER TWO

2.0 Objectives

2.1 General Objective

To determine the microbiological profile of causative organisms of COM at UTH.

2.2 Specific Objectives

1. To identify the causative organisms of COM
2. To relate the identified organisms to patient specific factors (Age, sex, residency)
3. To find out the antimicrobial sensitivity of the organisms causing COM.

CHAPTER THREE

3.0 Methodology

3.1 Study Design

Study Design and Setting

This was a cross-sectional study carried out at UTH.

Sampling

Convenience sampling of patients presenting with COM to UTH was done. In spite of the high patient burden of the condition, few cases are operated and thus convenience sampling was used to ensure that more patients were captured.

3.20 Inclusion and Exclusion Criteria

3.21 Inclusion Criteria

- Patients presenting with COM to UTH and are operated on.
- Informed written consent

3.22 Exclusion Criteria

- Patients presenting with COM to UTH but were not operated on were not included in the study.
- Patients who refused to consent were not enrolled into the study.

Sample size

The sample size was calculated using the formula:

$$N = \frac{Z^2 \times P(1-P)}{(E)^2}$$

N = Sample required

Z = Z statistic for a given level of confidence = 1.96 when using a 95% CI

P = the expected prevalence of the condition in the population being studied

E = confidence interval, 0.05= accuracy range (+/- 5%)

Giving: $N=\frac{1.96x1.96x0.067\ (1-0.067)}{0.05x0.05}$

=96 participants

At a prevalence rate of 6.7%,(from neighbouring Malawi with similar conditions to Zambia) a sample size of 96 participants was calculated.

Over the years, different studies have been undertaken to determine the microbiological causes of COM. Between 1984 and 2006, various studies in different centers have been done with sample sizes ranging from 14 to 100 as outlined in the literature review.

With this precedence, in this study, a sample size of *50 participants* was targeted but only *43 participants were enrolled and analysed*. (Green, 1984, Yamashita, 1989, Zuluga, 2000, Simpson, 2001, Zuluga, 2006).

Curettings after a sequestrectomy from patients who met the above criteria were subjected to microbiological tests to determine the causative organisms and antimicrobial sensitivity. The tests included microscopy, culture and sensitivity.

CHAPTER FOUR

4.1Descriptive data of Participants

4.1.1 Social Demographic Data of participants

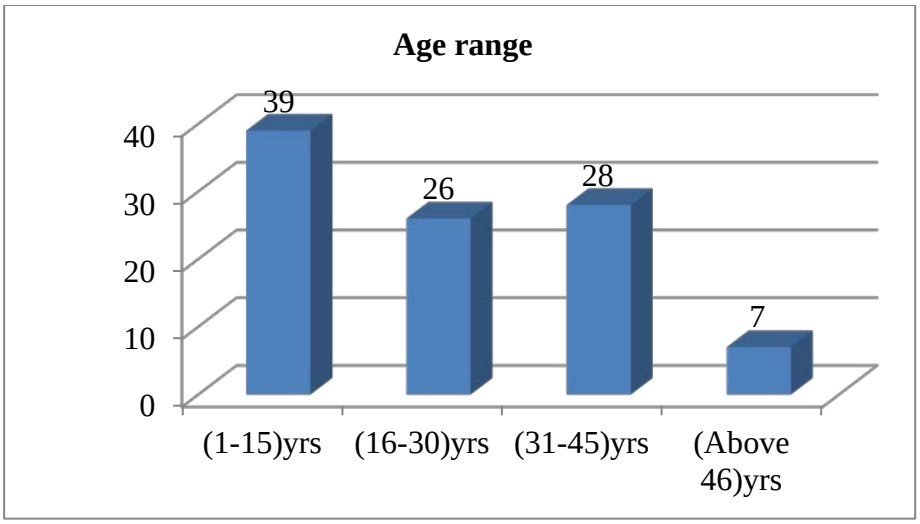
In this study on the microbiological causes of COM atUTH, 43 patients were enrolled from May 2014 to January 2015. The targeted sample size of 50 could not be reached due to reduced theater time after a rule to operate COM cases only from the emergency theater (Phase V) and not Main Theater (Phase III) was passed. This was a major hindrance coupled with shortage of reagents for analyzing the specimens during the period of the study. Additionally, as a control measure for confounding factors, only patients from within Lusaka were considered in the study. All the enrolled patients had their collected specimens examined and data analysed.

