BRIEF COMMUNICATION

BACTERIOLOGY OF COMPOUND (OPEN) FRACTURE WOUNDS AT 'TIKUR-ANBESSA' SPECIALIZED HOSPITAL, ADDIS ABABA UNIVERSITY, ETHIOPIA

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ABSTRACT

Background: *Infection of open fractures depends on the microbial environment, fracture handling, and host factors. Sound knowledge of the bacteriological epidemiology and antimicrobial susceptibility helps to rationally select prophylactic antibiotics.*
Objectives: *To isolate and identify the bacterial agents present on compound (open) fracture wound and to determine the antimicrobial susceptibility pattern.*
Setting: *Addis Ababa University, 'Tikur Anbessa Specialized Hospital.*
Methods: *Between 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 (200 wounds) who visited the orthopedic department of 'Tikur Anbessa' Hospital. Wounds were graded using Gustilo-Anderson's classification. The detailed bacteriological profile of the wound swabs collected by Levine's technique is documented. All of the wound specimens were processed for microscopic examination, culture, and sensitivity testing.*
Results: *Of the 191 patients, 82.7% were male whose average age was 31.55 years (age range 4 to 75 years). Most of the open fractures were caused by road traffic injuries (37.2%) and occurred in the lower extremity bones (60.0%). Twenty-three percent of the open fractures were Gustilo-Anderson grade I, 41.5% grade II, 14.0% grade IIIA, 5.5% grade IIIB, and 16.0% grade IIIC. 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. Eighty-two (41%) of the wounds were culture-positive, of which 51.2% showed mono-microbial growth while 48.8% showed polymicrobial growth. 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 for which they showed (60-80%) intermediate level resistance. Fifty-one percent of the gram-negative bacterial isolates were identified as multiple drug resistant.*
Conclusion: *Staphylococcus aureus was the commonest isolate followed by Acinetobacter species, E. coli and Pseudomonas species. Gentamicin, ciprofloxacin, and norfloxacin were the most effective drugs against the tested gram positive and gram-negative bacteria. 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 antibiotics.*

Keywords: *Compound fracture, Open fracture wounds, Bacterial isolates, Antimicrobial susceptibility testing.*

INTRODUCTION

Open or compound fractures are fractures that communicate with the outside environment through skin wounds (**1**). They are usually caused by high-energy trauma (**2**). A previous study conducted in our hospital (**3**), revealed that over a quarter of the patients with chronic osteomyelitis had anteceding trauma of which 93% was a compound fracture. The main causes of open fracture include road traffic injury (RTI), fall from a height, gunshots, assault, machine injury, and others (**4**). Approximately, 3-4% of all fractures are open fractures (**5**), and the development of infection is favored by devitalization of bone and soft-tissue. Loss of skeletal stability is a major com-

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plication, especially in grade III open fractures (**6**). Deep fracture-site infections can lead to chronic osteomyelitis, non-union, loss of function, or even limb loss.

According to Gustilo-Anderson (G-A), open fractures are classified into three major types (Type-III has three subtypes), based on the mechanism of the injury, the degree of soft-tissue damage, the configuration of the fracture, and the level of contamination (**7**). Seventy percent of the wound contamination is believed to occur at the time of injury (**8**). The contaminating bacteria originate from both the skin and the environment. In some cases, the organism is not present at the time of injury, and the wound becomes infected later. The constantly changing local wound ecology and sampling variations have led to the proposition of different ideas by different authors in the orthopedic 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 (**9**). Open fractures of the tibial shaft (especially, that of the distal third of the tibia from RTI) are common injuries with very often-severe comminution, devitalization and contamination due to its superficial location and the subcutaneous characteristics of its anteromedial aspect (**10**).

Wound infecting pathogens differ from country to country, from hospital to hospital and even within the same institution (**9,11**). 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 (**6,8,12,13**). 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 (**14**).*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.

Numerous studies published on open fractures elsewhere contain varying controversies on issues relating to the management of the fractures. The present study has been conducted to determine the bacterial etiologies of open fracture wound infection and its antimicrobial susceptibility pattern (culture and sensitivity pattern) at 'Tikur Anbessa' Specialized Hospital, Addis Ababa, Ethiopia.

The findings of the study will provide valuable information for the management of open wound fracture infections with appropriate antimicrobial agents, and the sensitivity pattern of the isolates empowers the practitioner to rationally select antibiotics for prophylaxis or empiric treatment.

PATIENTS AND METHODS

From November 2007 to May 2008, a total of 330 patients (26.5%of whom were fracture patients) were clinically diagnosed to have compound (open) fractures. The sample size (n) was calculated to be 200 by taking the prevalence of open and/or complicated fractures (13.7%) from previous studies (4). Out of 330 patients, 191 (57.9%) signed an informed consent. The 191 patients, who had a total of 200 open wound fractures with or without overt signs of infection, were enrolled in the study. Seven patients had two wound sites while one had three.

