To study the changing pattern of bacterial flora and their sensitivity patterns in grade IIIB open fractures of long bones: a prospective study at a Tertiary Centre in North India

1. Dr. Gursagardeep Singh  
   MS Orthopaedics  
   Email id: drgursagardeepsidhu@gmail.com  

2. Dr. Mandeep Singh  
   MS Orthopaedics  
   Email id: drmandeep86@gmail.com  

3. Dr. Anshul Dahuja  
   Assist. Prof Dept. of orthopaedics GGS Faridkot Punjab India  
   Email id: anshuldahuja@gmail.com  

4. Dr. Rashmeet Kaur  
   Assist. Prof Dept. of radiodiagnosis GGSMCH Faridkot Punjab India  
   Email id: dr.rashmeet@gmail.com  

5. Dr. Radhe Shyam  
   Prof. and Head Dept. of Orthopaedics GGSMCH Faridkot Punjab India  
   Email id: dr.rsg29@gmail.com  

6. Dr. Haramritpal Kaur  
   Associate professor, Dept. of Anaesthesia GGSMCH Faridkot  
   Email id: amritk_dr@yahoo.co.in  

7. Dr. Shipra  
   Assistant professor Dept. of Microbiology GGSMCH Faridkot  
   Email id: shipragalhotra@gmail.com  

Corresponding author: Dr. Anshul Dahuja: Assistant professor, Department of orthopaedics GGSMCH Faridkot India  
   Email id: anshuldahuja@gmail.com  

Abstract: Primary goal in management of open fractures is prevention of infection of bone and soft tissue by early debridement, irrigation of wound and administration of broad-spectrum antibiotics with stabilization of fractures. The pattern of organisms found in open fractures is important in the selection of antibiotics for prophylaxis and empirical treatment. In this prospective study, 50 consecutive patients of all ages, both the sexes, with open fracture of grade IIIB as per Gustilo-Anderson classification were evaluated for bacterial isolates. Higher rate of infection was found in patients with farm injuries and leg was most common.
site. The most common gram negative and gram positive bacteria isolated were E.coli and S.aureus respectively (19.5% each). On analysis of the predebridement, post-debridement, and third culture, positive pre-debridement culture showed maximum growth of Gram-positive bacteria. However, majority of these patients were found to have growth of different organism in their post-debridement culture reports.

Key words: Antibiotics, Bacteria, Fracture, Wound.

Introduction
Open fractures are usually caused by high-energy trauma and are characterized by variable degrees of soft tissue and skeletal injury, both of which impair local tissue vascularity.[1] All open fracture wounds should be considered contaminated because of the communication of the fracture site with the outside environment.[2] The resulting contamination of the wound with microorganisms, coupled with the compromised vascular supply to the region, leads to an increased risk of infection as well as to complications in healing.[3] Wound contamination occurring in traumatic wounds can be exogenous or endogenous. It may be classified as primary or secondary depending upon contamination occurring at the time of injury or shortly after injury and occurring 24 hours or longer after injury respectively.[4] Organisms isolated from cultures obtained from various fracture sites are from numerous genera. However, the consistent isolation of particular aerobic or anaerobic microorganisms, point to the possibility of skin, fecal or environment contamination of fracture wounds. 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.[3-5] 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. Wound infecting pathogens differ from country to country and from one hospital to another within the same country due to the difference in bacterial prevalence in different environments. Therefore, bacteria that infect open fracture wounds and antimicrobial therapy used vary from hospital to hospital.[6] In this study, we studied the pattern of bacterial isolates in all cases of open fractures of long bones and their antimicrobial susceptibility pattern.

