## Bacterial flora and their antibiotic susceptibility patterns in open fracture wounds at a tertiary hospital of north India

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### **Abstract**

The changes in pathogenic microbiological flora and the emergence of bacterial resistance have created major problems in the management of open fractures. A better understanding is required of the patterns or predilection of organisms and thus anticipating infection by a particular organism. Hundred and seven open fractures wounds of long bones were studied prospectively over a period of one year. Wound swabs were obtained and sent for cultures at regular intervals i.e.; at Pre-debridement, intra-operative, post debridement, at first dressing and then every week. The infecting organism and its antibiotic susceptibility were noted. Most of the infections were caused by Gram-negative organisms 64.7%, commonest being *Pseudomonasspp* (36%) which was resistant to most antibiotics and showed maximum sensitivity to piperacillin (85%). A shift in the bacterial flora was noted after the 2<sup>nd</sup> week from Gram-negative to predominantly Gram-positive organisms. Among the Gram-positive organisms, 93.5% were *Staphylococcus aureus*, 58.6% of which were methicillin resistant. Use of a broad spectrum antibiotic during the initial phase of management could prevent a change in the bacterial flora in later stages and thus decreasing chances of bacterial resistance. However the final selection of antibiotic should be tailored according to the type of fracture, level of contamination, soft tissue status, and most importantly the prevailing infection and culture sensitivity patterns in the hospital.

Key Words: Bacterial flora, Open fractures, Antibiotic

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### INTRODUCTION

Open or compound fractures are fractures that communicate with the outside environment through a skin wound. According to Gustilo-Anderson classification open fractures are classified in to three major types (type III has three subtypes) based on mechanism of injury, the

degree of soft tissue damage, the configuration of the fracture, and the level of contamination. Treatment for open fractures is a challenge. Prevention of infection is one of the prime objectives in management of open fractures<sup>1</sup>. Sepsis occurring in between 2% and 25% of all open fractures, leads to significant morbidity. Seventy percent of open fracture wounds are believed to be contaminated at the time of injury. Deep fracture-site infections can lead to chronic osteomyelitis, non – union, loss of function, or even limb loss<sup>2</sup>. 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. 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. Wound infecting pathogens differ from country to country<sup>3,4</sup>. The source of an infecting organism

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may be one of the following: a) endogenous, from patient's own flora; b) exogenous, from another patient or a member of the hospital staff or from the inanimate environment of the hospital; c) environment (air, food, water, soiled linen, hospital waste etc) d) Contamination of wounds at the time of injury<sup>5</sup>. This study was designed to determine the microbiology of the open fracture wounds at the time of initial treatment and development of subsequent infections. An attempt has also been made to study the bacterial flora in relation to various factors that affect the biology and outcome of open fractures which could help us in anticipating infection by a particular organism and thus providing appropriate antibiotic therapy.

### MATERIALS AND METHODS

This prospective study was done over a period of one year in the Department of Orthopaedics at a tertiary care hospital in north India. Patients brought to our trauma centre with open fractures of long bones were included and were classified according to the Gustilo and Andersonclassification (6). Patients with Gustilo's grade IIIC fractures requiring an amputation were excluded from the study. A wound swab was obtained at the time of presentation, tetanus prophylaxis, and irrigation of the wound was done. A third generation cephalosporin was usually started in Gustilo's grade I and II fractures and an amino glycoside was usually added in grade III open fractures. Primary wound debridement was done in the operation theatre under appropriate anaesthesia with appropriate stabilization of the bone. Wound cultures were obtained prior to and after debridement. Regular wound care was done and wound cultures were sent after 24hours of debridement at the time of first dressing and then subsequently every week. Any change in the intravenous antibiotics was recorded.

Microbiological Methods: For all wound cultures two swab sticks were obtained in a culture tube. One swab was used to obtain smear by Gram staining. The second swab was used for inoculation on Blood agar and Mac Conkey agar and the plates were incubated at 37 degrees Celsius overnight. The swabs were assayed for the predominant organisms found in culture and the microbial sensitivity/resistance patterns according to standard techniques<sup>7</sup>. Antibiotic susceptibility testing was done by Disc diffusion method and measuring diameter of zone of inhibition as described by Kirby Bauer method on Mueller Hinton Agar (MHA).