Wound Bed Preparation

After initial assessment by an orthopedic resident and the Radiology Department, wound beds were prepared before specimen collection by using Levine's technique. This was the most valid of the three methods of wound specimen collection, namely the z-technique, the exudates method and Levine's method (Gardner SE, 2006) where the wound surface was cleansed of surface exudates and contaminants with a moistened sterile gauze and a sterile normal saline solution. Dressed wounds were cleansed with non-bacteriostatic sterile normal saline after removing the dressing.

Sample Collection, Handling and Transport

As part of Levine's technique, the end of a sterile cotton-tipped applicator was rotated over a 1 cm^2 area for 5 seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue. 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 their arrival at the trauma resuscitation area, and also from inpatients and outpatients attending the fracture follow-up clinic.

The sampling time of the 200 compound 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. The delay in sampling was not intentional.

Three swabs were taken from each compound frac-

ture 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 the culture were considered positive when the same microorganism was isolated in at least two of the three samples.

Specimens were placed in Amies transport medium (Oxoid Ltd, UK) and transported to the main bacteriology laboratory of the Department of Bacteriology, Immunology and Parasitolgy within an hour. Some of the specimens collected at night were kept at 4^{o}C overnight until analysis.

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 revealed the types and relative numbers of microorganisms, and served to assess the quality of clinical specimen and to interpret culture findings.

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, and Hampshire, England). The plates were incubated in aerobic, microaerophilic, and anaerobic atmosphere at 37^{o}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, H_2S production, citrate utilization, motility test, urease test, carbohydrate utilization tests, and other tests using API 20E identification kits (Biomerieux, 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.

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^{o}C until the turbidity of the suspension was 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 (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%).

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 these strains were obtained from The Ethiopian Health and Nutrition Research Institute (EHNRI).

These findings and the demographical data collected using questionnaire were documented. Data entry and analysis were done using EpiInfo-2002 software. The level of significance was set at 0.05, 95% confidence interval.

RESULTS

Of the 191 patients, 158 (82.7%) were male and 33 (17.3%) were female, with the male- to- female ratio of 4.8:1. The age and sex distribution of the patients involved in this study is presented in **Table 1 and Figure 1**. The average age was 31.55 years (ranging 4 to 75 years). Only 36 (18.8%) were admitted for fracture stabilization and/or wound care, mainly due to shortage of orthopedic beds (**Table 2**). Fourty-six (23.0%) of the fractures were grade I, 83 (41.5%) grade II, 28 (14.0%) grade IIIA, 11(5.5%) grade IIIB, and 32 (16.0%) grade IIIC as shown in **Figure 1**.

Table 1 -Distribution of age and cause of injury in patients with compound fracture wounds presenting to 'Tikur Anbessa' Specialized Hospital. (November 2007 - May 2008)

Age(yrs)	RTI	Assaults	BI	HHO	MI	FA	Others[x]	Total (%)
0-12	6	-	-	3	-	-	-	**9 (4.7)**
13-24	22	7	11	10	5	2	1	**58 (30.4)**
25-36	23	7	9	7	13	5	-	**64 (33.5)**
37-48	10	8	6	3	4	-	2	**33 (17.3)**
49-60	8	4	2	1	-	1	3	**19 (9.9)**
>60	2	3	-	-	1	1	1	**8 (4.2)**
Total	71 (37.2)	29 (15.2)	28 (14.7)	24 (12.6)	23 (12)	9 (4.7)	7 (3.7)	**191 (100)**

Key: *RTI= Road traffic injury, BI= Bullet injuries, HHO= Hit by heavy object, MI= Machine injury, FA= Fall accident.*

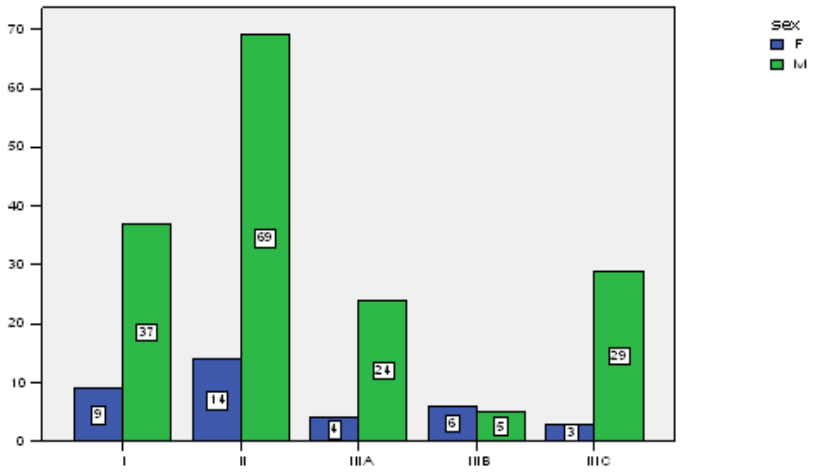

Figure 1 - Gustilo and Anderson grading of compound fractures seen at "Tikur Anbessa" Specialized Hospital between November 2007 and May 2008

