

Bacteriology of Open Fracture Wounds
in Tikur Anbessa University Hospital,
Addis Ababa, Ethiopia



By

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<u>Table of Contents</u>	<u>Pages</u>
Acknowledgements	i
Table of Contents	ii
List of Tables	iv
List of Figures	v
Abbreviations	vi
Abstract	vii
CHAPTER I: INTRODUCTION	1
1.1. Background.....	1
1.2. Literature Review	2
1.2.1 Grading of open (compound) fractures	2
1.2.2 Open fracture wound microbiology.....	3
1.2.3 Epidemiology of open fracture wound infection.....	10
1.2.4 Clinical features of infected open fracture wounds.....	11
1.2.5 Immunity	11
1.2.6 Laboratory diagnosis	12
1.2.7 Treatment and prevention of wound infection in open fractures	13
1.2.8 Antimicrobial resistance.....	16
1.2.9 Significance of the study	17
1.3. Objectives of the Study	18
CHAPTER II: PATIENTS AND METHODS	19
2.1. Study Design,Area, and Period	19
2.2. Source Population.....	19
2.3. Bone Imaging	20
2.4. Wound Bed Preparation	20
2.5. Sample Collection, Handling and Transport	21
2.6. Microscopic Examination.....	21
2.7. Culture and Identification.....	21
2.8. Antimicrobial Susceptibility Testing.....	22

2.9. Reference Strains.....	23
2.10. Data entry and Analysis.....	23
2.11. Ethical Considerations.....	24
CHAPTER III: RESULTS	25
3.1. Source Population.....	25
3.2. Gustilo and Anderson (G-A) Grading of Open Fractures	27
3.3. Fracture Patterns	28
3.4. Clinical Features	29
3.5. Wound Management and Time of Sample Collection	29
3.6. Microscopic Examination.....	30
3.7. Culture	30
3.8. Use of Antimicrobials and Culture Outcome	32
3.9. Antimicrobial Susceptibility.....	32
CHAPTER IV: DISCUSSION	38
LIMITATIONS OF THE STUDY	44
CONCLUSIONS AND RECOMMENDATIONS	45
REFERENCES	46
APPENDICES.....	54-60
Appendix I: Questionnaire	54-58
Appendix II: Consent Form (Amharic and English Versions).....	59-60

List of Tables

Pages

Table 1.1 Modified Gustilo and Anderson classification of open fractures3

Table 3.1 Time of arrival, address and pattern of admission of 191 patients.....25

Table 3.2 Demographic data of the patients with open fracture wounds.....26

Table 3.3 Skeletal sites and frequency of the fractured bones28

Table 3.4 Bacteria isolated from the 200 compound fracture wounds.....31

Table 3.5 Susceptibility Patterns of Gram-positive Bacterial Isolates33

Table 3.6 Susceptibility Patterns of *Clostridium* spp. isolates34

Table 3.7 Susceptibility Patterns of Gram-negative Bacterial Isolates36

List of Figures

Pages

Figure 1.1 Scanning electron microscopy of a staphylococcal biofilm8

Figure 1.2 Tobramycin-PMMA beads applied in an open fracture wound.....15

Figure 3.1 Age and sex distribution of the patients with compound fracture wounds ..27

Figure 3.2 The frequency of G-A grades of the compound fractures.....28

Figure 3.3 The skeletal sites of the bones involved in the open fractures29

ABBREVIATIONS

API	Analytical Profile Index
ATCC	American Type Culture Collection
BAMC	Brooke Army Medical Center
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical Laboratory Standards Institute
CoNS	Coagulase Negative Staphylococci
DNase	Deoxyribonuclease
EHNRI	Ethiopian Health and Nutrition Research Institute
FRPC	Faculty Research Publications Committee
G-A	Gustilo and Anderson
MDR	Multiple Drug Resistance
NCCLS	National Committee for Clinical Laboratory Standards
PMMA	Polymethylmethacrylate
RDNS	Royal District Nursing Services
RTA	Road Traffic Accident
SCFA	Short-chain Fatty Acid
SOPD	Surgical Outpatient Department
SPSS	Statistical Package for Social Science
TAUH	Tikur Anbessa University Hospital

ABSTRACT

Open fractures are those exposed to the outside environment through a skin wound. They are at risk of complications such as infected non-union and other co-morbid conditions. Sixty to seventy percent of compound fractures are believed to be contaminated with bacteria at the time of injury from both skin and environment. Infection of open fractures depends on the microbial and host factors. In Ethiopia, a high incidence of open fracture wound infection is suspected though the magnitude of the problem is not known. No documented report on bacterial isolates from open fracture wounds and their drug resistance pattern. During a period of November 2007 and May 2008, a cross-sectional prospective study was conducted to determine the bacteriology of open fracture wounds of 191 informed and consented patients who visited the orthopedic surgery department of Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The modified Gustilo and Anderson (G-A) grading of open fractures based on severity and extent of soft-tissue injury was used to categorize the open fractures. The clinical features of the open fracture wounds were assessed and wound swab specimens were collected using Levine's technique from each patient. All of the wound specimens were processed for microscopic examination, culture and sensitivity testing. The causes of the fractures varied, but most of the open fractures were caused by car accidents (37.2%) and occurred in lower extremities bones (60.0%). Of the 191 patients, 82.7% were males and 17.3% were females ($p < 0.05$) resulting in an overall male to female ratio of 4.8:1. The average age of the patients was 31.55 years (age range 4 to 75 years). According to G-A grading, 23.0% of the fractures were grade I; 41.5% were grade II; 14.0% were grade IIIA; 5.5% were grade IIIB and 16.0% were grade IIIC. Of the 200 wound specimens examined by gram stain, 30.5% were positive for the presence of bacteria. Out of the 200 wound specimens cultured, 82 (41%) were positive for bacteria. Of the culture-positive wounds, 51.2% showed mono-microbial growth and 48.8% showed polymicrobial growth. In general, a total of 162 bacterial pathogens were isolated from the open fracture wounds sampled. *Staphylococcus aureus* was the dominant isolate (14.8%) followed by *Acinetobacter* spp. (11.4%). The gram-positive and gram-negative bacteria accounted for 34.0% and 66.0%, respectively ($p < 0.05$). All gram-positive bacterial isolates showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and penicillin to which they showed intermediate level of resistance (60-80%). Most gram-

positive isolates, 29/55 (52.7%) showed multiple drug resistance (resistance to three or more drugs). All *Clostridium* spp. were susceptible to tetracycline, doxycycline, and kanamycin and showed low level of resistance (<60%) against chloramphenicol, clindamycin and penicillin. All gram negative bacterial isolates showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and amoxicillin (60-80%, intermediate level resistance). Fifty-one percent of the gram negative bacterial isolates were identified as multiple drug resistant. In conclusion, the present study showed that road traffic accident was the commonest cause of open fractures. Most fractures occurred in lower extremities. *Staphylococcus aureus* was the commonest isolate associated with open fracture wound infection. Gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested gram positive and gram negative bacteria. The findings of this study will give valuable information for establishing empiric therapeutic approaches for the management of open fracture wound infections. In addition, the findings underscore the need for routine microbiological investigation of open fracture wounds and monitoring antimicrobial resistance pattern for the use of prophylactic and therapeutic antimicrobials.

Key words: Open fracture wounds, Bacterial isolates, Antimicrobial susceptibility testing

CHAPTER I: INTRODUCTION

1.1. Background

Open or compound fractures are fractures that communicate with the outside environment through a skin wound (Hauser *et al.*, 2006). They are usually caused by high-energy trauma (Zalavras *et al.*, 2007). The causes of open fractures vary widely including road traffic accident (RTA), fall from a height, gunshot, assault, machine injury, and others (Taye and Munie, 2003; Ikem *et al.*, 2004; Ahmed and Chaka, 2006a). These fractures continue to be common, approximately 3-4% of all fractures being open fractures (Anglen, 2005; Petrisor *et al.*, 2008).

The wound which is usually wide, contaminated and devitalized may often expose the fractured bone resulting eventually in complications such as wound infection, delayed and non-union as well as osteomyelitis (Gebrechristos, 2002). Complications of open fractures increase with age of the patient and complexity of the injury (Stewart *et al.*, 2005). Whether a fracture will heal without complications depends more on the conditions of the soft-tissues surrounding the bone (Karlalani *et al.*, 2001; Ikem *et al.*, 2004; Necmioglu *et al.*, 2005). The anatomic location of the fracture may also help determine the risk of infection (Bowen and Widmaier, 2005).

Therefore, the development of infection favored by devitalization of bone and soft-tissue and loss of skeletal stability is a major complication in open fractures, especially in grade III open fractures (Ostermann *et al.*, 1993; Bowen and Widmaier, 2005; Quinn and Macias, 2006). Deep fracture-site infections can lead to chronic osteomyelitis, non-union, loss of function, or even limb loss (Hauser *et al.*, 2006).

By definition, open fracture wounds are contaminated (Gustilo, 1979; Ostermann *et al.*, 1995; Cat and Hall, 2007). Sixty to seventy percent contaminations is believed to occur at the time of injury (Gustilo and Anderson, 1976; Lee, 1997; Cat and Hall, 2007). The contaminating bacteria originate from both skin and environment (Bowler *et al.*, 2001; Ikem *et al.*, 2004). In some cases the organism is not present at the time of injury, and the wound becomes inoculated with it later (Lee, 1997).

The dynamics of bacterial populations in soft-tissue wounds and bone differ greatly over time (Zuluaga *et al.*, 2002). The constantly changing local wound ecology and sampling variations led to the proposition of different ideas by different authors in the orthopaedic literature. Based on the types of organisms causing infection compared with those seen on early wound cultures, several authors have proposed that many infections of open fracture wounds are nosocomial (Lee, 1997).

Wound infecting pathogens differ from country to country and from one hospital to another within the same country due to the difference in bacterial prevalence in different environments (Lee, 1997; Mulu *et al.*, 2006). Therefore, bacteria that infect open fracture wounds and antimicrobial therapy used vary from hospital to hospital (Lee, 1997). Moreover, wound infection rates vary between institutions and even among surgeons in the same institution (Taye, 2005).

In Ethiopia, there is no published information about the bacteriology of open fracture wounds. Therefore, the present study was undertaken to determine the bacterial etiologies of open fracture wound infection and their antimicrobial susceptibility pattern in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. The findings of the study will provide valuable information for the management of open fracture wound infections with appropriate antimicrobial agents.

1.2. Literature Review

Numerous studies have been published on the subject of open fractures with dogma and controversy dominating in different management issues of the open fractures (Carsenti-Etesse *et al.*, 1999; Cole and Bhandari, 2005).

1.2.1 Grading of open (compound) fractures

The concept of Gustilo and Anderson (G-A) classification of open fractures was proposed by Gustilo and Anderson, (1976), and further refined or modified by Gustilo *et al.*, (1984). Accordingly, open fractures are classified into three major types (of which type III has three subtypes), based on the mechanism of injury, the degree of soft-tissue damage, the configuration of the fracture, and the level of contamination (Gustilo *et al.*, 1987; Gustilo

et al., 1990). The classification system was being continuously refined to the currently used modified version as outlined in Table 1.1. The modified G-A classification although originally intended to grade open tibial fractures, currently it is used to grade all open fractures (Hauser *et al.*, 2006; Quinn and Macias, 2006; Cat and Hall, 2007).

Table 1.1. Modified Gustilo and Anderson classification of open fractures (Adapted from Okike and Bhattacharyya, 2006)

Grade	Definition	Infection Rates (%)
I	Wound <1 cm; minimal contamination, comminution, and soft-tissue damage	0-2
II	Wound >1 cm; moderate soft-tissue damage, minimal periosteal stripping	2-5
IIIA	Severe soft-tissue damage and substantial contamination; coverage adequate	5-10
IIIB	Severe soft-tissue damage and substantial contamination; coverage inadequate	10-50
IIIC	Arterial injury requiring repair	25-50

Open fractures more than 8 hours old at presentation were classified as a special category of type III fractures (Zalavras and Patzakis, 2003). The sensitivity of the modified G-A classification becomes limited as fractures become more severe and it has also been shown that there is interobserver variability (Bowen and Widmaier, 2005). It is note worthy that some lower-grade fractures may be upgraded at debridement and reversely some grade IIIC fractures may be converted to IIIB-like fractures by revascularization. Therefore, it is important that open fractures be classified not in the emergency room but in the operating room after surgical exploration and debridement have been completed to minimize risk of misclassifications (Okike and Bhattacharyya, 2006).

1.2.2 Open fracture wound microbiology

a. Overview

A normal intact skin prevents microbial populations in general and potential pathogens in particular from colonizing and invading underlying tissue (Bowler *et al.*, 2001). But, a loss of skin integrity by wounding provides a moist, warm, and nutritious environment for microbial colonization and proliferation which may be facilitated further by the presence of

foreign material and devitalized tissue in a traumatic wound (Bowler *et al.*, 2001; Shittu *et al.*, 2003; Taye, 2005).

