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Study of microbiological flora and role of primary bacterial cultures in management of open fractures of long bones

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Background: Microbiology of open fracture wounds is constantly changing. A clear understanding of the bacterial flora that could be expected is needed in order to administer a rational and effective antibiotic treatment for open fractures. The role and efficacy of primary bacterial cultures in management of open fractures is also debatable and needs further evaluation.

Methods: One hundred patients with one hundred and seven open fractures of long bones were studied prospectively, from March 1st 2001 till February 28th 2002. Wound swabs were obtained at pre-debridement, intra-operative, post debridement, 1st dressing/after 24hrs intervals, subsequently every week and sent for cultures. The infecting organism, its antibiotic susceptibility and its correlation between cultures at different stages was noted.

Results: An infection rate of 43.9% was noted. Most of the initial wound cultures, showed growth of Gram-negative organisms (76%), commonest being Pseudomonas (36%) and Acinetobacter (20.7%). However, majority of infections after 2nd week were caused by Gram-positive organisms. Staphylococcus aureus (93.5%) was the predominant Gram-positive organism.

None of the organisms grown on admission and pre-debridement cultures eventually caused infection; however, 28% of cases with negative cultures eventually got infected. Post debridement cultures were positive in none. Among the cultures obtained at 1st dressing 40% of organisms grown eventually caused infection whereas 60% showed growth with different organism.

Conclusions: A shift in the bacterial flora occurs in compound fracture wounds from Gram-negative to Gram-positive organisms after the 2nd week. Cultures obtained at admission, predebridement, post debridement and at 1st dressing or after 24hrs are not reliable indicators of subsequent wound infection.

Keywords: Open fractures, bacterial flora, wound cultures

Introduction

Having preceded man on earth, bacteria continue to exert their "territorial imperative" [1]. Bacterial infection of bone and soft tissues has undesirable consequences for the patient and the physician [2]. A changing etiologic pattern of infections and the antibiotic susceptibility of bacteria have been reported. This includes a decrease in the incidence of Gram positive infections and a relative increase in Gram negative infections and superimposed or secondary infections [3]. The cause of this change has not been clear but they seem to be related to widespread use of antibiotics, changes in the host resistance and a number of iatrogenic factors. Based on the types of organisms causing infections compared with those seen on initial wound cultures, several authors have proposed that many infections of open fracture wounds are nosocomial [4, 5].

The goal of treating infections is to establish a precise microbiologic diagnosis prior to the institution of any type of therapy. Obtaining preoperative cultures from open fracture wounds before the definitive debridement has long been taught to medical students and orthopaedic residents as part of open fracture management. The information provided by the test should allow the clinician to alter treatment to improve outcome in some way or, at least, to prognosticate regarding the severity or clinical course of the condition. The necessity for sequential or multiple cultures obtained from open fracture wounds and their interpretation is controversial [6, 7].

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In the present study we have attempted to study the changing patterns of bacterial flora in compound fractures and define the role of primary bacterial cultures in their management. Thus, formulate better and more effective protocol for management of wounds with compound fractures.

Materials and Methods

100 patients with 107 open fractures were studied prospectively in the Department of Orthopaedics at Christian Medical College and Hospital, Ludhiana from March 1st 2001 till February 28th 2002. All open fractures were classified according to Gustilo's classification. (Gustilo *et al.*, 1984). Patients with Gustilo's IIC fractures going for an amputation were excluded from the study.

A wound swab was obtained at the time of presentation, tetanus prophylaxis, intra-venous antibiotics were given and irrigation of the wound was done in the casualty/OPD. Primary wound debridement was done in the operation theatre under appropriate anesthesia with appropriate stabilization of the bone.

Wound cultures were obtained prior to debridement and after debridement. Regular wound care was done as per expected protocol. Wound cultures were sent after 24 hours of debridement or at first dressing after debridement and then subsequently every week.