MATERIAL AND METHODS
In this prospective study, 50 consecutive patients of all ages, both the sexes, with open fracture of grade IIIB as per Gustilo-Anderson classification, admitted in the department of Orthopaedics at Guru Gobind Singh Medical College and Hospital, Faridkot from January 2016 to June 2017 were selected to study the pattern of bacterial isolates.[2] Ethical approval had been taken from the Institutional Ethical committee (BFUHS/2k15-TH/12747) Inclusion criteria: All patients with Gustilo grade IIIB fractures of long bones admitted in the Department of Orthopaedics were included in this study. Exclusion criteria: All Patients with Gustilo grade I, II, IIIA, IIIC were excluded from the study. For all grade IIIB fractures of long bones a wound swab was obtained at the time of presentation, before debridement, after debridement, after 24 hours of debridement/first dressing and in second week. Reports of cultures at admission, pre-debridement, post-
debridement, first dressing and second week were recorded. Wounds were regularly monitored in the ward during the stay of patient in hospital. Wounds were managed as per expected protocol. Any signs of infection were recorded as positive bacterial culture along with discharge from the wound. Tetanus prophylaxis, intravenous antibiotics were given and initial irrigation of the wound was done in the casualty or OPD. Primary wound debridement and appropriate bone stabilization was done in the operation theater under appropriate anaesthesia. Wound debridement was done as per requirement and any change in antibiotic was noted. Antibiotic sensitivity was performed using Kirby Bauer Disc Diffusion method. Results were reported according to Clinical and Laboratory Standard Institute (CLSI) guidelines.[7] Statistical methods: Microsoft excel + SPSS 20.0 was used for data compilation and appropriate analysis. Descriptive and summary statistics were used to evaluate the data. Various tests of significance were used wherever required.

Results
Majority of our patients were in age group of 21-40 years with male predominance (as shown in graphs 1-6). Road side accidents were most common mode of presentation with leg was the most common involved site of injury and most of patients presented with in <6 hours of injury. Infection rate was found higher in age group of 41-60 years with male predominance (as shown in graph 7-8). But we found no statistical significance between infection rate and age. Higher rate of infection was found in patients with farm injuries and leg was most common site where infection rate was higher (as shown in graph 9-10). The most predominant gram-negative bacteria isolated was E. Coli (19.5%), followed by klebsiella (16.7%) and third most common was Pseudomonas (13.9%). Other gram-negative bacteria isolated were as Citrobacter (11.1%), Acinetobacter (8.3%), Enterobacter and Proteus (5.5% each). The most common Gram-positive bacteria isolated was S. aureus (19.5%) (as illustrated in graph 11-12).

Discussion
In our study, on analysis of the pre-debridement, post-debridement, and third culture, positive pre-debridement culture showed maximum growth of Gram-positive bacteria. However, majority of these patients were found to have growth of different organism in their post-debridement culture reports. One of the major problem and complication in the treatment of open fractures is infection which leads to significant morbidity. Prevention of wound sepsis remains a major objective in management of open fractures. The reported infection rates in grade IIIB fractures range from 10-50% which poses challenge to every surgeon who treats them. In our study we had taken 50 patients with grade IIIB fracture of long bones. We aimed to study changing pattern of bacterial flora in grade IIIB fracture of long bones in context to site, mode of injury, and various other modalities as described in results before. We have also attempted to study antibiotic susceptibility of bacterial flora and various factors that hamper the infection rate in order to make better and more effective protocols for management of open wound and compound fractures.
Age and gender incidence:
In our study the mean age of patients was 36.98 (range 7-70) years old. And amongst this population, 41 (82%) were males and 9 (18%) females which was similar to previous studies. [8,9,10]

Mode of injury:
In our study road side accidents predominated as the most common mode of injury, involving 38 (76%) patients followed by fall from height 5 (10%), then by assault (6%), industrial and farm injuries (4% each). Roth et al reported most common mode of injury in their population studied to be road side accidents (68%) followed by fall from height (6.7%) then fire arm injuries (15%) and lastly they found assault (3.5%) as least common mode of injury in their study. [3] similar findings were observed by multiple other studies. [8-11]

Site of injury:
Gustilo et al observed leg to be most common site of injury accounting for 63.2% of cases similar to Roth et al (52.1%) and Dellinger et al (50.6%).[2,3,6] Fernandes at al also observed most common site of injury to be leg in 62.2% of cases studied.[10] In our study leg was the most common site of injury, involving 26 (52%) patients followed by thigh in 11 (22%) patients, followed by arm 7 (14%), and lastly by forearm 6 (12%).

Infection rate in relation to duration of presentation after injury:
Roth et al found that interval from injury to initial debridement did not alter the infection rate. Patient who were debrided within 3 hours of injury had an 11% infection rate, 3-6 hours had 14% and > 6hrs had 15% infection rate.[3] Dellinger et al and Merritt et al found no correlation between the time interval between injury and surgery. [6,11] In our study, we found no correlation between duration of presentation and infection rate.