Table 2- Time of arrival, address and pattern of admission of patients with open fracture wounds presenting to "Tikur –Anbessa Specialized Hospital, (November 2007 - May 2008)

Variable		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 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**). Most of the fractures (60.0%) occurred in lower extremities and the remaining (40.0%) in upper extremities as shown in Table-3. The different causes of the open fractures were presented in **Table 1**. Out of the 200 open fracture wounds, 55 (27.5%) were with overt signs of clinically important infection (erythema, pain, drainage, fever >38.5ºC and foul odor). Only 26 (13%) of the wounds were irrigated and surgically debrided . As 61 (30.5%) were positive for the presence of bacteria, different bacterial morphologies were observed. Eighty-two (41%) were culture positive, and of these 42 (51.2%) showed mono-microbial growth while 40 (48.8%) showed polymicrobial growth.

A total of 162 bacteria were isolated from the culture -positive wounds as shown in **Table 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. (9.9%), distribution of other bacteria is l listed on the table. 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. Of the 191 patients, 56 (29.3%) were treated with cloxacillin alone or in combination with other antimicrobials (mainly ceftriaxone) before the 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.

Table 3 - Site(s) of compound fracture(s) in extremities presenting to "Tikur Anbessa" Specialized Hospital (November 2007 to May 2008)

Bones	Tibia/ Fibula	Hand/ metacarpals	Radius/ Ulna	Femur	Foot/ metatarsals	Humerus	Ankle joint	Elbow joint	Patella	**Total (%)**
Count (%)	80 (37.9)	49 (23.2)	26 (12.3)	22 (10.4)	19 (9.0)	8 (3.8)	4 (1.9)	2 (0.9)	1 (0.5)	**211 (100)**

The susceptibility patterns of Gram-positive bacteria (n = 47) other than *Clostridium* spp. isolated from the compound fracture wounds against 14 antimicrobial agents were shown in **Table 5**. All isolates showed low level of resistance (<60%) to all antibiotics tested, except for ampicillin and penicillin to which they showed an intermediate level of resistance (60-80%). Most Gram-positive isolates, 29/55 (52.7%) showed multiple drug resistance (to three or more drugs) .Susciptibility pattern of gram negatives is shown in **Table 6**. The susceptibility pattern of *Clostridium* spp. (n=8) is presented in **Table 7**. All are susceptible to tetracycline, doxycycline, and kanamycin. Low level of resistance (<60%) was observed against chloramphenicol, clindamycin and penicillin. Amoxicillin-clavulanic acid, chloramphenicol, erythromycin, gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested

Table 4- Bacteria isolated from 200 compound fracture wounds investigated at "Tikur Anbessa " Specialized Hospital, Addis Ababa, Ethiopia (November 2007 to May 2008)

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

Gram-positive bacteria with the exception of *Clostridium* spp. All Gram positive 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). Gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested Gram-negative bacteria.

Table 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**
Staphylococcus aureus (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
Bacillus cereus (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	-	-
Streptococcus pyogenes (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
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	-	-	-	-	-	-
Micrococcus 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**

KEY: **S = Sensitive *I = Intermediate *R = Resistant. AMP: Ampicillin; AMC: Amoxicillin-clavulanic acid; C: Chloramphenicol; E: Erythromycin; CN: Gentamicin; OB: Cloxacillin; MET: ethicillin; P: Penicillin; AML: Amoxicillin; TE: Tetracycline; SXT: Trimethoprim-sulphamethoxazole; CRO: ceftriaxone; NOR: Norfloxacin; CIP: Ciprofloxacin*

DISCUSSION

The majority of the patients (68.6%) with open fractures as shown in Table 1 and Figure 1, were in the productive age group. Males were affected more than females in this study. This might be explained by the fact that traditionally, in this country, mainly males are involved in some occupations such as the transportation industry, machinery operation and construction works. The leading cause of open fractures in our setting (especially Addis Ababa) is RTI which alone contributed to 37.2% of the causes of open fractures in this study (Table 1). Similar findings have been reported from Nigeria and India (**15**).Assault or interpersonal violence which is the second most important cause of open fractures affected 15.2% of the patients. This finding corresponds to the with finding reported from north Gondar administrative zone, north west Ethiopia (**16**). Most of the bullet injuries which caused open fractures in 14.7% of our patients were also part of the interpersonal violence. Most of the fractures (60.0%) occurred in lower extremities (Table 3); this is consistent with an Iranian study (**17**).

