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Full Length Research Paper

Microbial susceptibility of bacteria isolated from open fracture wounds presenting to the err of black-lion hospital, Addis Ababa University, Ethiopia

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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 environmental, microbial and host factors. In developing countries like 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. In set-ups where immediate culture and sensitivity tests are difficult, sound epidemiological knowledge of microbes helps in rationally selecting antibiotics for prophylaxis and empiric treatment as well. To profile the antimicrobial susceptibility pattern (Culture and sensitivity) of the bacterial isolates from open fracture wounds to the commonly used antibiotics. Addis Ababa University, Black-Lion ('Tikur Anbessa') Hospital-BLH, is the country's highest tertiary level referral and teaching Hospital. The Hospital has a newly established separate ER to receive trauma patients. 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 (200 wounds) who visited the orthopedic department of 'Tikur Anbessa' Hospital, - a tertiary University Hospital, Addis Ababa, Ethiopia. Wounds were graded using Gustilo-Anderson's classification. The detailed bacteriological profile of the wounds swabs collected by Levine's technique is documented. All of the wound specimens were processed for microscopic examination, culture and sensitivity testing. A total of 162 bacterial pathogens were isolated from the 200 open fracture wounds sampled. *Staphylococcus aureus* was the dominant isolate (14.8%) followed by *Acinetobacter* spp. (11.4%). Of the culture-positive wounds, 51.2% showed mono-microbial growth (single bacterial type) and 48.8% showed polymicrobial (more than one bacterial type) growth. The gram-positive and -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 resistants (MDR). *Staphylococcus aureus* was the commonest isolate associated with open fracture wound contamination. Gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested gram- positive and -negative bacteria and should be considered in empirical antibiotic selection. 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.

Key words: Compound fracture, open fracture wounds, bacterial isolates, antimicrobial susceptibility testing.

INTRODUCTION

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). A study conducted in the same Hospital (Biruk and Wubshet, 2007), revealed that over a quarter of patients with chronic osteomyelitis had 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 (Ahmed and Chaka, 2006). Approximately 3 - 4% of all fractures being open fractures (Anglen, 2005), and the development of infection favored by devitalization of bone and soft-tissue and loss of skeletal stability are a major complication, especially in grade III open fractures (Quinn and Macias, 2006). 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 injury, the degree of soft-tissue damage, the configuration of the fracture, and the level of contamination (Gustilo and Anderson, 1976). Seventy percent wound contamination is believed to occur at the time of injury (Cat and Hall, 2007) (Table 1a).

Open fractures more than 8 h old at presentation were classified as a special category of type III fractures (Zalavras and Patzakis, 2003). The contaminating bacteria originate from both skin and environment. In some cases the organism is not present at the time of injury, and the wound becomes inoculated later. 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. The constantly changing local wound ecology and sampling variations 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 (Lee, 1997). 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 (Soontornvipart et al., 2003). Wound infecting pathogens differ from country to country, from(Lee, 1997; Taye, 2005). The majority of infections in open fractures are caused by Staphylococci (*S. aureus* one hospital to another-even vary in the same institution and Coagulase negative staphylococci) and gram-negative bacilli, which include *Acinetobacter* spp., *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp. and others (Quinn and Macias, 2006; Cat and Hall, 2007; Okike and Bhattacharyya, 2006; Courvalin, 2005).

A gram-stained smear from a wound swab requires less than 10 min preparing (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). Blood, MacConkey and chocolate agar can be used as primary isolation media for gram-positive and -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). 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). 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). 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. Numerous studies have been published elsewhere on the subject of open fractures with dogma and controversies dominating in different

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Table 1a. Modified Gustilo and Anderson classification of open fractures (Adapted from Okike and Bhattacharyya, 2006).

Grade	Definition	Historical 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

issues of the open fractures. But from Ethiopia, there is no published information about the bacteriology of open fracture wounds.

MATERIALS AND METHODS

During the period from November, 2007 - May, 2008, a total of 330 (26.5% of 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. Out of 330 patients, 191 (57.9%) informed and consented patients with a total of 200 open fracture wounds with or without overt signs of infection were enrolled in the study. Seven patients had two wound sites and one had three. Fifty five (27.5%) wounds were with overt signs of infection. The patients were assessed by history taking, physical examination and bone imaging result by attending doctor. After a standard bone imaging, wound bed preparation, sample collection (Swab) and transportation to the main lab, microscopic examination, organism identification, culture and sensitivity testing were performed serially. 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).