Infection rate in relation to gender and age:
Meritt et al and Bowen et al found no statistical significance between infection rates of male and female.[11,12] In our study also we have found no statistical significance of infection rate between different gender and increasing age.

Infection rate in relation to mode of injury:
Meritt reported infection rate as 100% in farm injuries, motor cycle accidents 32%, gunshot wounds 22%, automobile accidents 7% and others 9%. [11] Similarly, in our study we have found 100% infection rates in farm injuries, followed by fall from height, then industrial injuries. It is because of the fact that farm injuries are more exposed to soil and dust and hence these have higher chances of infection.

Infection rate in relation to site of injury:
In our study, we also found higher infection rate in leg followed by thigh and last in arm and nil in forearm similar to earlier studies. [3,6,10]
Bacterial flora isolated:
Gustillo and Anderson isolated gram-negative bacteria in larger number of patients as compared to gram positive i.e. they found enterobacter colonies in 26% of cases followed by E. coli in 16% of cases then they found proteus in 13% of cases and klebsiella and pseudomonas were isolated in 10% and 6% cases. While in category of gram-positive bacteria S. aureus was isolated in 13% of cases.[13]

Dellinger et al isolated gram-positive bacteria in 36.5% study population in which S. aureus was in 34.5% of study population followed by β hemolytic streptococci in 1.8% of population. Out of gram negative bacteria, most common organism isolated was enterobacter (18.2%), followed by pseudomonas (10.9%), then by E. coli and klebsiella (1.8% each). They also isolated anaerobes in 3.6% of population.[6]

Lakshminarayan et al isolated S. aureus in 40.9% of population and β hemolytic streptococci in 2.3% of population while E. Coli (15.9%) constituted majority of gram-negative bacteria followed by growth of pseudomonas (13.6%), Proteus (4.6%) and Klebsiella (10.2%) of population.[14] Johnson et al isolated S aureus in 18.2% of population in gram-positive category. In gram negative bacteria Acinetobacter was most common (19.7%) followed by Pseudomonas (12.1%). Enterobacter growth was found in 9.09% and the least growth was found of E. coli and klebsiella (4.54% each).[15] Agrawal et al reported the most common gram-negative bacteria as E Coli (34.2%), followed by Pseudomonas (26.1%). Klebsiella growth was found in 8% cases followed by Proteus (6.3%). In gram positive group S. Aureus growth was found in 18.9% of population followed by β hemolytic streptococci.[5]

In our study, the most predominant gram-negative bacteria isolated was E. Coli (19.5%), followed by klebsiella (16.7%) and third most common was Pseudomonas (13.9%). Other gram-negative bacteria isolated were as Citrobacter (11.1%), Acinetobacter (8.3%), Enterobacter and Proteus (5.5% each). The most common Gram-positive bacteria isolated was S. aureus (19.5%). Clearly microbiology of open fracture wounds favours hospital acquired bacteria and isolates depend on microbiologic environment of institution where study is being conducted.

Role of wound cultures:
Lee et al reported the prognostic value of bacterial cultures. Only 8% of organisms grown on predebridement cultures eventually caused infection; 7% of cases with negative predebridement cultures became infected. Of cases that did become infected, predebridement cultures grew the infecting organism only 22% of the time. However, the cultures taken late after debridement were more accurate in predicting infection rate.[16]

Lingaraj et al (2015) in their study also found that cultures taken early in the predebridement period have no value in predicting post-debridement wound infection.[17] As in above mentioned studies, we also found the similar results that cultures taken on admission, pre-debridement, immediate after debridement are less specific in predicting the infection as compared to the cultures taken on first dressing, second week. We observed that compound tibia fractures are more prone to infection because of severe comminution, contamination, and
devitalization due to superficial location, subcutaneous characteristic, delay in providing early coverage, and moreover delay in getting proper medical care at right time, which coincides with the findings of earlier studies.[18-20] Postdebridement culture can be best used to lay down proper antibiotic protocol according to the sensitivity pattern found in our hospital for all patients with open fractures.

**Incidence of infection:**
Different studies had shown infection rate between 29% to 52%. [2,6,15,18] In our study we found incidence of infection to be 42% which is comparative to study undertaken by Gustilo et al.