### RESULTS

Out of hundred and seven open fractures wounds, pathogenic bacteria were found in 43.9% cases. Most of the patients (27.1%) were in the age group of 20-30 years with a mean age of 33.93. The male female ratio was 9:1. Road traffic accident accounted for highest percentage of injuries (77.6%). Most of patients (46.6%) in our study had grade IIIB open fractures. The bacterial profile at different stages of wound care is shown in Table 1. The bacterial profile in relation to various factors like grade and site of fracture and duration of presentation after injury are sited in Tables 2 and 3. The antibiotic susceptibility of various pathogenic bacteria is detailed in table 4. Most of the initial wound cultures showed growth of Gram-negative organisms (71.4%) as compared to their only 40% growth in the final cultures. On the other hand Gram-positive organisms were present in only 28.6% of patients at admission as compared to their 60% growth in the final culture. This change occurred after the 2nd week as evident from the graph (Fig.1).

Table 1: Bacteria	I profile at	different stages	of wound care
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	l l	At	P	re-	Po	st-	•	st	and ,	week		nal	T	otal
ORGANISM	adm	ission	debrio	dement	debrid	ement	dre	ssing	2	week	г	паі	10	Jiai
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No growth	100	93.5	101	94.4	107	100	86	80.4	70	65.4	72	67.3		
Acinetobacter	2	1.9	2	1.9	0	0.0	7	6.5	11	10.3	2	1.9	24	20.7
Pseudomonas	1	0.9	0	0.0	0	0.0	8	7.5	9	8.4	9	8.4	27	23.3
E-coli	1	0.9	2	1.9	0	0.0	5	4.7	9	8.4	2	1.9	19	16.4
Proteus	1	0.9	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0	2	1.7
S-aureus	1	0.9	1	0.9	0	0.0	0	0.0	7	6.5	20	18.7	29	25.0
Klebsiella	0	0.0	0	0.0	0	0.0	1	0.9	1	0.9	1	0.9	3	2.5
Enterobacter	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.9	1	0.9
Beta-hemolytic streptococci	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.9
Non-hemolytic streptococci	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.9	1	0.9
Mixed growth	0	0.0	0	0.0	0	0.0	3	2.5	7	6.1	2	1.9	12	10.1

**Table 2:** Bacterial flora in different grades of open fractures

ORGANISM	GRADE I	GRADE II	GRADE IIIA	GRADE IIIB	GRADE IIIC
Acinetobacter	NO.	NO.	NO.	NO.	NO.
Pseudomonas	0	1	2	17	4
E-coli	0	2	4	16	5
P roteus	0	0	0	16	3
S-aureus	0	0	0	2	0
Klebsiella	1	1	6	18	3
Beta-hemolytic streptococci	0	0	1	2	0
Non-hemolytic streptococci	0	0	1	0	0
Mixed	0	0	1	0	0
Total	0	0	1	11	0
	1	4	16	82	15

Table 3: Bacterial flora in relation to duration and site of injury

ORGANISM	<6 HRS	6-12 HRS	12-48 HRS	>48 HRS	ARM	FOREARM	THIGH	LEG
Acenitobacter	20	1	2	1	0	0	7	17
Pseudomonas	17	5	1	4	6	0	3	18
E-coli	5	6	3	5	1	0	3	15
Proteus	0	0	0	2	0	0	1	1
S-arueus	6	9	10	4	2	1	7	19
Klebsiella	2	1	0	0	0	0	1	2
Beta-hs	1	0	0	0	0	0	1	0
Non-hs	0	0	0	1	0	1	0	0
Mixed	4	7	0	1	0	0	4	8
TOTAL	55	29	16	18	9	2	27	80

Table 4: Antibiotic susceptibility patterns (%)

Antibiotic	Acinetobacter	Pseudomonas	E-coli	Prot eus	Methicillin resistant S-aureus	Non-Methicillin resistant S- aureus	Klebsiella	Enterobacter
Ceftriaxone	33	41	47	50	29	50	0	100
Cefotaxime	71	81	47	100	53	75	0	100
Cefoperazone	21	52	21	100	23	75	67	100
Ceftazidime	46	56	42	50	23	50	0	100
Cefuroxime	4	18	16	0	18	42	33	100
Ciprofloxacin	17	41	37	50	35	25	0	100
Cephalaxin	4	4	11	0	18	83	0	0
Netromycin	92	33	90	0	59	50	33	0
Ofloxacin	75	60	84	100	76	83	66	100
Pefloxaxin	4	26	5	0	0	3	33	0
Gentamycin	22	15	26	0	0	16	0	0
Amikacin	50	44.	74	0	41	75	66	0
Cloxacillin	0	0	0	0	5.8	25	0	0
Pipracillin	46	85	5	0	5.8	16	33	100
Tobramycin	25	23	16	0	0	10	0	0
Sparfloxacin	33	26	11	0	0	8	33	100
Augmentin	29	11	42	50	82	83	33	0
Sisomycin	17	22	0	0	0	8	0	0
Lincomycin	4	4	0	0	59	66	0	0
Vancomycin	4	4	0	0	100	100	0	0