The G-A I, II, and IIIC were the predominant types of the open fractures in this study (Figure 2). Grade II open fractures were the most dominant ones (41.5%). This is similar to a study reported by Osman, (**16**). Wound and bone infections mainly occurred in higher grades of open fractures.

Some (27.5%) of the compound fracture wounds in this study showed overt signs of infection. These wounds, especially those with foul odor, yielded a significant amount of bacterial isolates, particularly the polymicrobial ones. In the present investigation, only 13% of the wounds were irrigated and urgically debrided. Gram stain which revealed 61 (30.5%) was positive for bacterial isolates of different morphologies. The total bacterial isolation rate from the compound fracture wounds in this study was 41%. This is slightly lower than the finding reported in Ile-ife, Nigeria, which showed that the isolation rate was 45.8% (**15**).

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. One should not infer that only those wounds with positive cultures are at risk. Specimens taken from clinically infected wounds that yield no growth suggest the possibility of a false-negative result (**18**). 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* . This is in agreement with previous studies conducted at different places in Ethiopia (**3, 14, 19,20**). *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 at Brooke Army Medical Center, USA. 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. The explanation for this may partly be due to problems in our method of isolating this organism, or it could be due to the success of vaccination campaigns which have made invasive *H. influenzae* infections rare.

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. This is in agreement with a study done in USA (**21**). The Gram-negative (60%) to Gram-positive (40%) bacterial proportion in our findings disagrees with reports from Minnesota, USA (40% vs. 60%) (**7**), Indian tertiary care hospital, India (47% vs. 53%) and Gondar teaching hospital, Ethiopia (29% vs.71%) (**22**).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 injuries. It is also noted that bacterial prevalence differs in different environments (**9**). In this study, 51.2% of the culture-positive wounds showed mono-microbial growth, and 48.8% showed polymicrobial growth. Similarly, Johnson *et al.*, 2007, reported that gram-positive bacteria were less frequently recovered, and 37% were polymicrobial infections. Culturing wound swabs for both aerobic and anaerobic microorganisms is recommended (**2**). They were isolated from a polymicrobial mixture with a facultative anaerobic bacteria.

The isolation of anaerobic bacteria in this study was a difficult task because of poor laboratory set up for anaerobic culture. In general, the profile of the bacterial isolates in our study comparatively agrees with findings that have been observed in many studies

(**23**). 29.3% of the patients were treated with cloxacillin alone or in combination with other antimicrobials (mainly ceftriaxone) before the collection of samples, and of these, 71.4% had positive culture results. The possible explanation for high culture positivism could mainly be bacterial resistance for prophylactically administered antimicrobials. In addition, this also shows that the rational use of some antibiotics alone or in combination, requires periodic evaluation and the establishment of an antimicrobial policy for prophylaxis and treatment guidelines in the Ethiopian setting.

All Gram-positive bacterial isolates with the exception of *Clostridium* spp. showed a low level of resistance (<60%) to all antimicrobials tested, except for ampicillin and penicillin to which they showed an intermediate level of resistance (60-80%). All Gram-negative bacterial isolates showed a low level of resistance (<60%) to all antimicrobials tested except for ampicillin and amoxicillin (60-80%, intermediate level resistance). In general gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested Gram-positive and Gram-negative bacteria. This is in agreement with reports from Ile-Ife, Nigeria (**20**), and Ahwaz University of Medical Sciences teaching hospitals, Iran (**23**).

All *Clostridia* were found to be susceptible to most antimicrobial agents tested as shown in Table 7. Similar findings have been reported elsewhere (**18**). In this study, MDR (resistance to three or more drugs) 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.

CONCLUSION AND RECOMMENDATIONS

Most of the open fractures (60.0%) occurred in lower extremities (usually from RTI) and (41.5%) were grade II. More has to be done to decrease the incidence of RTI. In general, 41% of the compound fracture wounds were culture positive, *S. aureus* being the dominant isolate. The Gram-positive and Gram-negative bacteria accounted for 34.0% and 66.0%, respectively. Ciprofloxacin, norfloxacin, and gentamicin were the most effective drugs against the tested Gram-positive and Gram-negative bacteria.

Based on our findings, we recommend that the value of the Gram stain as a quick and inexpensive additional or alternative test is also worthy of consideration. Anaerobic organisms remain important isolates and culture facilities should be improved in order to provide additional information on anaerobic bacteriology of compound fracture wounds at "Tikur Anbessa" Specialized Hospital.

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