For all wound cultures two swab sticks were obtained in a culture tube. One to obtain smear by Gram staining and second for inoculation on both aerobic and anaerobic media. For aerobic bacteria inoculation was done on blood agar and Mac Conkey agar and the plates were incubated at 37 degrees C overnight. For anaerobic bacteria blood agar was inoculated and incubated at 37 degrees over 48 hours. For anaerobic bacteria, which were difficult to isolate Brewers enrichment broth was also inoculated and incubated overnight. Subcultures were then obtained on blood agar anaerobically and incubated at 37 degrees over 48 hours.

Reports of cultures were recorded. Wounds were regularly monitored in the ward and any change in the intra-venous antibiotics was recorded. Repeated wound debridement was done as per requirement and pre and post debridement cultures were sent and followed and their results were analyzed.

Antibiotic sensitivity of the isolate was done by the Kirby Buaer technique.

Results

Most of the patients were in the age group of 20-30 years. The male female ratio was 9: 1. Road traffic accident was the commonest mode of injury i. e. 83(77.6%).

A total infection rate of 43.9% was noted.

Bacterial Flora

Most of the infections were caused by were caused by Gram-negative organisms 75(64.7%), 31(26.7%) were Gram positive organisms and 10(8.6%) cultures showed a mixed growth. Pseudomonas 27(36%) and acinet o bacter 24(20.7%) were the predominant Gram-negative organisms. Among the Gram-positive organisms, majority i. e. 29(93.5%) were Staphylococcus aureus, 17(58.6%) of which were methicillin resistant (see table1).

Table 1: Bacterial flora

Organism	Total	%
Acinetobacter	24	20.7
Pseudomonas	27	23.3
E-coli	19	16.4
Proteus	2	1.7
S-aureus	29	25.0
Klebsiella	3	2.5
Enterobacter	1	0.9
Beta-hs	1	0.9
Non-hs	1	0.9
Mixed growth	12	10.1
Total	119	

Bacterial flora in relation to duration after injury

Acinetobacter and pseudomonas were the predominant organisms isolated in patients reaching us within 6 hrs of injury. Staphylococcus aureus was the predominant organism isolated in patients presenting within 6-48 hrs of injury. Unusual pathogens like E-coli and proteus were isolated in increasing frequency with increasing duration of injury (see table no. 2, pic 2).

Table 2: Bacterial flora in relation to duration after injury

Organism	<6 hrs		6-12 hrs		12-48 hrs		>48 hrs	
	No.	%	No.	%	No.	%	No.	%
Acinetobacter	20	36.4	1	3.4	2	12.5	1	5.5
Pseudomonas	17	30.9	5	17.2	1	6.3	4	22.2
E-coli	5	9	6	20.7	3	18.8	5	27.8
Proteus	0	0.0	0	0.0	0	0.0	2	11.1
S-aureus	6	10.9	9	31	10	62.5	4	22.2
Klebsiella	2	3.6	1	3.4	0	0.0	0	0.0
Beta-hs	1	1.8	0	0.0	0	0.0	0	0.0
Non-hs	0	0.0	0	0.0	0	0.0	1	5.5
Mixed	4	7.2	7	24.1	0	0.0	1	5.5
Total		55		29		16		18

Bacterial flora in different sites of open fractures

Commonest site of injury was the leg 60(55.5%). Acinetobacter and Staphylococcus aureus were the predominant organisms isolated in open fractures of the leg and thigh. In open fractures of the arm, most common organism isolated was Pseudomonas (66.7%). There were only 2 open fractures of the forearm, so the number is too less to be statistically relevant (see table no.3).