**Role Of blood investigations:**
In our study on using chi square test value to find significance of investigation with infection rate. Increased TLC, ESR and TSA (Total Serum Albumin) were found to be significant with infection rate. On the contrary, low Hb was not found to be associated with higher infection rate.

**Antibiotic senstivity:**
Laxminarayan etal. reported the most commonly isolated organisms were Staphylococcus aureus (40.90%), Escherichia coli (15.9%), Pseudomonas aeruginosa (13.6%). 12.5% of Staphylococcus aureus were methicillin resistant.[14] All Staphylococci were susceptible to vancomycin, linezolid and teicoplanin. All gram-negative bacilli were sensitive to colistin and tigecycline. Agrawal etal reported Cefoperazone and Ceftriaxone to be the most effective antibiotics against gram-negative bacteria while Cefaperazone was equally effective against S. aureus.[5] Emerging resistance was found against amoxicillin, ampicillin and the aminoglycosides. In our study we had more growth of gram-negative bacteria as compared to gram positive bacteria. In gram negative group E. Coli was predominant followed by Klebsiella then by Proteus. Most common gram-positive growth was of staphylococcus aureus. Gram positive bacteria were found highly sensitive to linezolid and vancomycin, moderately sensitive to clindamycin, erythromycin, cefotaxin. Gram negative bacteria were found to be sensitive to colistin and imipenam and they were moderately sensitive to aminoglycosides. Vancomycin, imipenem, meropenem, teicoplanin and Linezolid were highly effective across both gram negative and gram-positive organisms. 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.

**CONCLUSION**
In view of the bacterial species leading to infections we have to deal more frequently with hospital acquired bacteria than with environmental bacteria from the accident site. The initial debridement and early antibiotic prophylaxis certainly have contributed to the low incidence of environmental bacteria, whereas the high incidence of hospital-acquired bacteria should be targeted in future. Although the bacteria were sensitive to higher antibiotics, but these antibiotics should be used with utmost care in order to prevent the resistance to the respective drug.
Prevention of infection in open fractures need to be further improved by a combination of specific antibiotics and surgical wound management yielding at definite wound closure as soon as possible by all means. Limitations: Small sample size, single center trial. Conflict of interest: none

Acknowledgement: Department of microbiology and social & preventive medicine.

References:
10. Fernandes MC, Peres LR, Neto AC, José Queiroz Lima Neto, Turbio FM, Matsumoto MH. Open fractures and the incidence of infection in the surgical debridement 6 hours after trauma acta ortop bras. 2015;23(1):38-42

**Graph 1: Age distribution of patients**
GRAPH 2: Distribution of patients according to gender

- Male: 41
- Female: 9
- Total: 50

Graph 3: Distribution of patients according to mode of injury

- RSA: 38
- Fall from height: 5
- Farm: 2
- Assault: 3
- Industrial: 2
- Others: 0
- Total: 50
Graph 4: Distribution of patients according to site of injury

![Bar chart showing the distribution of patients by site of injury. Leg: 26, Thigh: 11, Forearm: 6, Arm: 7, Total: 50.]

Graph 5: Distribution of patients according to duration of presentation after injury

![Bar chart showing the distribution of patients by duration of presentation. <6: 26, 6-12: 12, 12-48: 7, >48: 5, Total: 50.]

Duration (Hours)
Graph 6: Infection rate in relation to duration of presentation after injury

Graph 7: Infection rate in relation to different age group
Graph 8: Infection rate in relation to gender

Graph 9: Infection rate in relation to site of injury
GRAPH 10: Infection rate in relation to mode of injury

Infection rate in relation to mode of injury

Graph 11: Distribution of bacterial flora in relation to isolation at different stages

Distribution of bacterial flora in relation to isolation at different stages

- Admission
- Pre-debridement
- Post-debridement
- First dressing
- Second week
- Total
Graph 12: Distribution of bacterial flora in relation to duration of presentation after injury

![Bar chart showing distribution of bacterial flora by duration of presentation after injury.](image)

Graph 13: Infection rate in relation to haemoglobin

![Bar chart showing infection rate by haemoglobin levels.](image)
Table 14: Infection rate in relation to TLC

Graph 15: Infection rate in relation to TSA