Wound contaminating microorganisms may originate from the environment, the surrounding skin and endogenous sources including primarily the gastrointestinal, oropharyngeal, and genitourinary mucosae (Lee, 1997; Bowler *et al.*, 2001; Ikem *et al.*, 2004). To date, it is widely accepted that aerobic or facultative bacterial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and β -hemolytic streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds (Bowler *et al.*, 2001). *S. aureus*, β -hemolytic streptococci, and enteric Gram-negative bacilli were reported to be frequently responsible for wound infections and osteomyelitis (Lawrence *et al.*, 1978).

Culture and isolation of anaerobic bacteria was undermined in different studies but they were encountered in significant proportion (on average, one-third of the total number of microbial species in colonized wounds, and approximately 50% in infected wounds) of the microbial population in both acute and chronic wounds (Bowler *et al.*, 2001).

Wounds with anaerobic conditions like sufficiently hypoxic and reduced environment, especially deep wounds, are susceptible to colonization by a wide variety of endogenous anaerobic bacteria such as *Bacteroides*, *Prevotella*, *Porphyromonas*, and *Peptostreptococcus* spp., *Clostridium* spp. and others (Bowler *et al.*, 2001; European commission, 2006; Hauser *et al.*, 2006). Acute wound flora is similar to that of intact skin with aerobic and some anaerobic species whereas older infected wounds with associated tissue necrosis and deep structure involvement usually contain polymicrobial mixtures of aerobic and anaerobic pathogens (Kingsley, 2001; Dow, 2003).

Bacterial flora of open wounds is seldom static; it is usually changing due to the interaction between open wounds and bacteria at four successive levels: contamination, colonization, critical colonization and infection (Saini *et al.*, 2004; Fletcher, 2005; Howell-Jones *et al.*, 2005). Contamination and colonization by microbes may not inhibit healing (RDNS Research Unit, 2002; Fletcher, 2005; Howell-Jones *et al.*, 2005). 'Critical colonization' with a bacterial load $>10^5$ bacteria per gram of tissue is the stage at which bacteria begin to adversely affect or delay wound healing (Dow, 2003; Howell-Jones *et al.*, 2005; White and

Cutting, 2007). Most often, either too many replicating microbes or too many species in wound base having a detrimental effect on the host are important in the development of infection (Kingsley, 2001; Fletcher, 2005; White and Cutting, 2007). In complex extremity wounds, a critical level of bacteria that causes infection is $\geq 10^4$ CFU/g of tissue (Bowler *et al.*, 2001).

b. Risk factors in wound infection

Any wound is at some risk of becoming infected, but the progression to an infected state may involve a complex interaction of multiple factors related to bacteria, local environment and host defense mechanisms with the host response playing a key role (Bowler *et al.*, 2001; Taye, 2005; White and Cutting, 2007).

The factors related to multiplication of microorganisms in any wound infection include type, size, depth and site of wound, the level of blood perfusion to the wound tissue, the extent of nonviable exogenous contamination, the microbial load, and the combined level of virulence expressed by the types of the microorganisms and the antimicrobial efficacy of the host immune response (Bowler *et al.*, 2001; Kingsley, 2001). Those related to the patient are: sex and age, overweight, nutritive status, hypovitaminoses, chronic illness, infections, corticosteroid and immunosuppressive therapy (Kingsley, 2001; Soldatovic *et al.*, 2004; Bowen and Widmaier, 2005). For example, a suppressed immunity due to extreme ages, various diseases or nutritional status plays a very important role in the receptivity of infection with *Klebsiella* spp. (Purghel *et al.*, 2006).

The rate of infection of open fractures is affected by the fracture characteristics (especially, the location of the fracture with the infection rate for open tibial fractures being twice that for open fractures in other locations) and nature of treatment (antibiotic therapy variables) (Bowen and Widmaier, 2005; Stewart *et al.*, 2005; Zalavras *et al.*, 2007). The critical infective dose required to cause clinical infection is reduced in the presence of dead tissue and foreign bodies (Gristina and Costerton, 1985; Merritt and Dowd, 1987; Taye, 2005). They provide an environment suitable for microbial adherence, multiplication and point of entry to surrounding tissue, making it more prone to infection (Kingsley, 2001).

Lower extremities open fractures are more common today (Cole and Bhandari, 2005). Open fractures of the tibial shaft (especially, that of the distal third of the tibia) are common injuries with very often severe comminution, devitalization and contamination due to its superficial location and the subcutaneous characteristics of its anteromedial aspect (Clancey and Hansen, 1978; Ikem *et al.*, 2004). These fractures have the highest risk of infection and the most severe morbidity with established infection. Those in the upper extremities, particularly the hand, are less likely to become infected, and their established infections are generally easier to treat (Quinn and Macias, 2006).

Orthopaedic implants such as plates and screws influence susceptibility of wound tissues to infection through several mechanisms, including corrosion, adherence of biofilm, isolation from the immune response, resistance to antimicrobial therapy and compromise of blood supply making a conducive environment to chronic bacterial proliferation (Gristina and Costerton, 1985; Hendricks *et al.*, 2001; Soontornvipart *et al.*, 2003). Clinical reports have clearly shown that the presence of a biomaterial (a suture, internal fixation device, and others) and compromised tissue (dead bone or traumatized soft-tissue) make the adjacent tissues susceptible to both immediate and delayed infection (Gristina and Costerton, 1985). Factors that decrease the patient's general medical health are thought to increase the risk of complications after an open fracture (Bowen and Widmaier, 2005). However, a major risk factor for local infection is the extent of the soft-tissue and periosteal damage associated with the fracture (Khosravi *et al.*, 2009).

c. Common etiologic agents of open fracture wound infections

The majority of infections in open fractures are caused by Staphylococci (*S. aureus* and Coagulase negative staphylococci) and Gram-negative bacilli which include *Acinetobacter* spp., *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp. and others (Okike and Bhattacharyya, 2006; Quinn and Macias, 2006; Cat and Hall, 2007). Deep wound infection is most commonly caused by *S. aureus* and CoNS such as *S. epidermidis* (Fletcher *et al.*, 2007). *S. aureus* is the most common pathogen in orthopedic patients constituting about 60-70% of infecting organisms (Gustilo, 1979; Dietz *et al.*, 1991; Hendricks *et al.*, 2001). It has special receptors for adherence onto the bone being the most common pathogen in osteomyelitis (Ostermann *et al.*, 1993; Biruk and Wubshet, 2007).

Nosocomial organisms have emerged as the main source of open fracture infections in the developed world (Okike and Bhattacharyya, 2006). Pseudomonads and *Enterobacter* spp. are associated with hospital-acquired infection rather than initial contamination of the open fracture in the field (Ostermann *et al.*, 1993; Garazzino *et al.*, 2005). *Acinetobacter* spp. has recently emerged as a nosocomial pathogen and multiple drug resistant one which survives in dry environments is the most important one (Davis *et al.*, 2005; Dhawan *et al.*, 2005). *Acinetobacter* spp. are ubiquitous in the environment and transmitted through hands, clothing, contaminated surgical instruments, and air conditioning or ventilation devices (Davis *et al.*, 2005; Purghel *et al.*, 2006).

Fungi (especially, *Aspergillus* spp. and *Candida* spp.) may invade deep tissue and the bloodstream leading to complications, mainly mixed with bacterial infection (Mousa, 1999). Zygomycetes are found readily in soil, vegetation, and decaying matter and zygomycosis is the third leading cause of invasive fungal infection after candidiasis and aspergillosis (Koonce *et al.*, 2009). Nosocomial fungal infections can occur when patients' conditions deteriorate (Bolignano and Criseo, 2003). The presence of a prosthetic device or surgical wound may well predispose patients, even otherwise healthy individuals, to such infections (Cimerman *et al.*, 1999). A traumatically injured patient is more susceptible to local bacterial and fungal infection (Koonce *et al.*, 2009).

In general, Gram-positive bacteria [*S. aureus*, CoNS, *Streptococci* spp., *Clostridium* spp., *Bacillus cereus*, *Enterococci* spp., Diphtheroids, and others], Gram-negative bacteria [*Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, *Serratia marcescens*, Bacteroides, *P. aeruginosa*, *Stenotrophomonas maltophilia*, *Moraxella* spp., *Acinetobacter* spp., *Proteus* spp., *Escherichia coli*, *Aeromonas hydrophilia*, and others], and Fungi [Yeast, *Aspergillus*, and others] are commonly encountered in open fracture wound infection (Lee, 1997; Ikem *et al.*, 2004; Davis *et al.*, 2005).

d. Clinical relevance of microbial interactions and enhanced pathogenicity

The majority of open wounds are polymicrobial (Gristina and Costerton, 1985; Merritt and Dowd, 1987; Bowler *et al.*, 2001). Polymicrobial infections have been noted to be more virulent than infections caused by a single organism (Gristina and Costerton, 1985; Ikem *et al.*, 2004; Fletcher, 2005). For example, the presence of four or more groups of bacteria in

an open wound delays its healing (Scanlon, 2005). The ability of bacteria to act in concert to enhance pathogenicity is referred to as synergy (Hendricks *et al.*, 2001). Biofilms are stable mixed communities of microorganisms including aerobes and anaerobes which work synergistically exchanging nutrients and metabolites (Dow, 2003; Fletcher, 2005). The adherent mode of bacterial growth within a biofilm is ubiquitous and is the natural state of bacterial existence in biomaterial-related infections. There is a causal relationship between the natural, adherent biofilm mode of bacterial existence and the persistence of these infections (Gristina and Costerton, 1985). The pathogenicity of *S. aureus* was shown to be increased in the presence of anaerobic bacteria (Hendricks *et al.*, 2001). Aerobic bacteria promote anaerobic growth by inducing tissue hypoxia and reduced redox potential (Dow, 2003). Tissues with mixed Gram-negative and Gram-positive flora have an increased risk of infection since the presence of the Gram-positive organisms markedly increases the infection rate with the Gram-negative organisms (Merritt and Dowd, 1987).

Biofilms have decreased sensitivity to both antimicrobial agents and the host immunological mechanisms (Fletcher, 2005; Harris and Richards, 2006; Purghel *et al.*, 2006). Biofilm bacteria (such as *P. aeruginosa*, *S. aureus*, *S. epidermidis*) are typically enveloped in an extracellular polymeric substance or network which connects cells with one another and to the substratum (Soontornvipart *et al.*, 2003). For instance, polysaccharide intercellular adhesin found in many strains of *S. aureus* is responsible for the production of the extracellular polysaccharide matrix that makes up the biofilm (Figure 1.1). In addition, the biofilm enhances the expression of *S. aureus* virulence factors, such as the α -toxin which play an integral part in biofilm formation (Harris and Richards, 2006).



Figure 1.1 Scanning electron microscopy of a staphylococcal biofilm (Adapted from Harris and Richards, 2006).

e. Anaerobic conditions and anaerobic bacteria in open fracture wounds

Anaerobic conditions develop in deep wounds, in tissue necrosis and following simultaneous infection with other bacteria (European commission, 2006). Devitalized soft-tissues increase the risk of infection by acting as a culture medium for bacteria, by directly inhibiting leukocytic phagocytosis, and by providing an anaerobic environment that further inhibits leukocyte function. Necrotic muscle is the major medium for bacterial growth, and its presence increases the risk of anaerobic infection (Quinn and Macias, 2006). Wounds with a sufficiently hypoxic and reduced environment are susceptible to colonization by different types of anaerobic bacteria (Bowler *et al.*, 2001). Clostridial infections of open fractures were commonplace in wartime prior to antibiotic use, and they may still occur sporadically in heavily contaminated wounds where treatment is delayed or the wounds are closed primarily (Hauser *et al.*, 2006). *Clostridium perfringens* is the most commonly isolated *Clostridium* spp. (Health Protection Agency, 2006).

f. Virulence factors

Virulence factors expressed by one or more microorganisms in a wound out competes the host's natural immune system in infection (Bowler *et al.*, 2001; Harris and Richards, 2006). Adherence constitutes the first step of bacterial colonization (Carsenti-Etesse *et al.*, 1999; Hauser *et al.*, 2006). Gram-positive bacteria are known to adhere better to foreign material which may be predictive of their pathogenicity in relation to foreign material and in bone infections (Carsenti-Etesse *et al.*, 1999). Many of the bacteria that colonize the surfaces of clinical biomaterials grow in thick, adherent biofilms. Their adherence is probably a virulence factor (Gristina and Costerton, 1985).