Sample collection, handling and transport

As part of Levine's technique, the end of a sterile cotton-tipped applicator was rotated over 1 cm^2 area for 5 s with sufficient pressure to express fluid and bacteria to surface from within the wound tissue (Levine et al., 1976; Gardner et al., 2007). Of 200 open fracture wounds, 127 (63.5%) were deep and wide wounds. 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.

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 h of the injury; 43 (21.5%) of them were swabbed between 9 and 24 h; and 78 (39.0%) of them were swabbed after 24 h 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.

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 analyses.

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.

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°C for 24 - 48 h. 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°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 -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. C*lostridium* 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 h 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 the strains were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

These findings and the demographical data collected using questionnaires were documented. 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. 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.

RESULTS

Demography

(Of the 191 patients, 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 age and sex distribution of patients involved in this study is presented in Table 1b and Figure 1. The average age of the patients was 31.55 years (age range 4 - 75 years). Only 36 (18.8%) had been admitted for fracture stabilization and/or wound care mainly due to shortage of available orthopedic beds (Table 2). 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 2.

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%) occurred in upper extremities as shown in Table 3. The different causes of the open fractures were presented in Table 1b. Out of the 200 open fracture wounds, 55 (27.5%) were with overt signs of clinically important infection (erythema, pain, drainage, fever >38.5°C and foul odor). Foul odor was typical feature of older wounds. Only 26 (13%) of the wounds were irrigated and surgically debrided. 61 (30.5%) were positive for the presence of bacteria and different bacterial morphologies were observed. 82 (41%)(<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 (to three or more drugs) .The susceptibility pattern of *Clostridium* spp. (n = 8) is presented in Table 4. All are susceptible to were culture positive and of these 42/82 (51.2%) showed mono-microbial growth and 40/82 (48.8%) showed polymicrobial growth. A total of 162 bacteria were isolated from the culture-positive wounds as shown in Table 5. *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 well listed on the table. Anaerobic bacteria, *Clostridium* spp. (*C. perfringens* and *C. tetani*) were also isolated. The gram-positive and -negative bacteria account-ted for 55/162 (34.0%) and 107/162 (66.0%), respectively ($p < 0.05$). 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 ($p > 0.05$). The susceptibility patterns of gram-positive bacteria (n = 47) other than *Clostridium* spp. isolated from the compound fracture wounds against 14 antimicrobial agents were used. The sensitivity details of gram positive and negative bacteria are shown on Tables 6 and 7 respectively. All isolates showed low level of resistance 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 clavulanic acid, chloramphenicol, erythromycin, gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested gram-positive (G +ve) bacteria with exception of *Clostridium* spp. All G +ve 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

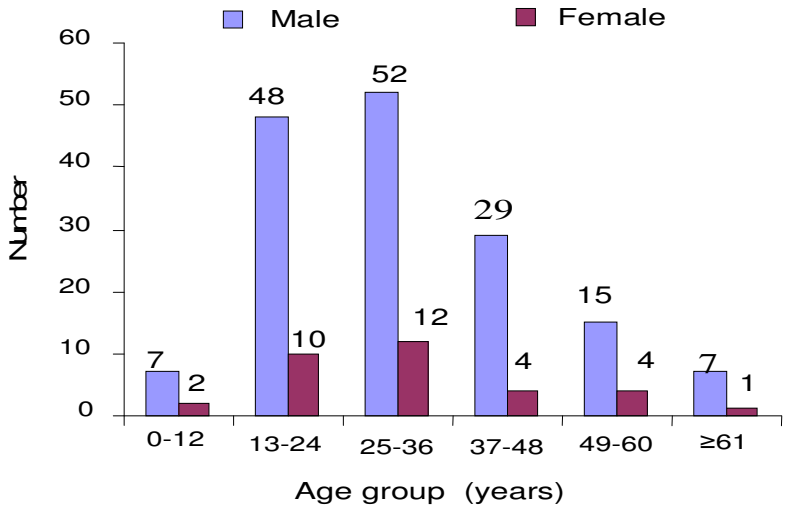

Figure 1. Age and sex distribution of the patients with compound fractures investigated for wound infection at TAUH, Addis Ababa, Ethiopia (November 2007 to May 2008).