Table 4 :Sex distribution of COM patients

Sex	**Frequency**	**Percentage (%)**
Male	**30**	**70**
Female	**13**	**30**

From the collected data, 30 (70%) of those enrolled in the study were males while 13(30%) were female as shown in *table 3***.** The age ranged from 1 year to 63 years.

Figure 3 Age Range



It was noted that COM was most prevalent in children, with an age range 1-15 years accounting for 39%, which was the majority as shown in *figure 3* above.

Table 5 Area of residency

Area	Frequency	Percentage
High Density	**36**	**84**
Low Density	**7**	**16**

All the enrolled patients came from within Lusaka and were simply divided into high and low density population areas. The majority of those enrolled came from high population density areas (84%). These are areas of low social economic status.

4.2 Descriptive Data of COM of participants

The commonest site of COM was noted to be in the lower limb in 13(37%) of the children **(***Table 5***).** In the adults, the study found that COM was mostly a result of infection of open fractures and implants following internal fixation. In 72% of the patients, COM was of haematogenous onset, with no known risk factors as illustrated in *figure 4.*

Table 6 Age in relation to site of COM

Age(years)	**Site**		
	Upper Limb	**Lower Limb**	**Totals**
1-15	**4**	**13**	**17**
16-30	**3**	**8**	**11**
31-45	**4**	**8**	**12**
Above 46	**1**	**2**	**3**

Figure 4 : Primary cause of COM

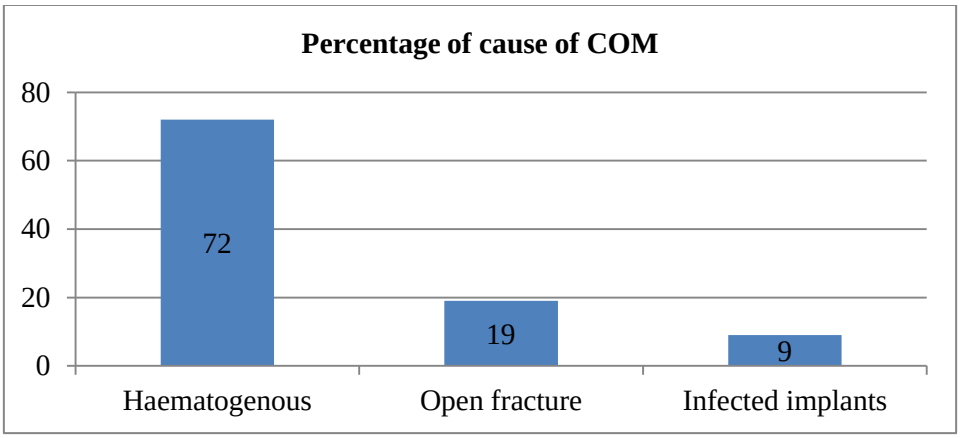
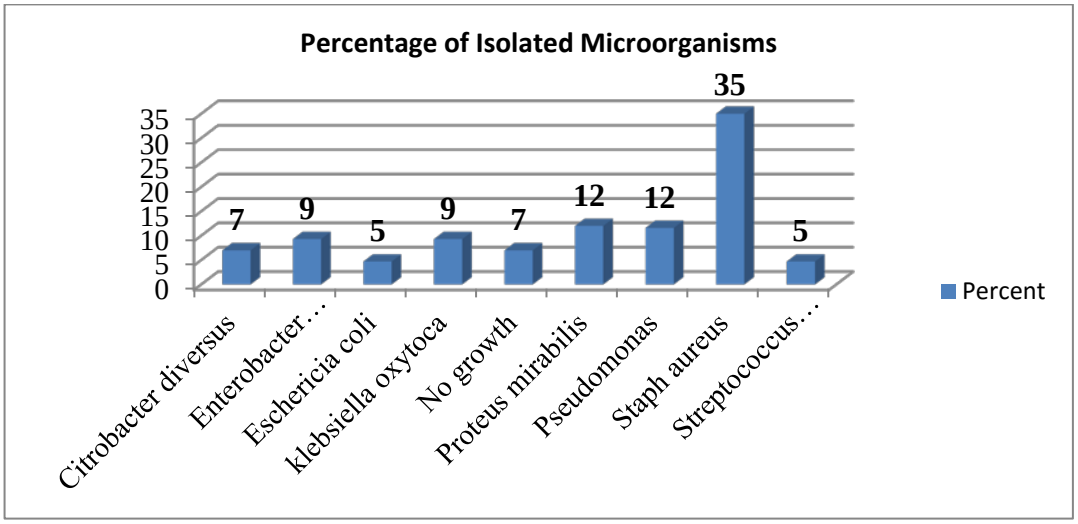