Succinate (a short-chain fatty acid, or SCFA linked to chronic wound malodour) produced by aerobes and anaerobes may increase the risk of infection by impairing host-cell function (White and Cutting, 2007). *S. aureus* produces a large number of enzymes and toxins which can be classified as extracellular toxins (α -, β -, γ -, δ -haemolysin, and Panton-Valentine leukocidin, enterotoxin A-E), extracellular proteases (metalloprotease, serine proteases V8 (SspA)), and exfoliative toxins A and B (Harris and Richards, 2006; Purghel *et al.*, 2006). The golden color of *S. aureus* due to carotenoid pigments is also associated with its virulence (Harris and Richards, 2006). Almost all of species of *S. aureus* produce

β -lactamase (Purghel *et al.*, 2006). Many pathogens including *P. aeruginosa* induce inappropriate or premature apoptosis of immune cells such as macrophages and neutrophils (White and Cutting, 2007). It has long been noticed that occasional green coloration in chronic wounds is associated with the presence of *P. aeruginosa* and delayed healing. The green pigment, pyocyanin, has been shown to inhibit many cell functions and impair host defenses through apoptosis (White and Cutting, 2007).

1.2.3 Epidemiology of open fracture wound infection

Environmental and skin contamination of open fracture wounds is responsible for the type of microorganisms in the wounds (Alonge *et al.*, 2002). Carsenti-Etesse *et al.*, (1999), demonstrated that there is comparable distribution of Gram-positive and Gram-negative bacteria upon arrival at the emergency department and at the start of the operation; grade I and II fractures possessing bacteria similar to that usually observed in cutaneous flora.

Valenziano *et al.*, (2002), collected culture swabs from 32 grade I, 51 grade II, and 34 grade III consecutive open fractures of the extremities at the time of arrival at the trauma resuscitation area, prior to any antimicrobial administration. They found that 89/117 (76%) of the open fracture wounds were culture-negative and 28/117 (24%) only grew skin flora. However, 7/117 (6%) of the wounds developed infections finally. Of 7 infected open fracture wounds, 5/7 (71%) did not demonstrate any growth on primary cultures. In conclusion, they demonstrated that nosocomial pathogens such as *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Enterococcus* spp. are the culprits that lead to wound infection.

In another study reported by Sen *et al.*, (2000), it was observed that from 20 patients, in 4/20 (20%) the bacteria that were initially found became the final infective organisms. In 2/20 (10%) patients, initial *Acinetobacter* contamination ended in mixed bacterial infection. In other 2/20 (10%) patients having initial mixed bacterial contamination, infection occurred due to single bacteria. Only in one case were the infective bacteria different from the initially grown. The predominantly polymicrobial growth in the initial contamination had been followed by either polymicrobial or *Klebsiella* infection. Of 20 cases, 9/20 (45%) developed wound infections and the pathogens cultured were mixed flora in 4, *Klebsiella* in 3, and *Pseudomonas* and *S. aureus* in one each.

In a study by Johnson *et al.*, (2007), only 1/35 (2.9%) patient developed subsequent infection due to an organism isolated from the original cultures and the subsequent infections were much more likely to be due to Gram-positive organisms. Further, they stated that recurrent infections tend to be due to staphylococcal organisms. In general, the pathogens that were identified most frequently were *Acinetobacter*, *Enterobacter* spp., and *Pseudomonas aeruginosa*.

1.2.4 Clinical features of infected open fracture wounds

Wound infection is a serious complication associated with open extremity fractures (Gustilo and Anderson, 1976; Ostermann *et al.*, 1995; Valenziano *et al.*, 2002). Possible clinical indicators of wound infection include delayed wound healing, purulent discharge, green, yellow or brown exudates, increased amount of exudates, offensive odour, inflammation and erythema including cellulitis (may be except in persons with diabetes), hypergranulation of tissue, elevated body temperature, lethargy, increased or unusual pain (may be except in persons with diabetes), confusion, elevated blood glucose level in persons with diabetes and leukocytosis (Heier *et al.*, 2003; Anglen, 2005; Mansell, 2005). The common signs of wound infection are erythema, pain, drainage, and fever $>38.5^{\circ}\text{C}$ (Patzakis and Wilkins, 1989; Heier *et al.*, 2003; Anglen, 2005).

1.2.5 Immunity

The effects of immune modulation secondary to systemic trauma or to the fracture injury itself, combined with the continued presence of injured or devitalized tissues make open fracture wounds liable to invasion by nosocomial bacteria. Under such conditions, opportunistic organisms resistant to antimicrobials will always invade the tissues (Hauser *et al.*, 2006). With impaired blood supply in the zone of injury, the body's immune system is compromised (Azam *et al.*, 2007).

During the first 2 hours, the host defense works to decrease the overall bacterial load. During the next 4 hours the number of bacteria remains fairly constant, with the bacteria that are multiplying and those that are being killed by the host defense being about equal. After the first 6 hours, invading organisms (bacteria), in the presence of abundant necrotic tissues, replicate in logarithmic fashion to establish a clinical infection. Therefore, a contaminated

wound is considered infected after 12 hours. The time limit is shorter in a severely contaminated wound with severe soft-tissue injury (Azam *et al.*, 2007).

1.2.6 Laboratory diagnosis

The standard for determination of whether a bacterial infection is present is isolation and identification of bacteria on Gram stain or by culture (Patzakis and Wilkins, 1989; Dietz *et al.*, 1991; Zalavras *et al.*, 2007). It is known that the moist swab provides a direct and simple method for ascertaining infection (Bowler *et al.*, 2001). Probably, sampling a part of wound with the most dramatic signs of infection is the best approach (Dow, 2003).

Wound specimens should be cultured for both aerobic and anaerobic microorganisms (Bowler *et al.*, 2001; Zalavras *et al.*, 2007). A single microorganism in a Gram-stained smear prepared from wound swab and swabs that yield more than 30 colonies on culture plate both reliably predict a microbial load of $>10^5$ CFU/g of tissue (Bowler *et al.*, 2001). A failure to isolate infection causing organism from infected wound may be due to poor microbiological technique or a sampling error, particularly in the case of anaerobes (Lee, 1997; Bowler *et al.*, 2001).

a. Collection and transport of wound swab

Wound swab from the depth of fracture wound should be collected using a sterile cotton applicator. The specimen can be transferred into transport media and delivered to the microbiology laboratory as soon as possible (Cheesbrough, 2004).

b. Microscopic examination

A Gram-stained smear from a wound swab requires less than 10 minutes to prepare (Levine *et al.*, 1976). Visualization of bacteria on the smear by microscopic examination indicates that 10^6 or more bacteria per swab are present and reliably predicts a microbial load of $>10^5$ CFU/g of tissue (Levine *et al.*, 1976, Bowler *et al.*, 2001).

c. Culture

Blood, MacConkey and chocolate agar can be used as primary isolation media for Gram-positive and Gram-negative bacteria, respectively. The wound specimen can be inoculated on these media and incubated appropriately at 35-37°C overnight in appropriate gaseous atmosphere. Blood agar is mainly used for isolation of *S. aureus*, *S. pyogenes* and *S. pneumoniae*. MacConkey agar is appropriate for isolation of Gram-negative bacteria such as *E. coli*, *Proteus* spp. and *P. aeruginosa*, whereas chocolate agar is used for isolation of *H. influenzae*, particularly if the specimen is obtained from children (Cheesbrough, 2004).

1.2.7 Treatment and prevention of wound infection in open fractures

Patients who sustain high-energy extremity trauma in general and lower-limb trauma in particular benefit from treatment or care of trauma-center (Stewart *et al.*, 2005; MacKenzie *et al.*, 2008). All patients presenting with an open fracture require initial stabilization, tetanus prophylaxis, systemic antibiotic therapy, prompt surgical debridement and copious irrigation, fracture stabilization, timely wound closure, thorough rehabilitation, and adequate follow-up (Okike and Bhattacharyya, 2006). The generally appreciated golden period that open soft-tissue injuries should be dealt with is a maximum of 8 hours of their occurrence (Ahmed and Chaka, 2006b).

A meticulous surgical technique can alter the degree of endogenous contamination and make the local factors unfavorable to infection playing a central role in prevention (Taye, 2005). Therefore, thorough operative debridement should be considered the standard of care for all open fractures (Okike and Bhattacharyya, 2006; Azam *et al.*, 2007). Preservation of the bone's blood supply plays a key role in the resistance of fracture wounds to infection (Hauser *et al.*, 2006; Quinn and Macias, 2006). In infection prevention it is necessary to pay attention to the monitoring of the patient, operation rooms, the qualification of the surgical staff, appropriate surgical techniques, the duration of the operative procedure, postoperative care of the patient, and appropriate use of antibiotics in prophylaxis (Soldatovic *et al.*, 2004; Purghel *et al.*, 2006; Fletcher *et al.*, 2007). Improvements in air flow and ultraviolet lighting in operation rooms reduce not only bacterial counts but also rates of wound infection (Taye, 2005; Purghel *et al.*, 2006; Fletcher *et al.*, 2007). The number of bacteria shed by operating

room personnel can be decreased by using air exhaust systems or completely covering bacteria-shedding areas including ears and beards (Fletcher *et al.*, 2007).

Clinical management of open fractures improved from the life preservation era of limb amputation to the functional preservation era of rehabilitation of the involved extremity (Okike and Bhattacharyya, 2006; Cat and Hall, 2007). Preventing the devastating infection of bone and soft-tissues is through early surgical debridement and irrigation, immediate antibiotic administration, partial wound closure, soft-tissue transfer and delayed wound closure (for grade III injuries) and fracture stabilization (Hauser *et al.*, 2006; Zalavras *et al.*, 2007; Petrisor *et al.*, 2008). The antimicrobials should be of broad spectrum to cover both Gram-positive and Gram-negative bacteria (Lee, 1997; Johnson *et al.*, 2007; Zalavras *et al.*, 2007). They should be given as soon as possible, preferably within 3 hours after the injury because delay >3 hours increases the risk of infection (Holtom, 2006; Quinn and Macias, 2006; Zalavras *et al.*, 2007). The use of antibiotics must be restricted to 24 hours for grades I and II, and to 48-72 hours after the last surgical procedure for grade III, though this duration is somewhat empiric (Anglen, 2005; Hauser *et al.*, 2006; Quinn and Macias, 2006). According to the existing trauma literature, the only effect of more prophylactic antibiotics for more than 24 hours is a higher incidence of bacterial resistance (Velmahos *et al.*, 2002; Holtom, 2006).

There is currently controversy with regard to the specific antimicrobial agent(s) to be given after open fracture (Okike and Bhattacharyya, 2006). However, there is adequate evidence to suggest that antimicrobial agents with activity against *S. aureus* (i.e., first-generation cephalosporin) would be appropriate for type I and II open fractures whereas administration of antimicrobials with additional broader Gram-negative coverage (aminoglycosides, usually gentamicin) in conjunction with the Gram-positive antimicrobials is recommended for severely contaminated type III open fracture wounds (Holtom, 2006; Quinn and Macias, 2006; Cat and Hall, 2007). The lowest infection rate was seen with the combination of antimicrobials that offered both Gram-positive and Gram-negative coverage; especially in children who receive early antibiotic therapy following an open fracture (Patzakis and Wilkins, 1989; Skaggs *et al.*, 2005). Penicillin, or metronidazole or ampicillin should be added when there is a high risk of anaerobic bacterial infection (Holtom, 2006; Okike and

Bhattacharyya, 2006; Quinn and Macias, 2006). Interventions, such as nutritional support and cessation of smoking, will help optimize the patient's condition (Zalavras *et al.*, 2007).

Tetanus prophylaxis should be provided to any patient who does not have documentation of vaccination (Stewart *et al.*, 2005; Quinn and Macias, 2006; Cat and Hall, 2007). Anaerobic infections (especially clostridial myonecrosis) should be considered in injuries occurring on farms and in the case of a vascular injury where ischemia results from reduced oxygenation of tissue (Holtom, 2006; Okike and Bhattacharyya, 2006; Cat and Hall, 2007).

Conservative treatment with short course of anti-Gram-positive prophylaxis may be adequate for low-risk open fractures (Heier *et al.*, 2003; Soldatovic *et al.*, 2004). Antibiotic use in gunshot injuries is controversial but there is a consensus about it in high-velocity gunshot wounds (Necmioglu *et al.*, 2005; Holtom, 2006). Prophylactic antibiotics have no clinical value in the treatment of open finger fractures and are not recommended to be routinely used in the management of acute hand injuries unless high-energy gunshot wounds (Suprock *et al.*, 1990; Hauser *et al.*, 2006).

Certain patients (like those with a type-IIIB open tibial fracture) may benefit from local antibiotic therapy (Ostermann *et al.*, 1993; Okike and Bhattacharyya, 2006). This is done by application of antibiotic-laden-polymethylmethacrylate beads (Figure 1.2).



Figure 1.2 Tobramycin-polymethylmethacrylate beads applied in a type-IIIB tibial open fracture wound (Adapted from Ostermann *et al.*, 1995)

Polymethylmethacrylate (PMMA) beads combined with systemic antimicrobials may provide added benefit to control the local infection by increasing local antibiotic concentrations (often 10 to 20 times higher than concentration provided by systemic administration) (Holtom, 2006). This approach minimizes systemic toxicity and seals the wound from the external environment with seemingly low adverse reaction rates (Wininger and Fass, 1996; Purghel *et al.*, 2006; Cat and Hall, 2007). The use of a carrier that will release the antibiotic for a longer period and consequently prolong its effect would be helpful for the treatment of chronic osteomyelitis (Lesic *et al.*, 2004). With this regard, the use of PMMA bead chains is well established (Ostermann *et al.*, 1993).