Table 1b. Distribution of age, sex and cause of injury in patients with compound fracture wounds (November, 2007 - May, 2008).

Age group (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)

Table 1b. Contd.

Age group (yrs)*	Sex	Car accident	Assault	Bullet injury	Heavy object	Machine injury	Fall accident	Others[ᵡ]	Total (%)
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)	

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

Variable		Time of arrival after Injury			Total (%)
		= 8 h	>8 h	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)

Table 3. Site(s) of compound fracture(s) in extremities presenting to TAUH (November 2007 to May 2008).

Bones	Tibia/ Fibula	Hand/metacarpals	Radius/Ulna	Femur	Foot/ metatarsals	Humerus	Ankle joint	Elbow joint	Patella	Total (%)
Count	80	49	26	22	19	8	4	2	1	211
(%)	(37.9)	(23.2)	(12.3)	(10.4)	(9.0)	(3.8)	(1.9)	(0.9)	(0.5)	(100.0)

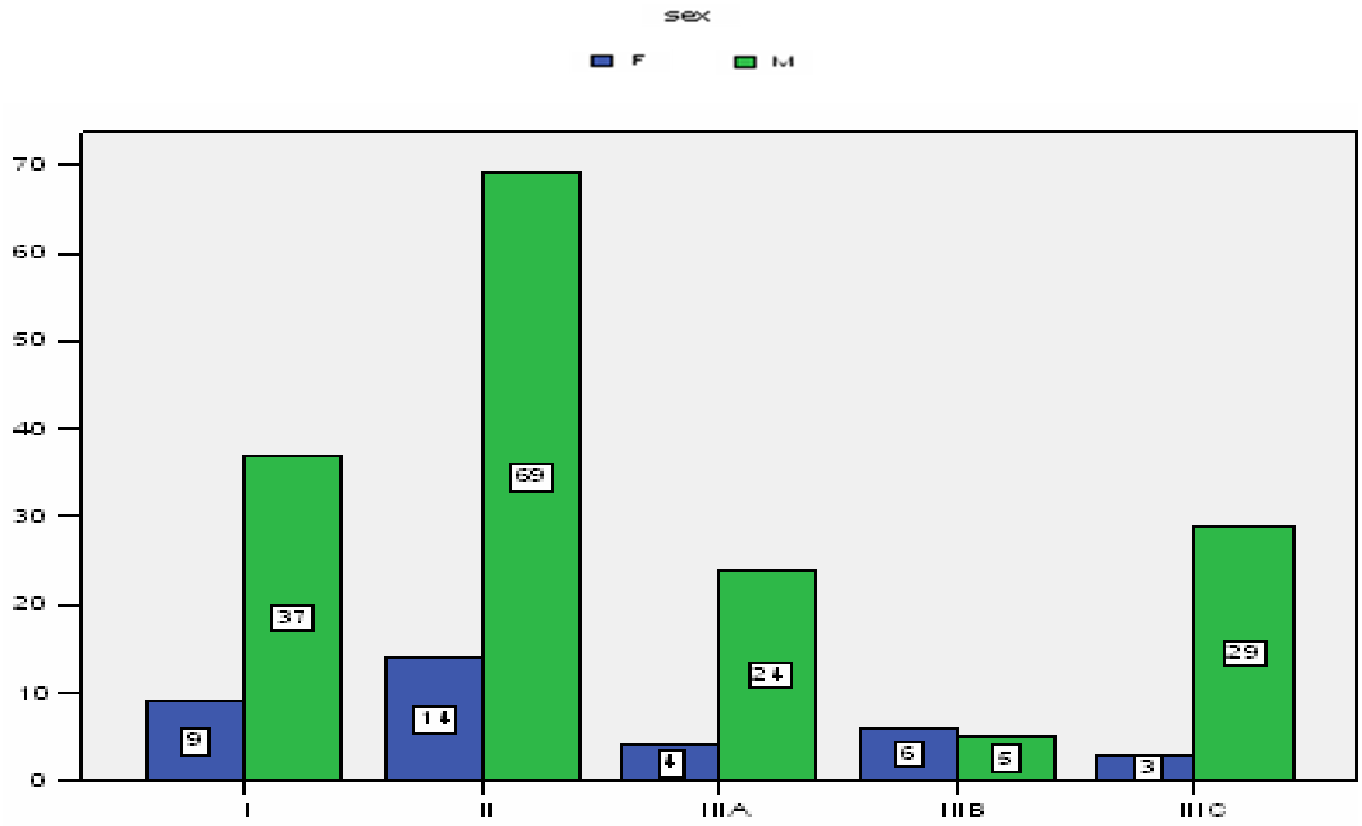

Figure 2. Gustilo and Anderson grading of compound fractures seen at TAUH between November 2007 and May 2008.