Figure 5 Percentage of isolated microorganisms



A profile of 8 different causative organisms was formulated from all the deep tissue cultures and staphylococcus aureus was isolated in 35% of the patients with COM while 7% cultured no growth.

Table 7 Antimicrobial Sensitivity of cultured organisms

Cultured organisms	Ceftazidime	Cefotaxime	chloramphenicol	**Imipenem**	**Ciprofloxacin**	Gentamycine	Tetracycline	Erithromycine	Cotrimoxazole	N/A
Staph aureus	0	0	0	8	8	1	1	1	0	0
Staph aureus, Klebsiellaoxytoca	0	3	0	3	3	0	0	0	2	0
Pseudomonas, Proteus mirabilis	1	0	0	1	1	0	0	0	0	0
No growth	0	0	0	0	0	0	0	0	0	3
Klebsiellaoxytoca	0	0	0	1	1	0	0	0	0	0
Strep pneumonae	0	0	1	2	2	0	0	0	0	0
Pseudomonas	0	0	0	4	0	0	0	0	0	0
Citrobacterdiversus	0	3	0	3	0	0	0	0	0	0
Proteus mirabilis	4	0	4	0	5	0	0	0	0	0
Enterobacteraerogens	0	0	0	4	0	0	0	0	0	0
Eschericia coli	0	0	0	0	0	2	2	2	0	0
Staph aureus, Pseudomonas	0	0	0	6	6	0	0	0	0	0
Totals	5	6	5	**32**	**26**	3	3	3	2	3

The profile of eight different cultured organisms showed highest sensitivity to imipenem and ciprofloxacin at frequencies of 32 and 26 respectively *(table 6).* All the patients had been on treatment with cloxacillin for different durations with no resolution of the COM symptoms, thus considered resistant to cloxacillin.

4.3 Statistical data of COM

Table 8 Association of COM with selected variables

Variable	P value
Age	**0.940**
Socioeconomy	**0.380**
Site of COM	**0.073**
Microorganism	**0.073**
Gender	**0.310**

Association of COM to various selected variables showed P values that were >0.05, thus considered statistically insignificant.

CHAPTER FIVE

5.0 Discussion

In this study done at UTH on the microbiological causes of bone infections, it was shown that COM is common among males at 70 %,mostly children of a median age of 7.5 years old caused by staphylococcus aureus, which was mostly sensitive to imipenem and ciprofloxacin. The age of patients and microorganism cultured confirm the results of studies done in UK and Nigeria (Alonge et al, 2002, Sheely, 2010)

Some of the challenges encountered during this study included; 1) reduced theater time leading to fewer patients than expected being enrolled; 2) lack of polymerase chain reaction (PCR) to identify organisms of low yield 3) shortage of reagents in the UTH microbiology laboratory during some periods of the study.

5.1 Sex Distribution

As literature suggests that COM is more common in males, or in young children, this study was similarly able to demonstrate 70% with the condition, with the 1-15 years age group contributing 39% (Miller, 2007). This has been attributed to the frequent injuries that children sustain and the respiratory infections resulting in septicemia that render them susceptible to bone infections (Carek P et al 2001). In this study, 37% of COM was found to be in the lower limbs, confirming the theory of trauma to bone or skin predisposing to COM as male children usually suffer such.