Table 3: Bacterial flora in different sites of open fractures

Organism	Arm		Forearm		Thigh		Leg	
	No.	%	No.	%	No.	%	No.	%
Acinetobacter	0	0.0	0	0.0	7	25.9	17	24.3
Pseudomonas	6	66.7	0	0.0	3	11.1	18	22.5
E-coli	1	11.1	0	0.0	3	11.1	15	18.8
Proteus	0	0.0	0	0.0	1	3.7	1	1.3
S-aureus	2	22.2	1	50	7	25.9	19	23.8
Klebsiella	0	0.0	0	0.0	1	3.7	2	2.5
Beta-hs	0	0.0	0	0.0	1	3.7	0	0.0
Non-hs	0	0.0	1	50	0	0.0	0	0.0
Mixed	0	0.0	0	0.0	4	14.8	8	10
Total		9		2		27		80

Table 4: Bacterial flora in different grades of open fractures

Organism	Grade I		Grade II		Grade IIIA		Grade IIIB		Grade IIIC	
	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter	0	0.0	1	25	2	12.5	17	20.7	4	26.7
Pseudomonas	0	0.0	2	50	4	25	16	19.5	5	33.3
E-coli	0	0.0	0	0.0	0	0.0	16	19.5	3	20
P roteus	0	0.0	0	0.0	0	0.0	2	2.4	0	0.0
S-aureus	1	100	1	25	6	37.5	18	22	3	20
Klebsiella	0	0.0	0	0.0	1	6.3	2	2.4	0	0.0
Beta-hs	0	0.0	0	0.0	1	6.3	0	0.0	0	0.0
Non-hs	0	0.0	0	0.0	1	6.3	0	0.0	0	0.0
Mixed	0	0.0	0	0.0	1	6.3	11	13.4	0	0.0
Total	1		4		16		82		15	

Bacterial flora in different grades of open fractures

Most of patients (50) in our study had grade IIIB fractures (46.6%). Only 1 patient developed infection among grade I fractures, who grew *Staphylococcus aureus*. Grade II and Grade IIIC fractures had maximum infection with *Pseudomonas* i. e. 50% and 33.3% respectively. Grade IIIA fractures showed predominance of *Staphylococcus aureus* (37.5%) and Grade IIIB with *Staphylococcus aureus* (22%) and *Acinetobacter* (20.7%) (See table no. 4).

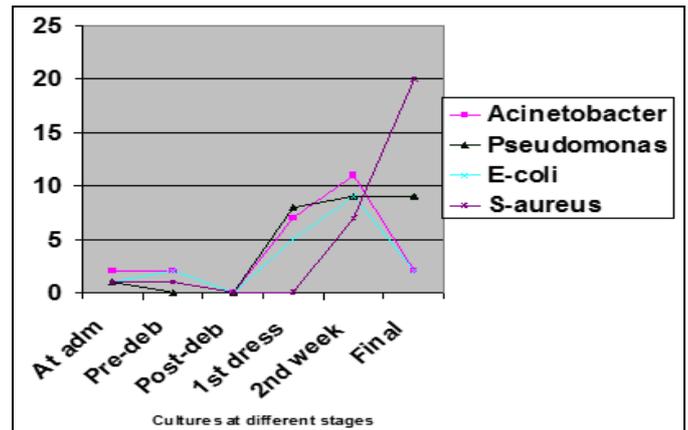


Fig 1

Bacterial Flora in relation to different stages of wound management and their changing patterns

Cultures on admission were positive in only 7(6.5%) patients and pre debridement cultures in 5.6% of patients. Most of the initial wound cultures showed growth of Gram-negative organisms (71.4%) as compared to only 40% growth in the final cultures. Maximum growth rate was seen in the 2nd week (fig; 1). On the other hand Gram-positive organisms i. e. *staphylococcus aureus* were present in only 28.6% of patients at admission as compared to 60% in the final culture. This clearly indicates a shift in the bacterial flora from Gram-negative to Gram-positive later in the course of wound management (pic 1). This change occurred around the 2nd week as evident from the graph (see fig. 1).