In conclusion, the management of open fractures should include appropriate evaluation of the patient and classification of open fractures, early application of antibiotic therapy targeting Gram-positive and Gram-negative organisms, and most importantly, thorough surgical debridement and copious irrigation with appropriate wound closure timing (Davis *et al.*, 2005; Stewart *et al.*, 2005; Cat and Hall, 2007). Lastly, a study by Fakoor and Pipelzadeh, (2007) highlighted beneficial effect of honey on the healing of infected open fracture wounds that were unresponsive to conventional antimicrobial treatment.

1.2.8 Antimicrobial resistance

Resistance to antimicrobial drugs in bacteria can result from two mutually non exclusive phenomena: mutations in housekeeping structural or regulatory genes and the horizontal acquisition of foreign genetic information (Courvalin, 2005). The rapid spread of antimicrobial resistance in a wide variety of bacteria is mainly due to the location of antimicrobial resistance genes on mobile genetic elements such as plasmids and transposons (Gebreselassie, 2002).

Enterobacter isolates resistant to expanded-spectrum cephalosporins are becoming a matter of concern for the possibility of transmitting antimicrobial resistance from one microorganism to another worldwide. Outbreaks of infections due to *Klebsiella pneumoniae* harboring plasmid encoded cephalosporinases and the spread of this resistance mechanism to bacterial species naturally susceptible to cephamycins have been reported (Garazzino *et al.*, 2005).

An infection engrafted on a biomaterial (thick, adherent biofilm) responds poorly to antimicrobial therapy and usually is not cured until the biomaterial is removed. Isolates may not be entirely representative of the microbial components of the biofilm due to the fact that the coherent properties of the adherent biofilms that are found on surfaces in these infections may prevent truly representative organisms from detaching in sufficient numbers to be detected completely and consistently by simple sampling and routine culture techniques. Therefore, antimicrobials that are chosen on the basis of the culture results will not be effective against all of the bacterial species in these biofilm infections (Gristina and Costerton, 1985).

1.2.9 Significance of the study

Patients with open fractures are likely to develop an infection at varying rates up to 50% (Ikem *et al.*, 2004). The rate is two to three times higher in developing countries than the developed ones (Taye, 2005).

In developing countries like Ethiopia, antibiotic resistance among bacteria is a serious problem with increasing drug resistant strains (Woldetenssaie, 2001; Gebreselassie, 2002; Taye, 2005). This might be a reflection of inappropriate use of the existing antimicrobials due to unavailability of guideline regarding selection of drugs (Mulu *et al.*, 2006). To help physician's choice of appropriate antimicrobials, up-to-date information on local prevailing strains (pathogens) and their drug sensitivity pattern is very crucial to treat patients (Lindtjorn *et al.*, 1989; Mulu *et al.*, 2006).

It is easy to count resistance rates of bacteria documented at the hospital laboratory. But, patients whose wound specimen is sent to the laboratory are those with chronic wound infections that have failed to respond to empirical treatment. As the result, biased sample with exaggerated magnitude of resistance is highly possible (Belihu and Lindtjorn, 1999).

In Ethiopia, there is no documented report on the bacteriology of open fracture wounds. Therefore, this study was undertaken to determine the bacterial etiologies of open fracture wound infection and their antimicrobial susceptibility pattern in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. Tikur Anbessa University Hospital is a tertiary referral hospital giving service for patients referred from different parts of the country.

OBJECTIVES OF THE STUDY

General objective

- To determine the bacteriology of compound (open) fracture wounds from patients visiting the Orthopaedic Surgery Department at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Specific objectives

- To isolate and identify the bacterial etiologic agents responsible for compound (open) fracture wound infection.
- To determine the antimicrobial susceptibility pattern of the bacterial isolates to the commonly used antimicrobial agents.

CHAPTER II: PATIENTS AND METHODS

2.1. Study Design, Area, and Period

During the period of November 2007 and May 2008 a cross-sectional prospective study was conducted at Orthopaedic Surgery Department of Tikur Anbessa University Hospital (TAUH), Addis Ababa, Ethiopia. TAUH represents the highest tertiary level for referred patients in the country. It has 560 beds and is located in Lideta sub-city, Addis Ababa, Ethiopia. The hospital has different departments and the orthopaedic surgery department is the only specialty training department offering orthopedic services in the country. It receives referred and some directly visiting patients from all parts of the country and provides emergency service. There are 67 beds for orthopaedic admissions (12% of the total), of which 49 are for adults and 18 for children. In addition to 18 children's beds which are largely reserved for elective surgery, all other children with orthopaedic emergencies are 'admitted' to the casualty ward. The department holds a fracture follow-up clinic four days a week.

2.2. Source Population

A total of 1247 patients with orthopaedic problem were seen at Orthopaedic Surgery Department of TAUH. Of these, 330 (26.5%) were clinically diagnosed to have compound (open) fractures. Out of 330 patients, 191 (57.9%) informed and consented patients with a total of 200 open fracture wound episodes with or without overt signs of infection were enrolled in the study. The patients were assessed by history taking, physical examination and bone imaging result by attending physician.

Working definition of open fracture wound infection

Open fracture is fracture that communicates with the outside environment through a skin wound (Hauser *et al.*, 2006).

A patient is considered to have an open fracture wound infection when clinical signs and symptoms of infection, such as fever $>38.5^{\circ}\text{C}$, erythema, tenderness, pain, and wound drainage are present along with either a positive Gram stain or a positive culture (Patzakis *et al.*, 1974; Heier *et al.*, 2003; Mansell, 2005). Delay in wound healing and offensive odour are also associated with infected wounds (RDNS Research Unit, 2002).

Sample Size Determination

The sample size (n) was calculated for a desired power of 0.8 and an α value of 0.05 by taking the prevalence of open and/or complicated fractures (13.7%) in the orthopaedic and major limb trauma patients who attended the 'surgical' emergency department of TAUH in a three-year period between December 2001 and November 2004 and who received treatment necessitating at least one further visit (Ahmed and Chaka, 2006a). The expected margin of error (d) was 0.05 and the confidence interval ($Z\alpha/2$) was 95%. Contingency for the unknown circumstance was 10%. The calculation for appropriate sample size was performed according to the following formula:

$$n = \frac{(Z\alpha/2)^2 \times P(1-P)}{d^2} = \frac{(1.96)^2 \times 0.137(0.863)}{(0.05)^2} = 181.7 + 10\% = 181.7 + 18.2 = 199.9 \approx 200$$

Where: Z = z value (1.96 for 95% confidence interval), p = percentage picking a choice, expressed in decimal (13.7% = 0.137)

Demographic, clinical and other relevant data were obtained by attending physician and were transferred to the questionnaire prepared for this study by the principal investigator (see appendix I).

2.3. Bone Imaging

Plain radiographs/Bone X-ray images were taken from each patient to confirm the fracture and determine the fracture patterns. Radiologist and/or orthopedic surgeon interpreted the imaging results.

2.4. Wound Bed Preparation

Wound beds were prepared before specimen collection by using Levine's technique (Levine *et al.*, 1976), where the wound surface is cleansed of surface exudates and contaminants with a moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with non-bacteriostatic sterile normal saline after removing the dressing. This technique is believed to be the best technique for swabbing open wounds and more reflective of tissue bioburden than swabs of exudate or swabs by other techniques (Gardner *et al.*, 2007).

Cleansing the wound prior to obtaining swab specimens was done in an effort to remove immediate surface contaminating organisms (bacteria). The culture was more likely to represent the microbiology in the deep wound compartment (Gardner *et al.*, 2007).

2.5. Sample Collection, Handling and Transport

As part of Levine's technique, the end of a sterile cotton-tipped applicator was rotated over a 1 cm² area for 5 seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue (Levine *et al.*, 1976; Gardner *et al.*, 2007). The applicators were applied deep into the wounds in order to avoid contaminants that are usually found on the surface of the wounds.

Samples were taken from some patients at the time of arrival at the trauma resuscitation area, and also from inpatients and outpatients attending fracture follow-up clinic.

Multiple (three) wound swabs were taken from each compound fracture wound at a point in time to reduce the chance of occurrence of false-negative cultures and to increase the chance of recovering bacterial pathogens. The results of culture were considered positive when the same microorganism was isolated in at least two of the three samples (swabs) as described by Bori *et al.* (2007).

Specimens were placed in Amies transport medium (Oxoid Ltd, UK) and transported to bacteriology laboratory within an hour. Some of the specimens collected during night were kept at 4°C overnight until analysis.

2.6. Microscopic Examination

Gram staining was performed from the wound swabs according to standard procedures. The morphological and Gram characters of the bacteria and the presence of bacterial spore in wound specimens were recorded. It reveals the types and relative numbers of microorganisms, and serves to assess the quality of clinical specimen and to interpret culture findings.

2.7. Culture and Identification

All wound specimens were inoculated on blood agar (for Gram-positive bacteria), mannitol salt agar (selective media for *S. aureus*), chocolate (for *Haemophilus* spp.) and MacConkey agar (for Gram-negative bacteria) (Oxoid, Ltd., Basingstoke, Hampshire, England). The

plates were incubated in aerobic, microaerophilic and anaerobic atmosphere at 37°C for 24-48 hrs. Candle jar was used for microaerophilic atmosphere. Anaerobic atmosphere was achieved by using gas generating kits (Oxoid). All positive cultures were identified by their characteristic appearance on their respective media, Gram staining reaction and confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2004). Members of the family enterobacteriaceae and other Gram-negative rods were identified by indole production, H₂S production, citrate utilization, motility test, urease test, carbohydrate utilization tests and other tests using API 20E identification kits (Biomérieux, France). For Gram-positive bacteria, coagulase, DNase, catalase, bacitracin and optochin susceptibility tests, and other tests were used.

The specimens were cultured semiquantitatively and colony counts were performed before identification. Colony count <5 was considered as contamination; 5-15, colonization; 16-30, critical colonization; and >30, infection. Cultures with <5 CFUs were considered as simple contaminants with the exception of *S. aureus* and Gram-negative rods (Dietz *et al.*, 1991).

2.8. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed for all isolates by disk diffusion method according to the criteria set by the Clinical and Laboratory Standards Institute (CLSI, 2006) (formerly known as National Committee for Clinical Laboratory Standards / NCCLS).

From a pure culture, 3-5 selected colonies of bacteria were taken and transferred to a tube containing 5 ml TSB and mixed gently until a homogenous suspension was formed and incubated at 37°C until the turbidity of the suspension becomes adjusted to a McFarland 0.5. A sterile cotton swab was used and the excess suspension was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar and blood agar (Oxoid, Ltd., Basingstoke, Hampshire, England). Mueller-Hinton agar was used for all Gram-negative and Gram-positive bacteria, except *Clostridium* spp. and Streptococci. The sensitivity test of *Clostridium* spp. and Streptococci was performed on blood agar.

The drugs tested were in the following concentrations: amoxicillin (AML) (25 µg), amoxicillin-clavulanic acid (AMC) (30 µg), ampicillin (AMP) (10 µg), ceftriaxone (CRO)

(30 µg), chloramphenicol (C) (30 µg), ciprofloxacin (CIP) (5 µg), clindamycin (DA) (2 µg), cloxacillin (OB) (5 µg), doxycycline (Do) (30 µg), erythromycin (E) (15 µg), gentamicin (CN) (10 µg), kanamycin (K) (30 µg), methicillin (MET) (5 µg), norfloxacin (NOR) (10µg), penicillin (P) (10 units), tetracycline (TE) (30 µg), and trimethoprim-sulphamethoxazole (SXT) (25µg).

Gram-positive bacteria other than *Clostridium* spp. were tested against amoxicillin, amoxicillin-clavulanic acid, ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, cloxacillin, erythromycin, gentamicin, methicillin, norfloxacin, penicillin, tetracycline, trimethoprim-sulphamethoxazole.

Clostridium spp. were tested against chloramphenicol, clindamycin, doxycycline, kanamycin, penicillin, and tetracycline.

All Gram-negative bacteria were tested against amoxicillin, amoxicillin-clavulanic acid, ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, norfloxacin, tetracycline, and trimethoprim-sulphamethoxazole.

The plates were then incubated in aerobic, microaerophilic and anaerobic atmosphere for 24-48 hrs with respect to the organism tested. Diameters of the zone of inhibition around the disc were measured using a graduated caliper in millimeters, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI (CLSI, 2006). The percentage of resistance was defined as high (>80%), intermediate (60-80%) and low (< 60%).

2.9. Reference Strains

P. aeruginosa (ATCC-27853), *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922) were used as a quality control throughout the study for culture and antimicrobial susceptibility testing. All the strains were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

2.10. Data entry and Analysis

Data entry was done by using EpiInfo-2002 software and analysis was done using both EpiInfo-2002 software and SPSS version 13.0 for windows. Pearson chi-square and Fisher exact tests analysis were used to compare categorical variables. The level of significance

was set at 0.05 in order to consider a p-value <0.05 as indicator of a statistically significant difference with 95% confidence.