5.2 Causes of COM

In this study, COM was found to be of haematogenous origin mostly (72%). This is accounted to the frequent respiratory illnesses and skin infections that may complicate into septicemia and the infection then seeding in the metaphyseal bone to cause COM (Carek P et al, 2001). Only 19% of the cases were attributed to open fractures and 9% secondary to infected implants. This underscores the good management protocol for open fractures and infection control in open reduction and internal fixation at UTH by the orthopaedic units.

5.3 Cultured Microorganisms and drug sensitivity

As shown by studies world over, staphylococcus aureus is the major causative organism of COM (Alonge et al, 2002, Sheely, 2010). Similarly, in this study, staphylococcus aureus was found in 35% of the participants. Additionally, staphylococcus aureus was found in combination with either Klebsiella oxytoca or Pseudomonas aerogenosa in 14 %. The other paired combination of microorganisms was Pseudomonas aerogenosa and Proteus mirabilis. Thus, as shown in other studies that polymicrobial infection is possible in COM, this study demonstrated this fact (Mader, 1996). Other microorganisms cultured in this study included Streptococcus pneumonia, Eschericia coli, Enterobacteraerogens and Citrobacterdiversus. However, no growth was cultured from 7% of the participants. This cannot be conclusively relied upon as there was no PCR to confirm if any microorganism of low yield was present despite literature indicating the possibility of COM with no growth.

Ciprofloxacin and imipenem demonstrated the highest sensitivity against the cultured organisms in this study, 26 and 32 respectively. The current practice at UTH is the long term use of cloxacillin in patients with COM. As demonstrated by this study on sensitivity of the organisms cultured, use of ciprofloxacin in COM may be more effective in treating COM. Use of ciprofloxacin in children carries the risk of damage to the articular cartilage in weight-bearing joints as demonstrated in experimental animals (Goldman, 2011). However, the benefits of treatment outweigh the risks, thus it can safely be used in treating COM in both adults and children.

5.4 Residential Category

Of the 43 participants in this study, 36 (84%) came from high density residential areas which are associated with low socioeconomic status, poor nutrition and poor hygiene. Another study done at UTH on factors influencing the outcome of acute haematogenous osteomyelitis, poor nutrition emanating from low socioeconomic status ranked highest in poor outcome (Chowa, 2003).

5.5 Statistical Tests

Association of COM to selected variables shown in *table 8*, shows P-values that are greater than 0.05. This can be accorded to 1) the sample size used in the study is small (n=43) and 2) the sampling method used in the study is Convenience sampling, which limits association of risks.

CHAPTER SIX

6.0 Conclusion

1. Staphylococcus aureus is the commonest causative organism of COM at UTH.

2. COM is common among males, especially in children and is highly associated with low social economic status.

3. Imipenem and Ciprofloxacin are the most sensitive drugs to the causative organisms of COM isolated at UTH.

6.1 Limitations

The projected sample size of 50 could not be achieved due to reduced theater time and the change of COM cases not to be operated in the main operating theater from mid-2014. Additionally, this study had a small sample size of 43 patients from within Lusaka, thus its findings can not be generalized as a representation of the microbiological profile of causative organisms of COM in Zambia.

6.2 Recommendations

1. From the sensitivity pattern of the cultured organisms, ciprofloxacin and imipenem should be used as first line drugs in the management of COM at UTH.
2. There is need for a country-wide survey for a profile of microbiological causes of COM in Zambia to guide in the national management of the condition with antibiotics.

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APPENDICES

APPENDIX A

PARTICIPANT INFORMATION SHEET

Title of Research-The Microbiological Causes of Chronic Osteomyelitis at University Teaching Hospital Lusaka

Principal Investigator: Dr. J. Mulenga

Introduction

You (or your child) are/is invited to participate in a research study. This form explains the research you are being asked to join. Please review this form carefully and ask any questions about this research study. If you would like more information or there is anything that you do not understand before you decide to join or not to in this study, feel free to ask. You may ask questions at any time after joining the study and at any time can withdraw from this study.