Table 4. Susceptibility patterns of *Clostridium* spp. isolated from open fracture wounds (November, 2007 - May, 2008).

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

Key: *S = Sensitive; *I = Intermediate; *R = Resistant;TE:Tetracycline;C:Chloramphenicol;DA:Clindamycin;DO:Doxycycline:P: Penicillin: K: Kanamycin.

Table 5. Bacteria isolated from 200 compound fracture wounds investigated at TAUH, A.A, 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 spp	15 (9.3)	3 (1.9)	18 (11.4)
Escherichia coli	12 (7.4)	5 (3.1)	17 (10.5)
Pseudomonas spp	9 (5.6)	7 (4.3)	16 (9.9)
Enterobacter spp	11 (6.8)	4 (2.5)	15 (9.3)
CoNS	12 (7.4)	- -	12 (7.4)
Klebsiella spp	7 (4.3)	5 (3.1)	12 (7.4)
Clostridium spp	6 (3.7)	2 (1.2)	8 (4.9)
Citrobacter spp[a]	2 (1.2)	4 (2.5)	6 (3.7)
Proteus spp[b]	5 (3.1)	1 (0.6)	6 (3.7)
Aeromonas spp[c]	1 (0.9)	4 (2.5)	5 (3.1)
Erwinia spp	- -	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 spp	1 (0.6)	- -	1 (0.6)
Alcaligenes spp	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)

(data not shown). Gentamicin, ciprofloxacin and norfloxacin were the most effective drugs against the tested gram-negative bacteria.

DISCUSSION

Majority of patients (68.6%) with open fracture as shown In Table 1b and Figure 1 were in the productive age group. Ikem et al. (2004) reported similar finding in a study conducted in Ile-Ife, Nigeria

Table 6. Susceptibility Patterns of gram-positive Bacteria Isolated from open fracture wounds (November, 2007 to May, 2008).

		Antimicrobial agents (%)													
Organisms		**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	-	-	-	-	-	-

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

Table 6. Contd.

Organisms		Antimicrobial agents (%)													
		AMP	AMC	C	E	CN	OB	MET	P	AML	TE	SXT	CRO	NOR	CIP
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.

Table 7. Susceptibility patterns of gram-negative bacteria isolated from open fracture wounds (November 2007 to May 2008).

Organisms		Antimicrobial agents (%)									
		AMP	AMC	C	CN	AML	TE	SXT	CRO	NOR	CIP
Acinetobacter species (n = 18)	S	16.7	22.2	50.0	88.9	16.7	55.6	88.9	22.2	38.9	77.8
	I	-	44.4	33.4	-	22.2	22.2	-	22.2	27.8	11.1
	R	83.3	33.4	16.7	11.1	61.1	22.2	11.1	55.6	33.4	11.1
Escherichia coli (n = 17)	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	-	-	-	-
	R	41.2	23.5	41.2	11.8	47.1	41.2	29.4	11.8	5.9	5.9
Pseudomonas species (n = 16)	S	6.3	18.8	18.8	75.0	6.3	25.0	31.3	25.0	87.5	93.8
	I	6.3	-	6.3	12.5	12.5	18.8	-	43.8	-	6.3
	R	87.5	81.3	75.0	12.5	81.3	56.3	68.8	31.3	12.5	-
Enterobacter species (n = 15)	S	-	33.3	60.0	80.0	-	33.3	66.7	66.7	93.3	86.7
	I	6.7	20.0	13.3	-	13.3	53.3	6.7	13.3	6.7	6.7
	R	93.3	46.7	26.7	20.0	86.7	13.3	26.7	20.0	-	6.7
Klebsiella species (n = 12)	S	-	66.7	75.0	75.0	-	58.3	75.0	66.7	83.3	75.0
	I	-	-	-	-	33.3	8.3	-	8.3	16.7	25.0
	R	100.0	33.3	25.0	25.0	66.7	33.3	25.0	25.0	-	-
Citrobacter species (n = 6)	S	16.7	50.0	83.3	83.3	16.7	83.3	66.7	83.3	100.0	83.3
	I	16.7	-	-	-	66.7	-	-	-	-	16.7
	R	66.7	50.0	16.7	16.7	16.7	16.7	33.3	16.7	-	-

Table 7. Contd.