2.11. Ethical Considerations

The M.Sc research project was approved by the Department of Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia. It was ethically cleared by the Faculty Research Publications Committee-II (FRPC-II) and endorsed by the Faculty Academic Commission. Official permission was obtained from the study site.

The study was explained to the patients/guardians, and written consent for participation in the study was obtained prior to collecting the wound specimens (see Appendix II).

CHAPTER III: RESULTS

3.1. Source Population

Regarding time of arrival to TAUH, 121 (63.3%) patients arrived within the golden period (8 hours), 67 (35.1%) arrived after 8 hours of their injury and the time of arrival of 3 (1.6%) patients was unknown (Table 3.1).

Table 3.1. Time of arrival, address and pattern of admission of 191 patients with open fracture wounds (November 2007 to May 2008)

		Time of Arrival after Injury			Total (%)
		≤8 hours	>8 hours	Unknown	
Address	A.A	88	17	3	108 (56.5)
	Oromiya	29	32	0	61 (32.0)
	Other	4	18	0	22 (11.5)
Total (%)		121 (63.3)	67 (35.1)	3 (1.6)	191 (100.0)
Admitted	Yes	16	19	1	36 (18.9)
	No	105	48	2	155 (81.2)
Total (%)		121 (63.3)	67 (35.1)	3 (1.6)	191 (100.0)

Most of the fractures were caused by high-energy trauma as follows: car accidents (RTA) 71 (37.2%), assault or interpersonal violence 29 (15.2%), bullet (gunshot) injuries 28 (14.7%), crushes by heavy objects 24 (12.6%), machine injuries 23 (12.0%), falls from a height 9 (4.7%) and others 7 (3.7%) (Table 3.2).

Seven patients had two open fracture wounds each, constituting 14 compound fracture wounds and one had three. Single open fracture per patient occurred in the remaining 183 patients. Out of the 200 open fracture wounds, 55 (27.5%) were with overt signs of infections (data not shown).

Table 3.2 Demographic data of the patients with open fracture wounds (November 2007 to May 2008)

Age group in yrs*	Sex	Car accident	Assault	Bullet injury	Heavy object	Machine injury	Fall accident	Others ^x	Total (%)
0-12	M*	4	-	-	3	-	-	-	7
	F*	2	-	-	-	-	-	-	2
	T*	6	-	-	3	-	-	-	9 (4.7)
13-24	M	15	7	9	10	5	1	1	48
	F	7	-	2	-	-	1	-	10
	T	22	7	11	10	5	2	1	58 (30.4)
25-36	M	15	6	9	7	10	5	-	52
	F	8	1	-	-	3	-	-	12
	T	23	7	9	7	13	5	-	64 (33.5)
37-48	M	7	8	5	3	4	-	2	29
	F	3	-	1	-	-	-	-	4
	T	10	8	6	3	4	-	2	33 (17.3)
49-60	M	5	3	2	1	-	1	3	15
	F	3	1	-	-	-	-	-	4
	T	8	4	2	1	-	1	3	19 (9.9)
≥61	M	2	3	-	-	1	-	1	7
	F	-	-	-	-	-	1	-	1
	T	2	3	-	-	1	1	1	8 (4.2)
Total	M	48	27	25	24	20	7	7	158 (82.7)
	F	23	2	3	0	3	2	0	33 (17.3)
	T	71	29	28	24	23	9	7	191 (100.0)
	(%)	(37.2)	(15.2)	(14.7)	(12.6)	(12.0)	(4.7)	(3.7)	

*M = Male *F = Female *T = Total yrs* = years Others^x = 2 each Blast injuries and Ox accidents; and 1 each Motor cycle injury, Cart injury and Cut by iron sheet

One hundred and ninety-one patients with a total of 200 compound fracture wounds were included in the study between November 2007 and May 2008. As shown in Table 3.2 and Figure 3.1, 158 (82.7%) were males and 33 (17.3%) were females ($p < 0.05$) resulting in an overall male to female ratio of 4.8:1. The average age of the patients was 31.55 years (age

range 4 to 75 years). Surprisingly, 131 (68.6 %) of the patients were in the age group of 20-50 years. Only 36 (18.8%) had been admitted for fracture stabilization and/or wound care mainly due to shortage of available orthopaedic beds (Table 3.1).

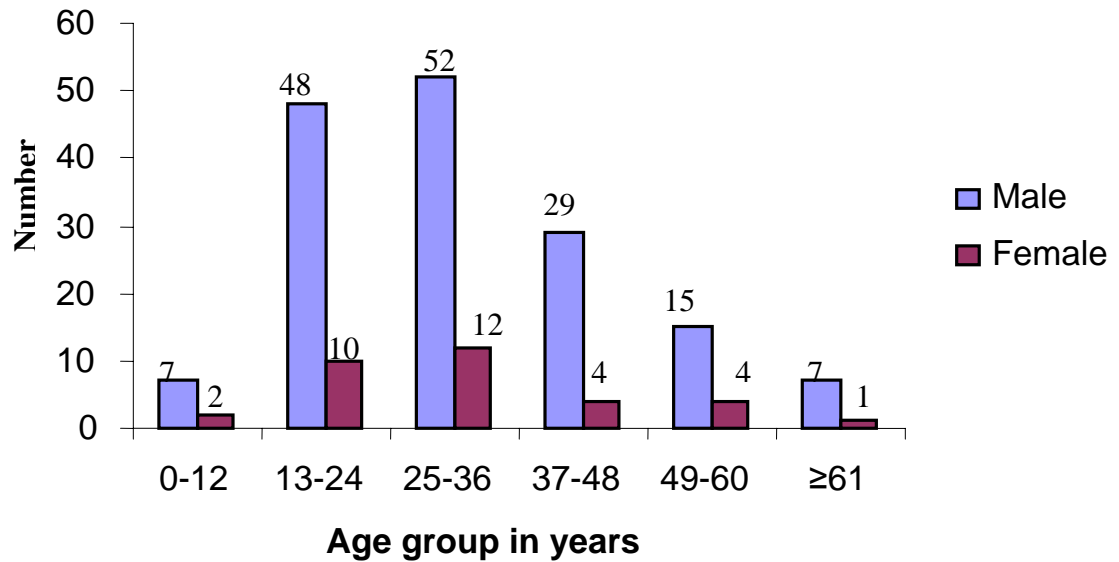


Figure 3.1 Age and sex distribution of the patients with compound fractures investigated for wound infection at TAUH, Addis Ababa, Ethiopia (November 2007 to May 2008)

3.2. Gustilo and Anderson (G-A) Grading of Open Fractures

The modified G-A classification of open fractures based on severity and extent of soft-tissue injury was used to categorize the open fractures. Accordingly, 46 (23.0%) of the fractures were grade I, 83 (41.5%) were grade II, 28 (14.0%) were grade IIIA, 11 (5.5%) were grade IIIB, and 32 (16.0%) were grade IIIC as shown in Figure 3.2.

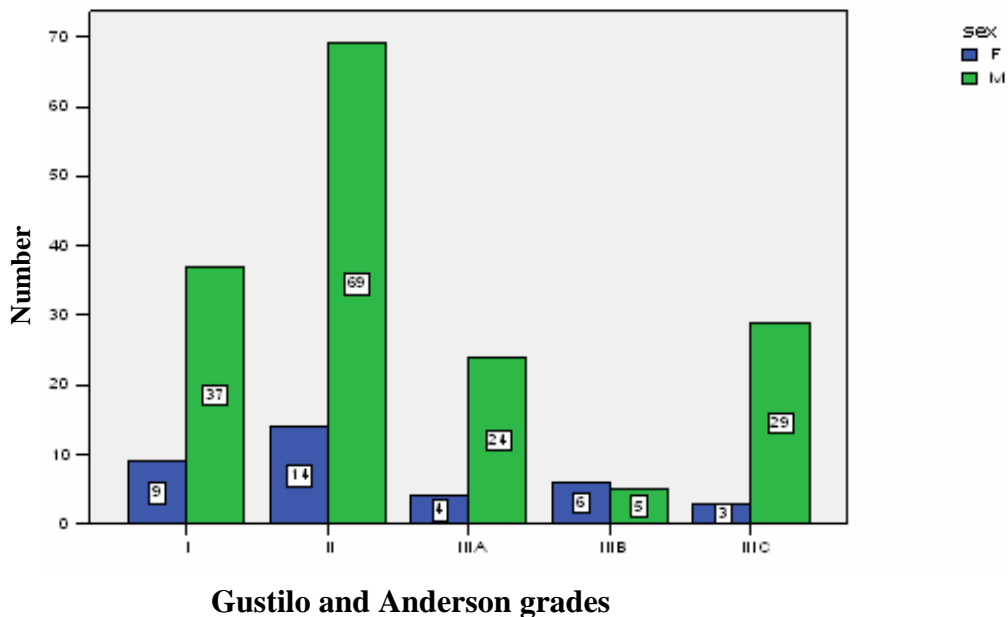


Figure 3.2. Gustilo and Anderson grading of compound fractures seen at TAUH (November 2007 to May 2008)

3.3. Fracture Patterns

Most fractures occurred in tibia/fibula (37.9%), followed by hands/metacarpals (23.2%), radius/ulna (12.3%), femur (10.4%), foot /metatarsals (9 %), humerus (3.8%), ankle joint (1.9%), elbow joint (0.9%) and patella (0.5%) (Table 3.3). Most of the fractures (60.0%) occurred in lower extremities and the remaining (40.0%) occurred in upper extremities as shown in Figure 3.3. The majority of the open fracture wounds occurred on the left side (52.5%). The different causes of the open fractures were presented in Table 2.1.

Table 3.3 Skeletal sites and frequency of the fractured bones (November 2007 to May 2008)

Fractured bones	Frequency	Percentage
Tibia/Fibula	80	37.9
Hand/metacarpals	49	23.2
Radius/ulna	26	12.3
Femur	22	10.4
Foot/metatarsals	19	9.0
Humerus	8	3.8
Ankle joint	4	1.9
Elbow joint	2	0.9
Patella	1	0.5
Total	211	100.0

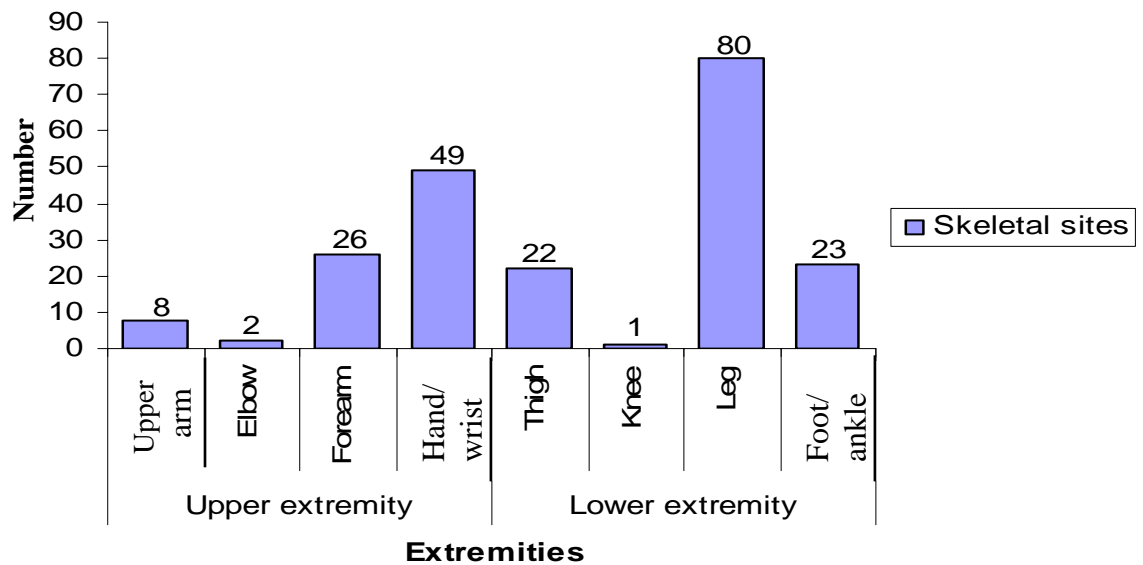


Figure 3.3. Patterns of fractures in upper and lower extremities (November 2007 to May 2008)

3.4. Clinical Features

Out of the 200 open fracture wounds, 127 (63.5%) were deep and wide wounds and 55 (27.5%) were with overt signs of clinically important infection (erythema, pain, drainage, fever $>38.5^{\circ}\text{C}$ and foul odour). Foul odour was typical feature of older wounds.

3.5. Wound Management and Time of Sample Collection

Only 26 (13%) of the wounds were irrigated and surgically debrided mainly in admitted patients. The rest were simply washed with sterile normal saline and iodine or H_2O_2 solutions and dressed during fracture stabilization at emergency room. In some of the patients (16.0%), the wounds were primarily closed by stitching either before their presentation or immediately after presentation. The majority of fractures (89.0%) associated with the soft-tissue wounds were stabilized in an acceptable time interval after injury but without opening window for wound care in most open fractures stabilized by plaster of Paris.