PURPOSE OF RESEARCH STUDY

The purpose of this study is to help us learn more about the germs and cause of infection in bones. In particular, we would like to relate the germs that cause the infection to the area of residence of the patient.

WHO CAN JOIN

All patients with a diagnosis of Chronic Osteomyelitis and scheduled for surgery at UTH may join. Both adults and children fitting this description are eligible.

You are therefore being asked to join this study because you meet this description. A total of 50 patients will be taking part in this study.

VOLUNTARY PARTICIPATION

Your participation in this research is completely voluntary. In the event that you join this research and then later decide you want to withdraw from the research, you will still receive the same quality of medical care available to you at this hospital. You should ask the principal investigator (whose details are given below) any questions you may have about this research study.

You may ask questions in the future if you do not understand something that is being done.

WHAT HAPPENS WHEN YOU JOIN THE STUDY

If you agree to join this study, we will ask you to give us some of your time to answer some questions. During this research, we will be looking through your medical records to assess the cause of infection in your bone/s.

After the operation is done, some of the bone/s, soft tissues and fluids removed will be collected and subjected to examination at the laboratory in UTH, Lusaka.

PAYMENT FOR PARTICIPATING

You will not be paid for participating in this study.

RISKS IN TAKING PART

There are NO perceived risks or disadvantages of taking part in this research study. If however, you should experience any discomfort or disadvantage as a result of taking part in this research study, you should make this known to the researcher promptly.

IMMEDIATE BENEFITS OF PARTICIPATION

If during the course of the study you are with some medical conditions that need to be addressed, you will be referred to relevant specialists.

CONFIDENTIALITY

Only the study investigators collecting the data and analyzing the specimens will have information on the answers you give to the questions asked.

You will not be named in any reports about this research. All the data collected will only be used for this research.

RESULTS OF STUDY

The study team will do their best to inform you of findings that potentially could improve your care. The results of this study will be published in a medical journal. All participants of the study will not be identifiable from the published results.

WHAT WILL HAPPEN IF I WANT TO STOP TAKING PART

As a participant in this study, you can withdraw at any time, without explanation. Results up to the period of your withdrawal may be used, if you are happy for this to be done. Otherwise you may request that they are destroyed and no further use is made to them.

Persons to Contact: If you want to talk to someone about this research study because you think you have not been treated fairly, or think you have been hurt by joining the study, or you have any other questions about the study, you should contact the principal investigator Dr. James Mulenga of the Department of Surgery at UTH on

cell phone number 0977-981640 or e e-mail mwenyajamus@yahoo.com and he will try to help you.

If however, you are still unhappy or have a complaint which you feel you cannot come to him with, then you should contact the University of Zambia Biomedical Research Ethics Committee (UNZABREC) on telephone number +2601 256067.

APPENDIX B

Consent Form

Title of research: The Microbiological Causes of Chronic Osteomyelitis at UTH.

Principal investigator: Dr. James Mulenga

1. I confirm that I have read and understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected.
3. I understand that I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish.
4. I understand that I will not be identified or identifiable in any report subsequently produced by the researcher.
5. I accept that taking part in this study is voluntary and confirm that any risks associated with this have been explained to me.
6. I agree to take part in the above study.

Participant's Name: …… Signature:…….
Date:…….....

Participant's thumb print…….

Witness: …… Signature:…….
Date:…….....

For further questions please contact Dr. James Mulenga, UTH, and 0977-981640 or e-mail mwenyajamus@yahoo.com

APPENDIX C

DATA SHEET

Age: …… Mobile number:…….....

Gender ……..

Town: ……Township: …….

Province: ……..

Hospital: ……..

Site/s: 1…… 2…… 3……

Cause: Haematogenous…….. Secondary to Open Fracture…….

Secondary to surgery (implants) ……

Co-Morbidity:……..

FBC WBC.……

HB…….

Sickle cell disease: YES ……NO……

Microbiology: Single organisms:…… Multiple organisms…….....

Sensitivity ……