Organisms		Antimicrobial agents (%)									
		AMP	**AMC**	**C**	**CN**	**AML**	**TE**	**SXT**	**CRO**	**NOR**	**CIP**
Proteus species (n = 6)	S	33.3	83.3	50.0	100.0	33.3	16.7	50.0	100.0	100.0	100.0
	I	-	-	-	-	33.3	-	-	-	-	-
	R	66.7	16.7	50.0	-	33.3	83.3	50.0	-	-	-
Aeromonas species (n = 5)	S	-	-	100.0	100.0	-	100.0	100.0	100.0	100.0	100.0
	I	20.0	-	-	-	40.0	-	-	-	-	-
	R	80.0	100.0	-	-	60.0	-	-	-	-	-
Erwinia species (n = 3)	S	66.7	66.7	100.0	100.0	66.7	100.0	100.0	100.0	100.0	100.0
	I	-	-	-	-	33.3	-	-	-	-	-
	R	33.3	33.3	-	-	-	-	-	-	-	-
Morganella morganii (n = 2)	S	-	-	-	100.0	-	50.0	50.0	100.0	50.0	50.0
	I	-	-	50.0	-	-	-	-	-	-	-
	R	100.0	100.0	50.0	-	100.0	50.0	50.0	-	50.0	50.0
Providencia rettgeri (n = 2)	S	50.0	-	100.0	100.0	50.0	-	100.0	100.0	100.0	100.0
	I	-	-	-	-	-	100.0	-	-	-	-
	R	50.0	100.0	-	-	50.0	-	-	-	-	-
Alcaligenes species (n = 1)	S	-	-	-	-	-	100.0	100.0	100.0	100.0	100.0
	I	-	-	-	-	-	-	-	-	-	-
	R	100.0	100.0	100.0	100.0	100.0	-	-	-	-	-
Stenotrophomonas maltophilia (n = 1)	S	-	-	100.0	100.0	-	-	100.0	-	-	-
	I	-	-	-	-	-	-	-	-	-	100.0
	R	100.0	100.0	-	-	100.0	100.0	-	100.0	100.0	-
Burkholderia cepaciae (n = 1)	S	-	-	100.0	100.0	-	-	100.0	-	-	100.0

Nigeria. Males were affected more than females in this study. 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, 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 the open fractures in this study (Table 1b). 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. Most of the fractures (60.0%) occurred in lower extremities (Table 3) consistent with Iranian study (Fakoor and Pipelzadeh, 2007).

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 et al., 2003), from Nigeria. Wound and bone infections mainly occur in higher G-A grades of open fracture.

Some (27.5%) of the compound fracture wounds in this study showed overt signs of infection. These wounds, especially those with foul odor yielded significant amount of the bacterial isolates, particularly the polymicrobial ones. This is comparable to a report from USA (Lawrence et al., 1978). Foul odor 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. Gram stain revealed 61 (30.5%) were positivism with 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% (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. One should not 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 (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 (Biruk and Wubshet, 2007; Gebreselassie, 2002; Belihu and Lindtjorn, 1999; Mulu et al., 2006). *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. This is in agreement with a study done in USA (Patzakis et al., 1974) (26). The gram-negative (60%) to gram-positive (40%) 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%) (25). 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. Culturing wound swabs for both aerobic and anaerobic microorganisms is recommended (Zalavras et al., 2007). 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. In general, the profile of the bacterial isolates in our study comparatively agrees with findings that have been observed in many studies (Khosravi et al., 2009). 29.3% of the patients had been treated with cloxacillin alone or in combination with other antimicrobials (mainly ceftriaxone) before collection of samples and of these, 71.4% had positive culture results. The possible explanation for high culture positivism 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. 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%). 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). 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 (Khosravi et al., 2009).

All *Clostridia* were found to be susceptible to most

antimicrobial agents tested as shown in Table 4. Similar findings have been reported elsewhere (Kingsley, 2001). 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.

CONCLUSIONS AND RECOMMENDATIONS

The gram-positive and -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 -negative bacteria.
Based on our 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 antibiotic regimen other wise. Periodic antimicrobial choice 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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