The sampling time of the 200 open fracture wounds after the time of injury was as follows: 79 (39.5%) of the wounds were swabbed within 8 hours of the injury; 43 (21.5%) of them were swabbed between 9 and 24 hours; and 78 (39.0%) of them were swabbed after 24 hours of the injury (data not shown). The delay in sampling was not intentional. It was

related either to delay of presentation of the patients to the hospital or due to some inconveniences made after the presentation of the patients.

3.6. Microscopic Examination

Of the 200 wound specimens examined by Gram stain, 61 (30.5%) were positive for the presence of bacteria. Different bacterial morphologies (Gram-positive cocci, Gram-positive bacilli, Gram-positive spore forming bacilli, Gram-negative cocci, Gram-negative coccobacilli, and Gram-negative bacilli) were observed.

3.7. Culture

Of the 200 wound specimens cultured, 82 (41%) were culture positive. Of these, 42/82 (51.2%) showed mono-microbial growth (single bacterial type) and 40/82 (48.8%) showed polymicrobial (more than one bacterial type) growth. In general, a total of 162 bacteria were isolated from the culture-positive wounds as shown in Table 3.4. *S. aureus* accounted for 14.8% of the total isolates followed by *Acinetobacter* spp. (*A. calcoaceticus-baumannii* complex) (11.4%), *E. coli* (10.5%), *Pseudomonas* spp. (*P. aeruginosa* and *P. fluorescens/putida*) (9.9%), *Enterobacter* spp. (*E. cloacae*, *E. aerogens*, *E. sakazaki* and *E. amnigenus*) (9.3%), CoNs and *Klebsiella* spp. (*K. pneumoniae*, *K. ornithinolytica* and *K. oxytoca*) each with an incidence of 7.4%. Other bacteria accounted for 29.3%. Anaerobic bacteria, *Clostridium* spp. (*C. perfringens* and *C. tetani*) were also isolated. The Gram-positive and Gram-negative bacteria accounted for 55/162 (34.0%) and 107/162 (66.0%), respectively ($p < 0.05$).

Table 3.4 Bacteria isolated from 200 compound fracture wounds investigated at TAUH, A.A, Ethiopia (November 2007 to May 2008)

Bacterial isolates	Lower extremities	Upper extremities	Total
	No. (%)	No. (%)	No. (%)
<i>Staphylococcus aureus</i>	15 (9.3)	9 (5.6)	24 (14.8)
<i>Acinetobacter</i> species	15 (9.3)	3 (1.9)	18 (11.4)
<i>Escherichia coli</i>	12 (7.4)	5 (3.1)	17 (10.5)
<i>Pseudomonas</i> species	9 (5.6)	7 (4.3)	16 (9.9)
<i>Enterobacter</i> species	11 (6.8)	4 (2.5)	15 (9.3)
CoNS	12 (7.4)	- -	12 (7.4)
<i>Klebsiella</i> species	7 (4.3)	5 (3.1)	12 (7.4)
<i>Clostridium</i> species	6 (3.7)	2 (1.2)	8 (4.9)
<i>Citrobacter</i> species ^a	2 (1.2)	4 (2.5)	6 (3.7)
<i>Proteus</i> species ^b	5 (3.1)	1 (0.6)	6 (3.7)
<i>Aeromonas</i> species ^c	1 (0.9)	4 (2.5)	5 (3.1)
<i>Erwinia</i> species	- -	3 (1.9)	3 (1.9)
<i>Bacillus cereus</i>	- -	2 (1.2)	2 (1.2)
Diphtheroids	2 (1.2)	- -	2 (1.2)
Enterococci (Group D)	1 (0.6)	1 (0.6)	2 (1.2)
Non-group A Streptococci	2 (1.2)	- -	2 (1.2)
<i>Morganella morganii</i>	2 (1.2)	- -	2 (1.2)
<i>Providencia rettgeri</i>	2 (1.2)	- -	2 (1.2)
<i>Streptococcus pyogenes</i>	- -	1 (0.6)	1 (0.6)
Viridans (α) Streptococci	- -	1 (0.6)	1 (0.6)
<i>Micrococcus</i> species	1 (0.6)	- -	1 (0.6)
<i>Alcaligenes</i> species	1 (0.6)	- -	1 (0.6)
<i>Stenotrophomonas maltophilia</i>	1 (0.6)	- -	1 (0.6)
<i>Burkholderia cepaciae</i>	- -	1 (0.6)	1 (0.6)
Actinobacillus	1 (0.6)	- -	1 (0.6)
Photobacterium-like bacteria	- -	1 (0.6)	1 (0.6)
Total	108 (66.7)	54 (33.3)	162 (100.0)

^a*C. braakii*, *C. koseri/farneri* and *C. freundii*; ^b*P. mirabilis* and *P. pennerii*; ^c*A. hydrophilia* and *A. sobria*

3.8. Use of Antimicrobials and Culture Outcome

Of the 191 patients, 56 (29.3%) had been treated with cloxacillin alone or in combination with other antimicrobials (mainly ceftriaxone) before collection of samples. Of the patients who received antimicrobial/s, 40/56 (71.4%) had positive culture results, while those who did not receive any antimicrobial had 42/135 (31.1%) positive culture results ($p < 0.05$).

3.9. Antimicrobial Susceptibility

a. Gram-positive bacteria

The susceptibility patterns of Gram-positive bacteria ($n = 47$) other than *Clostridium* spp. isolated from the compound fracture wounds against 14 antimicrobial agents are presented in Table 3.5. All isolates showed low level of resistance ($< 60\%$) to all antibiotics tested except for ampicillin and penicillin to which they showed intermediate level of resistance (60-80%). Most Gram-positive isolates, 29/55 (52.7%) showed multiple drug resistance (resistance to three or more drugs) (data not shown).

The susceptibility pattern of *Clostridium* spp. ($n=8$) is presented in Table 3.6. All are susceptible (100%) to tetracycline, doxycycline, and kanamycin. Low level of resistance ($< 60\%$) was observed against chloramphenicol, clindamycin and penicillin.

In general, amoxicillin-clavulanic acid, chloramphenicol, erythromycin, gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested Gram-positive bacteria with exception of *Clostridium* spp. (Tables 3.5).

Table 3.5. Susceptibility Patterns of Gram-positive Bacteria Isolated from open fracture wounds (November 2007 to May 2008)

Organisms		Antimicrobial agents (%)													
		AMP	AMC	C	E	CN	OB	MET	P	AML	TE	SXT	CRO	NOR	CIP
<i>Staphylococcus aureus</i> (n = 24)	S*	16.7	70.8	79.2	87.5	87.5	75.0	75.0	20.8	41.7	58.3	75.0	91.7	79.2	58.3
	I*	-	4.2	8.3	4.2	-	8.3	4.2	-	20.8	-	-	-	4.2	25.0
	R*	83.3	25.0	12.5	8.3	12.5	16.7	20.8	79.2	37.5	41.7	25.0	8.3	16.7	16.7
CoNS (n = 12)	S	58.3	91.7	75.0	83.3	83.3	66.7	66.7	41.7	58.3	66.7	91.7	66.7	83.3	66.7
	I	8.3	8.3	8.3	8.3	-	16.7	-	-	33.3	-	8.3	25.0	8.3	25.0
	R	33.3	-	16.7	8.3	16.7	16.7	33.3	58.3	8.3	33.3	-	8.3	8.3	8.3
<i>Bacillus cereus</i> (n = 2)	S	-	-	50.0	100.0	100.0	-	50.0	-	-	-	50.0	-	100.0	50.0
	I	-	-	50.0	-	-	-	-	-	-	100.0	-	-	-	50.0
	R	100.0	100.0	-	-	-	100.0	50.0	100.0	100.0	-	50.0	100.0	-	-
Diphtheroids (n = 2)	S	50.0	50.0	-	-	100.0	-	-	-	50.0	-	-	-	-	50.0
	I	-	-	-	-	-	-	-	-	-	50.0	50.0	-	50.0	-
	R	50.0	50.0	100.0	100.0	-	100.0	100.0	100.0	50.0	50.0	50.0	100.0	50.0	50.0
Enterococci (Group D) (n = 2)	S	100.0	100.0	50.0	-	50.0	50.0	50.0	50.0	100.0	50.0	100.0	-	-	-
	I	-	-	50.0	100.0	-	-	-	-	-	-	-	-	50.0	50.0
	R	-	-	-	-	50.0	50.0	50.0	50.0	-	50.0	-	100.0	50.0	50.0
Non group A Streptococci (n = 2)	S	50.0	50.0	50.0	100.0	-	-	-	-	50.0	50.0	-	-	50.0	-
	I	-	50.0	-	-	50.0	-	-	-	50.0	-	-	-	50.0	100.0
	R	50.0	-	50.0	-	50.0	100.0	100.0	100.0	-	50.0	100.0	100.0	-	-
<i>Streptococcus pyogenes</i> (n = 1)	S	100.0	-	100.0	-	100.0	-	-	-	100.0	100.0	-	-	-	-
	I	-	100.0	-	-	-	-	-	100.0	-	-	-	-	100.0	-
	R	-	-	-	100.0	-	100.0	100.0	-	-	-	100.0	100.0	-	100.0

		AMP	AMC	C	E	CN	OB	MET	P	AML	TE	SXT	CRO	NOR	CIP
Viridans (α) Streptococci (n = 1)	S	100.0	100.0	100.0	100.0	100.0	-	-	-	100.0	100.0	100.0	100.0	-	100.0
	I	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-
	R	-	-	-	-	-	100.0	100.0	100.0	-	-	-	-	-	-
<i>Micrococcus</i> species (n = 1)	S	100.0	100.0	100.0	100.0	100.0	-	100.0	-	100.0	100.0	100.0	100.0	100.0	100.0
	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	-	-	-	-	-	100.0	-	100.0	-	-	-	-	-	-
Total (n = 47)	S	38.3	72.3	72.3	78.7	83.0	57.5	61.7	23.4	51.1	57.5	72.3	68.1	70.2	55.3
	I	2.1	8.5	10.6	8.5	2.1	8.5	2.1	2.1	21.3	6.4	4.3	6.4	14.9	27.7
	R	59.6	19.2	17.0	12.8	14.9	37.2	36.2	74.5	27.7	36.2	23.4	25.5	14.9	17.0

*S = Sensitive *I = Intermediate *R = Resistant

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; C: Chloramphenicol; E: Erythromycin; CN: Gentamicin; OB: Cloxacillin; MET: Methicillin; P: Penicillin; AML: Amoxicillin; TE: Tetracycline; SXT: Trimethoprim-sulphamethoxazole; CRO: Ceftriaxone; NOR: Norfloxacin; CIP: Ciprofloxacin

Table 3.6 Susceptibility Patterns of *Clostridium* spp. isolated from open fracture wounds (November 2007 to May 2008)

Organisms		Antimicrobial agents (%)					
		TE	C	DA	DO	P	K
<i>Clostridium</i> species (n=8)	S*	100.0	75.0	62.5	100.0	87.5	100.0
	I*	-	-	-	-	-	-
	R*	-	25.0	37.5	-	12.5	-

*S = Sensitive

*I = Intermediate

*R = Resistant

TE: Tetracycline; C: Chloramphenicol; DA: Clindamycin; DO: Doxycycline; P: Penicillin; K: Kanamycin

b. Gram-negative bacteria

The susceptibility patterns of Gram-negative bacteria (n =107) isolated from the compound fracture wounds against 10 antimicrobial agents are presented in Table 3.5. All isolates showed low level of resistance (<60%) to all antimicrobials tested except for ampicillin and amoxicillin (60-80%, intermediate level resistance). Of the 107 Gram-negative isolates, 55 (51.4%) strains were also identified as multiple drug resistant (data not shown).

In general gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested Gram-negative bacteria (Tables 3.7).