Picture 1: Wound at 2 weeks with skin graft lysis-culture showing growth of *s-aureus*



Picture 2: A compound grade IIIB both bones leg who presented after 48 hrs of injury-wound showing growth of *E-coli*

Predictive value of cultures at different stages

Cultures on admission were positive in only 7(6.5%) patients and majority of patients 100(93.5%) had a negative culture. Of these 7(6.5%) patients, most of them i. e. 5(71.4%) grew Gram-negative organisms. Pre-debridement cultures were positive in 6(5.6%) patients and post-debridement cultures were positive in none. Cultures were positive in 19.6% of patients at 1st dressing, 34.6% of patients in the 2nd week and 32.7% at the final culture. None of the organisms grown on admission and predebridement cultures eventually caused infection. 28% of cases with negative cultures at these stages however, became infected. Among the cultures obtained at 1st dressing 40% of organisms grown eventually caused infection whereas 60% of cultures finally showed a different growth. 16.3% of cases with negative cultures became infected. The predictive value of the cultures at admission, pre debridement, post debridement and at 1st dressing for development of infection in the final culture was not found to be significant.

Discussion

In the present study we had majority of grade III open fractures, thus explaining the high incidence of infection rate i. e. 43.9%. We noted a predominance of infection with the Gram-negative organisms (64.7%), pseudomonas 27(36%) and acinetobacter 24(20.7%) being the predominant ones. Gram positive infections accounted for only 26.3% infections, staphylococcus aureus being the predominant organism. Acinetobacter was the commonest primary infecting organism in the lower extremity whereas pseudomonas was the predominant organism isolated in the upper extremity. The isolate however would depend on the microbiologic environment of the institution in which the study is conducted as evident from different reports by various authors [6-10], but identifying the patterns or predilection of organisms and anticipating infection by a particular organism in that institution might be worthwhile.

Another interesting observation was a change in the bacterial flora i. e. shift from Gram-negative to Gram-positive flora after the second week. This change can be attributed to the use of antibiotics during the initial phases of wound management which are more effective against Gram-negative organisms and but leaving behind the Gram-positive organisms to flourish later in the course of wound management. Also, debridement and irrigation change the ecology of local wound and finally another possibility is that the infecting bacteria are nosocomial. Based on this observation use of a broad spectrum antibiotic during the initial phase of wound management might prevent this change in bacterial flora and early coverage of the wounds would further decrease the incidence of nosocomial infections.

Cultures obtained at admission and prior to debridement were found to be of no value as predictors of the infecting organism as none of the organisms grown on these cultures eventually caused infection. Also a negative culture at these stages would not exclude the possibility of infection as 28% of cases with negative cultures got infected. Cultures obtained at 1st dressing/after 24hrs had a better prognostic value (40%), however it is still low and 16.3% of cases with negative cultures became infected. The results corroborate the observations of Merrit (1988) and Kreder (1976). Recent studies [5, 11] also show similar reports and recommend that sequential bacterial cultures in management of open fracture wounds are not helpful.

A likely explanation for the inadequacy of peri-operative cultures is sampling error. That is when taking the culture; the infecting pathogen is not obtained either because of poor technique or more likely, because the organism is present in small numbers, making it difficult to collect adequate samples. Other reason is the constantly changing local wound ecology due to various factors like surgical debridement and the use of antibiotics which will tend to select certain organisms that may be less susceptible or resistant. Finally since most of these infections are nosocomial, there is a change in the infecting organism in later stages of wound management depending on the microbiologic environment of the institution.

Conclusions

1) Most of bacterial infections in open fracture wounds are nosocomial and the isolated bacteria would depend upon the microbiologic environment of the institution; however there may be a predisposition of particular bacteria towards a particular site of compound fracture.

So early coverage should be achieved within 1 week and should be the primary goal in managing open fracture wounds

- 2) Antibiotic coverage should be more specific towards gram-positive organisms as there is usually a shift in bacterial flora which occurs in the second week from Gram-negative to Gram-positive flora.
- 3) Pre-debridement, intra-operative, post debridement and cultures after 24hrs or at 1st dressing are not reliable indicators of final infecting organism and should not be done.

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