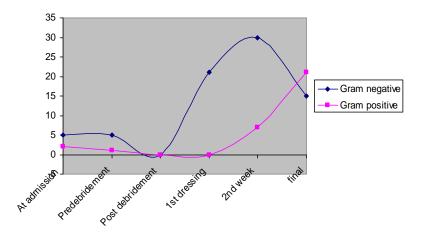


Figure 1: Graph showing bacterial flora in different stages of wound management

## **DISCUSSION**

Infection rates among open fractures, ranging from 2%-25% have reported in the past<sup>8</sup>. In this study we observed a high infection rate i.e. 43.9%. Some of recent studies by Ikem et al<sup>9</sup> and Sen et al<sup>10</sup> have similarly reported high incidence of infection i.e.; 45.8% and 45% respectively. The high infection rate in our study could be explained by the predominance of Grade III injuries and high velocity trauma which are likely to have a higher level of tissue contamination. Microbiology of open fractures has been changing since the late 70s. Coagulase-positive Staphylococcus aureus was predominant in the 70s and early  $80s^{11,12}$ . However, over past several decades the pattern of infection has been changing and gram negative bacteria are becoming more and more common<sup>3</sup>. Our study shows that gram negative infections continue to be a major threat and were isolated from 64.7% cases. Pseudomonas spp (23.3%) was the commonest gram negative bacteria isolated in our study. Gram-positive organisms accounted for 26.7% of infections and the predominant Gram positive organism was Staphylococcus aureus (93.5%), 58.6% of which were methicillin resistant. Recent studies by Akinyoola et al<sup>13</sup> and Ako-nai et al<sup>14</sup> similarly reported predominance of gram negative bacteria i.e.; 40.5% and 53.2% respectively. Akinyoola et  $al^{13}$  also observed pseudomonas spp (11.2%) to be the predominant gram-negative organism. However Ako-nai et al<sup>14</sup> found E-coli (12.8%) to be the commonest gramnegative organism. The use has become almost universal and widespread in management of open fractures. Recently it was hypothesized that this strategy may lead to selection of more virulent and also antibiotic resistant bacteria which subsequently would result in an increased infection rate over the years<sup>15</sup>. Our study clearly shows that Pseudomonas spp is resistant to most antibiotics as

also observed by Agarwal et al<sup>9</sup>. Various studies have been conducted till date, studying the bacterial flora in open fracture wounds but there is a dirth of studies which evaluate the flora in relation to various factors that affect the biology and outcome of open fractures. In our study, we had made an attempt to study the bacterial profile in relation to some of these factors which could probably help in selection of prophylactic antibiotics. We observed that Acinetobacter spp (23.3%) and Pseudomonas spp (54.5%) were more likely to be isolated from open fractures of the lower and upper limbs respectively. Staphylococcus aureus was predominant isolate among grade I (100%), IIIA (37.5%) and IIIB (22%) groups whereas Grade II (50%) and IIIC (33.3%) fracture wounds had maximum infection with *Pseudomonas spp.*. Fresh wounds reaching within 6hrs were more likely to grow Acinetobacter spp and Pseudomonas spp whereas unusual pathogens like E-coli and Proteus were isolated in increasing frequency with increasing duration of injury which could probably be the flora prevalent in the hospital were they had received initial treatment. A shift in the bacterial flora was also noted from Gram-negative to Gram-positive 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. Thus suppressing the 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. It could thus be concluded from our study that most of bacterial infections in open fracture wounds are acquired during the course of treatment and the isolated bacteria would depend upon the microbiologic environment of the institution; identifying the patterns or predilection of

organisms and anticipating infection by a particular organism in that institution might be worthwhile. Secondly a change in bacterial flora from Gram-negative to Gram-positive occurs usually in the second week. Broad spectrum antibiotic during the initial phase of wound management might prevent this change and early coverage of the wounds within the first week would further decrease the incidence of nosocomial infections. However, the final selection of antibiotic treatment should be determined by the previous experience of organisms isolated and sensitivity studies done from open fracture wounds in each institution. Setting up of infection control programmes in each institution could prove worthwhile especially in developing countries where they are still non-existent or in their infancy.

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