Table 3.7 Susceptibility Patterns of Gram-Negative Bacteria Isolated from open fracture wounds (November 2007 to May 2008)

Organisms	S	Antimicrobial agents (%)									
		AMP	AMC	C	CN	AML	TE	SXT	CRO	NOR	CIP
<i>Flavobacterium</i> <i>rettingeri</i> (n = 2)	I	-	-	-	-	-	100.0	-	-	-	-
Organisms	R	50.0	100.0	C	CN	50.0	TE	SXT	CRO	NOR	CIP
	S	16.7	22.2	50.0	88.9	16.7	100.0	100.0	100.0	100.0	100.0
	I	-	44.4	33.4	-	22.2	22.2	-	22.2	27.8	11.1
<i>Acinetobacter</i> <i>Alcaligenes</i> species (n = 18)	R	83.3	33.0	100.0	100.0	100.0	22.2	11.1	55.6	33.4	11.1
	S	29.4	52.9	58.8	76.5	23.5	47.1	70.6	88.2	94.1	94.1
	I	29.4	23.5	-	11.8	29.4	11.8	-	-	-	100.0
<i>Escherichia coli</i> <i>Stenotrophomonas maltophilia</i> (n = 1)	R	100.0	100.0	41.2	11.8	100.0	100.0	29.4	100.0	100.0	5.9
	S	6.3	18.8	100.0	75.0	6.3	25.0	100.0	25.0	87.5	100.0
	I	6.3	-	6.3	12.5	12.5	100.0	-	43.8	-	6.3
<i>Pseudomonas</i> <i>cepaciae</i> species (n = 16) (n = 1)	R	100.0	100.0	75.0	12.5	100.0	56.3	68.8	100.0	100.0	-
	S	-	33.3	60.0	80.0	100.0	100.0	66.7	66.7	100.0	86.7
	I	100.0	20.0	13.3	-	13.3	53.3	6.7	13.3	6.7	6.7
<i>Enterobacter</i> <i>Actinobacterius</i> species (n = 15) (n = 1)	R	93.3	46.7	100.0	20.0	86.7	13.3	26.7	20.0	-	6.7
	S	-	66.7	75.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	I	100.0	100.0	100.0	-	33.3	8.3	-	8.3	16.7	25.0
<i>Klebsiella</i> <i>Proteomabodus</i> like bacteria (n = 12) (n = 1)	R	100.0	33.3	25.0	25.0	66.7	33.3	25.0	25.0	-	-
	S	15.0	38.9	56.0	83.2	15.7	48.6	66.7	83.3	100.0	83.9
	I	49.3	14.0	10.3	3.7	54.3	19.6	0.9	13.1	7.5	16.7
Total (n = 107) species (n = 6)	R	65.7	49.9	35.6	15.1	60.7	31.8	39.0	24.3	11.2	4.7
	S	33.3	83.3	50.0	100.0	33.3	16.7	50.0	100.0	100.0	100.0
	I	-	-	-	-	33.3	-	-	-	-	-
<i>Proteus</i> species (n = 6)	R	66.7	16.7	50.0	-	33.3	83.3	50.0	-	-	-
	S	-	-	100.0	100.0	-	100.0	100.0	100.0	100.0	100.0
	I	20.0	-	-	-	40.0	-	-	-	-	-
<i>Aeromonas</i> species (n = 5)	R	80.0	100.0	-	-	60.0	-	-	-	-	-
	S	66.7	66.7	100.0	100.0	66.7	100.0	100.0	100.0	100.0	100.0
	I	-	-	-	-	33.3	-	-	-	-	-
<i>Erwinia</i> species (n = 3)	R	33.3	33.3	-	-	-	-	-	-	-	-
	S	-	-	-	100.0	-	50.0	50.0	100.0	50.0	50.0
	I	-	-	50.0	-	-	-	-	-	-	-
<i>Morganella</i> <i>morganii</i> (n = 2)	R	100.0	100.0	50.0	-	100.0	50.0	50.0	-	50.0	50.0

AMP AMC C CN AML TE SXT CRO NOR CIP

CHAPTER IV: DISCUSSION

Open fractures are always associated with soft-tissue injury (Carsenti-Etesse *et al.*, 1999; Zalavras *et al.*, 2007). Wound and bone infections mainly occur in higher G-A grades (Carsenti-Etesse *et al.*, 1999; Heier *et al.*, 2003; Stewart *et al.*, 2005). The complications of open fractures increase with age of the patient (Stewart *et al.*, 2005; Hauser *et al.*, 2006; Zalavras *et al.*, 2007).

There is no previous published report concerning the bacteriology of open/compound fracture wounds in Ethiopia. To address the problem, this study was undertaken to isolate and identify the bacterial etiologic agents in compound fracture wounds and their antimicrobial susceptibility pattern from orthopaedic patients visiting TAUH, Addis Ababa, Ethiopia.

In the present study, patients aged between 20 and 50 years (the active productive age group within the society) accounted the majority of cases (68.6%) with open fractures as shown in Table 3.2 and Figure 3.1. Ikem *et al.*, (2004), reported similar finding in a study conducted in Ile-Ife, Nigeria. Males were affected more than females in this study as shown in Figure 3.1. This is in agreement with a report by Fakoor and Pipelzadeh, (2007). This might be explained by the fact that traditionally, in this country, mainly males are involved in some occupations such as the transportation industry and construction works. Farmers and daily laborers are mostly men with very few exceptions. Males are commonly involved in assault or interpersonal violence. Consequently, more than 50% of the injury caused by road traffic accident (RTA) and interpersonal violence affected mainly males particularly those involved in economically important occupations in agricultural, transport, construction and other industries.

The leading cause of open fractures in our setting (especially Addis Ababa) is RTA which alone contributed to 37.2% of the causes of the open fractures in this study (Table 3.2). Similar findings have been reported from Nigeria (Ikem *et al.*, 2004) and India (Azam *et al.*, 2007). This might be explained by high number of vehicles and over crowded roads in Addis Ababa. Assault or interpersonal violence being the second most important cause of open fractures affected 15.2% of the patients. This finding coincides with finding reported from north Gondar administrative zone, North West Ethiopia (Osman *et al.*, 2003). Most of

the bullet injuries which caused open fractures in 14.7% of our patients were also part of the interpersonal violence.

In the present study, most of the fractures (60.0%) occurred in lower extremities (Table 3.3 and Figure 3.3). A study conducted in Iran also showed that 89.2% of patients with open fracture wounds had suffered from injury of lower extremities (Fakoor and Pipelzadeh, 2007).

The Gustilo and Anderson grades I, II, and IIIC were the predominant types of the open fractures in this study (Figure 3.2). Grade II open fractures were the most dominant ones (41.5%). This is similar to a study reported by Ikem *et al.*, (2004), from Nigeria.

Some (27.5%) of the compound fracture wounds in this study showed overt or recognizable signs of infection such as erythema, pain, drainage, and fever $>38.5^{\circ}\text{C}$. Almost all of these wounds, especially those with foul odour yielded significant amount of the bacterial isolates, particularly the polymicrobial ones. This is comparable to a report from USA (Lawrence *et al.*, 1978). Foul odour was typical feature of older wounds. This might be the sign of the wound infection either by anaerobic or polymicrobial mixture of bacteria.

In the present investigation, only 13% of the wounds were irrigated and surgically debrided mainly in admitted patients. The rest were simply washed with sterile normal saline and iodine or H_2O_2 solutions and dressed during fracture stabilization at emergency room. Meticulous wound management and irrigation with copious fluid are essential for the care of all patients with open fracture wounds. Necrotic tissue and other contaminating materials from the accident site should be debrided. The aim is to reduce the bacterial load and increase the chance of early wound closure (Gustilo *et al.*, 1990).

Of the 200 wound specimens examined by Gram stain, 61 (30.5%) were positive for the presence of bacteria with different morphologies. The presence of a bacterial cell in the Gram stain indicated the presence of bacterial pathogens in the specimens (Levine *et al.*, 1976).

The total bacterial isolation rate from the compound fracture wounds in this study was 41%. This is slightly lower than that (45%) reported from Chandigarh, India by Sen *et al.*, (2000) and 45.8% reported from Ile-ife, Nigeria by Ikem *et al.*, (2004). Different factors related to

wound bed preparation, sample collection, sample transportation and culturing technique might have an effect in the reduction of the bacterial isolation rate. In addition, those bacteria which were not uniformly isolated from the multiple (three) swabs were categorized as false-positive.

In this study colonial counts were considered to differentiate pathogens from contaminants. Colony count interpretation was as follows:- <5 CFUs (contamination); 5-15 CFUs (colonization); 16-30 CFUs ('critical' colonization); and >30 CFUs (infection) (data not shown). Cultures with <5 CFUs were ignored being considered as simple contaminants with the exception of *S. aureus* and Gram-negative rods. The finding of small number of colonies in these organisms may be due to inadequate or recent antibiotic treatment and the presence of high level of inhibitory substance in the culture media (Dietz *et al.*, 1991; Silletti *et al.*, 1997). On the other hand, small numbers of culturable organisms might signify clinically important infection in the presence of large foreign-body implants (Dietz *et al.*, 1991). One cannot infer that only those wounds with positive cultures are at risk (Patzakis *et al.*, 1974). Specimens taken from clinically infected wounds that yield no growth suggest the possibility of a false-negative result (Kingsley, 2001). In general, quantitative bacterial counts are useful in managing open fractures. If the quantitative bacterial count is greater than 10^5 at any one time, it should be taken as a predictor of infection. Then, further medical intervention should be considered prior to definitive fracture care and soft tissue coverage.

The main bacterial isolate in open fracture wounds in this study was *S. aureus* as shown in Table 3.4. This is in agreement with previous studies conducted at different places in Ethiopia that showed that *S. aureus* was the most prevalent bacterial isolates in most clinical samples including wound samples (Tewodros and Gedebou, 1983; Belihu and Lindtjorn, 1999; Ahmed and Chaka, 2005; Biruk and Wubshet, 2007; Woldetenssaie, 2000; Gebreselassie, 2002; Mulu *et al.*, 2006).

The predominating prevalence of *S. aureus* in wounds in general and compound fracture wounds have been also reported in other developing and developed countries like USA (Gustilo and Anderson, 1976; Clancey and Hansen, 1978; Davis *et al.*, 1986), France (Carsenti-Etesse *et al.*, 1999), UK, England (Bowler *et al.*, 2001; Kingsley, 2001; Health Protection Agency, 2007), Ile-Ife, Nigeria (Shittu *et al.*, 2003), Lagos, Nigeria (Onche and

Adedeji, 2004), Belgrade (Soldatovic *et al.*, 2004), India (Dhawan *et al.*, 2005), Brazil (Fontes *et al.*, 2005), Davos, Switzerland (Harris and Richards, 2006), Romania (Purghel *et al.*, 2006), and Iran (Khosravi *et al.*, 2009).

In the present investigation, *Acinetobacter* spp. including *A. calcoaceticus-baumannii* complex were the second most frequently isolated bacteria. Similar findings have been reported on war wound infection and infection of war-related fractures respectively in Brooke Army Medical Center (BAMC), Texas, USA (Davis *et al.*, 2005; Johnson *et al.*, 2007).

No *H. influenzae* was isolated in our study. It is known that *H. influenzae* cellulitis occurs in children predominantly between the ages of 1 and 16 (Health Protection Agency, 2006; Health Protection Agency, 2007). Nowadays because of a successful vaccination campaign invasive *H. influenzae* infections have become rare (Health Protection Agency, 2007).

In this study, the predominant (66.0%) isolates of the compound fracture wounds were Gram-negative bacteria compared to Gram-positive ones (34.0%) from culture-positive compound fracture wounds (Table 3.2). This is in agreement with a study done in USA (Patzakis *et al.*, 1974). A recent study by Khosravi *et al.* (2009) also showed that aerobic Gram-positive bacteria accounted for 33.5%, aerobic Gram-negative bacteria for 64.5% and anaerobes for 1.9%. Findings from another study done in Musgrave Park Hospital and the Ulster Hospital, Northern Ireland (McNally *et al.*, 1993) reported that most cultures had a Gram-negative isolate and multiple organisms were invariably found in culture-positive cases.

The Gram-negative (66%) to Gram-positive (34%) bacterial proportion in our findings disagrees with reports from Minnesota, USA (40% vs. 60%) (Gustilo and Anderson, 1976), Indian tertiary care hospital, India (47% vs. 53%) (Dhawan *et al.*, 2005) and Gondar teaching hospital, Ethiopia (29% vs. 71%) (Mulu *et al.*, 2006). The observed difference can be mainly explained by the high proportion of G-A grade III wounds with some older or chronic ones due mainly to the unusually high number of bullet injury. It is also noted that bacterial prevalence differs in different environments (Lee, 1997).

In this study, 51.2% of culture-positive wounds showed mono-microbial growth and 48.8% showed polymicrobial growth. Similarly, Johnson *et al.*, (2007), (BAMC, USA) reported

that Gram-positive bacteria were less frequently recovered and 37% were polymicrobial infections. A report from USA also showed that 40% specimen's yielded more than one organism (Lawrence *et al.*, 1978).

Culturing wound swabs for both aerobic and anaerobic microorganisms is recommended (Bowler *et al.*, 2001; Zalavras *et al.*, 2007). Anaerobic organisms remain important isolates where such cultures are feasible (Onche and Adedeji, 2004).

Few anaerobes, predominantly *Clostridium* spp. mainly *C. perfringens* were isolated from patients who developed gas gangrene and others. They were isolated from polymicrobial mixture with facultative anaerobic bacteria. Isolation of anaerobic bacteria in this study was a difficult task because of poor laboratory set up for anaerobic culture.

The profile of the bacterial isolates in our study comparatively agrees with findings that have been observed in Nigeria (Onche and Adedeji, 2004), India (Saini *et al.*, 2004; Dhawan *et al.*, 2005), Romania (Purghel *et al.*, 2006), and Iran (Khosravi *et al.*, 2009).

In this study, 29.3% of the patients had been treated with cloxacillin alone or in combination with other antimicrobials (mainly ceftriaxone) before collection of samples. Of these, 71.4% had positive culture results. The possible explanation for high culture positivity rate could be mainly due to bacterial resistance for prophylactically administered antimicrobial/s (Patzakis *et al.*, 1974; Hauser *et al.*, 2006). In addition, this also shows the rational use of some antibiotics alone or in combination, requires periodic evaluation and the establishment of antimicrobial policy for prophylaxis and treatment guidelines in the Ethiopian setting.

The study also provides insights into the susceptibility profile of bacteria isolated from open fracture wounds. In the present investigation, all Gram-positive bacterial isolates with the exception of *Clostridium* spp. showed low level of resistance (<60%) to all antimicrobials tested except for ampicillin and penicillin to which they showed intermediate level of resistance (60-80%) as shown in Table 3.5. All Gram-negative bacterial isolates showed low level of resistance (<60%) to all antimicrobials tested except for ampicillin and amoxicillin (60-80%, intermediate level resistance) (Table 3.7). In general gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested Gram-positive and Gram-negative bacteria (Table 3.5 and 3.7). This is in agreement with reports from Ile-Ife,

Nigeria (Ikem *et al.*, 2004), Lagos, Nigeria (Onche and Adedeji, 2004), and Ahwaz University of Medical Sciences teaching hospitals, Iran (Khosravi *et al.*, 2009).

All *Clostridium* spp. were found to be susceptible to most antimicrobial agents tested as shown in Table 3.6. Similar findings have been reported elsewhere (Carsenti-Etesse *et al.* 1999; Saini *et al.*, 2004).

In this study, MDR was defined as resistance to three or more drugs (Davis *et al.*, 2005). It was significantly high in both Gram-positive (52.7%) and Gram-negative (51.4%) bacteria. Particularly, 1.8% of *S. aureus* and 1.9% of *Acinetobacter* spp. isolates were resistant to all the tested antimicrobials. Similar observations were reported by Davis *et al.*, (2005) and Johnson *et al.*, (2007).

Many factors have contributed to such level of resistance, including misuse of antimicrobials by health professionals, unskilled practitioners and laypersons. In Ethiopia, it is a common practice that antimicrobials can be purchased without prescription, which leads to misuse of antimicrobials by the public thus contributing to the emergence and spread of antimicrobial resistance. Other causal factors can be poor drug quality, poor hospital hygienic conditions accounting for the spread of resistant bacteria, and inadequate surveillance, i.e. lack of information from routine antimicrobial susceptibility testing of bacterial isolates and surveillance testing of bacterial isolates and surveillance of antibiotic resistance, all of which are crucial for good clinical practice and for rational policies against antibiotic resistance (Okeke *et al.*, 1999).

Microbial drug resistance is a growing global problem. In Gram-negative bacteria, the most resistant pathogens are *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. and *P. aeruginosa*, with increasing trends observed for all major anti-Gram-negative agents (beta-lactams, fluoroquinolones and aminoglycosides) (Rossolini *et al.*, 2007).

Serious infections caused by Gram-positive bacteria are increasingly difficult to treat because of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae*. The more recent emergence of vancomycin intermediate and -resistant MRSA (VISA and VRSA) has further compromised treatment options (Menichetti, 2005). The detection of multidrug resistant isolates may further limit therapeutic options.

LIMITATIONS OF THE STUDY

- Fracture grading was mostly done in the emergency room being at risk of misclassifications due mainly to our poor facility.
- Wound size determination and measuring of its depth were not done due to different inconveniences.
- Few of the wound swabs were taken after the application of local anesthesia which may have antibacterial effect and cause misleading wound culture results.
- The work done on anaerobes was highly limited due to budget, time, and laboratory set up constraints.
- This study design lacked follow-up and did not include fungi.
- Blood cultures were not done due to lack of follow-up until patients develop septicaemia.
- Quantitative swab cultures were not possible mainly because they are more complex and expensive.
- Aetiology of osteomyelitis could not be assessed since swab cultures are ineffective in this respect.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, one hundred and ninety-one patients with a total of 200 compound fracture wounds were enrolled in the present study between November 2007 and May 2008. Road traffic accident was the major cause of open fractures. Most of the fractures (60.0%) occurred in lower extremities. Majority of the fractures (41.5%) were grade II according to modified G-A classification of open fractures. Signs of infection of the open fracture wounds such as erythema, pain, drainage with odour, and fever $>38.5^{\circ}\text{C}$ were associated with significant amount of the bacterial isolates, particularly the polymicrobial ones. Of the 200 wound specimens examined by Gram stain, 30.5% were positive for the presence of bacteria. In general, 41% of the compound fracture wounds were culture-positive. *S. aureus* was the dominant isolate. The Gram-positive and Gram-negative bacteria accounted for 34.0% and 66.0%, respectively. Anaerobic bacteria, *Clostridium* spp. were also isolated. Ciprofloxacin, norfloxacin and gentamicin were the most effective drugs against the tested Gram-positive and Gram-negative bacteria. MDR (resistance to three or more drugs) was significantly high in both Gram-positive (52.7%) and Gram-negative (51.4%) bacteria. Based on these findings the following recommendations were made: -

- Collecting multiple swabs at a point in time and culturing polymicrobial specimens are relevant in compound fracture wound microbiology.
- The value of the Gram stain as a quick and inexpensive additional or alternative test is also worthy of consideration.
- Routine cultures either before or after debridement should not be done in the absence of clinical signs of infection in open fracture wounds.
- Anaerobic organisms remain important isolates where such cultures are feasible and hence penicillin or metronidazole should be a component of the antimicrobial regimen otherwise.
- Fungal culture is worthy of attention in open fracture wound microbiology.
- Guidelines have to be developed to help standardize the care of orthopedic patients with open fractures.
- Anaerobic culture facilities should be improved in order to provide additional information on anaerobic bacteriology of compound fracture wounds in TAUH.

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Appendix I: Questionnaire

Questionnaire for investigation of the bacteriology of open fracture wounds in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia

I. Patient Identification

- 1. Serial No.....
- 2. Patient name.....
- 3. Age..... 4. Sex (M/F).....
- 5. Address.....
- 6. Occupation.....
- 7. Ward (for inpatients).....
- 8. Reason for admission of inpatients.....
.....
.....

II. Clinical Data

- 9. Date and time of injury.....
- 10. Date and time of arrival to the emergency room of SOPD.....
- 11. Time of first antibiotic administration in the Hospital.....
- 12. Bone/s involved

Upper extremities

Humerus: Right / left / both

Ulna: Right / left / both

Radius: Right / left / both

Hand bones: Right / left / both

Lower extremities

Femur: Right / left / both

Tibia: Right / left / both

Fibula: Right / left / both

Foot bones: Right / left / both

If other anatomic sites, specify.....
.....

13. Cause/s of fracture (circle appropriate letter):

- a. Gunshot (Bullet injury)
- b. Fall accident
- c. Car accident (RTA)
- d. Cycle accident (including motor cycle)
- e. Fight accident (assault)
- f. Industrial accident
- g. Collapsed building
- h. Other causes, specify.....

14. Duration of fracture.....
15. Associated with wound infection? Yes / No
16. Signs of wound infection: Swelling / Discharge / exudates / others.....

17. Other pertinent physical findings.....
18. History of antibiotic treatment? Yes / No
- If yes, specify the type of antibiotic/s used.....

III. Classification of open fracture during debridement

19. Date and time of surgical procedures.....
20. Gustilo and Anderson classification (circle the appropriate letter)
- a. Grade I: Clean wound of <1 cm in length without significant soft-tissue damage. The skin wound is usually caused by puncture of a bone fragment from within.
 - b. Grade II: Soft-tissue disruption of <10 cm without periosteal stripping. The skin wounds are usually caused by laceration by an external object.
 - c. Grade III A: Extensive soft-tissue destruction with adequate osseous coverage
 - d. Grade III B: Extensive soft-tissue destruction with periosteal stripping which requires a vascularized tissue transfer for soft-tissue coverage
 - e. Grade III C: Extensive soft-tissue destruction with neurovascular injury

that requires repair

21. Type of wound closure.....
22. Mode of fracture fixation (stabilization).....

IV. Radiological findings with sign of infection (circle the appropriate letter)

23. a. No
- b. Yes

- Bone resorption
- Periosteal elevation
- Presence of sequestrum
- Presence of involucrum
- Other findings, specify.....
.....
.....

V. Laboratory Data

24. Specimen: Wound / blood / both
25. Type of wound specimen: Swab / aspirated material / exudate / bone / others.....
26. Date and time of collection.....
27. Bacterial colony counts.....
28. Type of bacteria isolated.....
29. Antimicrobial susceptibility testing
- | | S (mm) | I (mm) | R (mm) |
|---------------|--------|--------|--------|
| • Ampicillin | ---- | ---- | ---- |
| • Amoxicillin | ---- | ---- | ---- |

• Amoxicillin-Clavulanic acid	----	----	----
• Ceftriaxone	----	----	----
• Kanamycin	----	----	----
• Ciprofloxacin	----	----	----
• Chloramphenicol	----	----	----
• Clindamycin	----	----	----
• Cloxacillin	----	----	----
• Erythromycin	----	----	----
• Gentamicin	----	----	----
• Penicillin	----	----	----
• Norfloxacin	----	----	----
• Doxycycline	----	----	----
• Trimethoprim-sulphamethoxazole	----	----	----
• Tetracycline	----	----	----
• Methicillin	----	----	----

S: Sensitive I: Intermediate R: Resistant

VI. Comments _____

Name of principal investigator _____

Signature _____ Date _____

Appendix II. Consent Form

I have been informed that this study is planned to determine the pattern of bacterial isolates and their antimicrobial sensitivity profile in open fractures. In addition, factors increasing the risk of infections in open fractures will be analyzed. I am also informed that the information I give is transferred to the questionnaire prepared for this study and will be kept confidential. It is therefore with the understanding of the objective of this study that I allow specimens required for the study (wound swab/discharge/biopsy) or blood specimens if septicaemia develops. I am also informed that laboratory results of culture and sensitivity will be reported to orthopaedic surgeon/resident attending me for appropriate treatment and management. Nevertheless, I was assured that I have full right to withdraw from this study and that it will never affect my right of getting appropriate treatment.

I _____ here by give my consent to be involved in this study giving the requested information and allowing samples (wound specimens/blood) as the doctors find it best for me.

Signature: _____ Date _____

የስምምነት መጠየቂያ ቅጽ

የዚህ ጥናት ዓላማ በአደጋ ምክንያት ከሚደርስ ስብራት ጋር ተያያዥነት ባላቸው ቁስሎች ላይ መመረዝን “infection” የሚያስከትሉ ባክቴሪያዎችን መለየትና በሀኪሞች ለሚታዘዙ መድኃኒቶች የሚያሳዩትን ተጋላጭነት “susceptibility” ማየት መሆኑን ተረድቻለሁ። በተጨማሪም የቁስሎችን መመረዝ የሚያባብሱ ነገሮችም በዚህ ጥናት እንደሚፈተሹ ተገንዝቧል። ከዚህም ባሻገር እኔ የሚሰጣቸውና ለዚህ ጥናት በተዘጋጀው የቃለ መጠይቅ ቅጽ የሚሞሉ መረጃዎች በምስጥር እንደሚጠበቁ ተነግሮኛል።

ስለሆነም የዚህ ጥናት ዓላማ ስለገባኝ ለላቦራቶሪ ምርመራ ከቁስሌ ላይ የሚወሰደውን ናሙና እና በደም ውስጥ የሚገኝ በሽታ በሚፈጠርብኝ ጊዜ የደም ናሙና ለመስጠት ፈቃደኛ መሆኔን እገልጻለሁ። የላቦራቶሪው ውጤት ለሚያክመኝ ሀኪም ሪፖርት እንደሚደረግና ተገቢውን ህክምና እንዳገኝ አስተዋፅኦ እንደሚያደርግ ተገልጾልኛል። በጥናቱ ያለመተባበርና እንዲሁም ከጥናቱ የመውጣት መብቴ የተጠበቀ መሆኑ የተገለፀልኝ ሲሆን ይህንንም በማድረግ ምክንያት የሕክምናዬ ሂደት የማይደናቀፍ መሆኑን በሚገባ ተረድቻለሁ።

እኔ _____ ሀኪሙ የተሻለ እንደሆነ እስካመነበት ድረስ የተጠየኩትን መረጃ እና ናሙናዎችን ለመስጠት መስማማቴን በፊርማዬ አረጋግጣለሁ።

ፊርማ _____

ቀን _____

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc candidate:

Yishak Abraham Leka

Signature

Date and place of submission

Supervisor

Daniel Asrat, MD, M.Sc, PhD

Signature

Date and place of submission

Addis Ababa, Ethiopia

Supervisor

Yimtubezinash W/Amanuel, MD, M.Sc, PhD

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