Original Research Article

Clinico-Microbilogical profile of Chronic Osteomyelitis in a Tertiary care Hospital of North India

ABSTRACT

Aims: Chronic osteomyelitis is an important clinical entity in patients with bone infections and is associated with great morbidity especially in developing countries. It is a persistent disease, difficult to treat and eradicate completely. Early identification and diagnosis of osteomyelitis has led to the improved management of osteomyelitis. This study was undertaken to determine the bacteriological profile of osteomyelitis and the antibiotic resistance pattern of various isolates obtained.

Study design: It was a prospective cross-sectional hospital based study

Place and Duration of Study: The present study was conducted in the Department of Microbiology, Government Medical College, Srinagar, from January 2019 to December 2019.

Methodology: In all, 208 patients with chronic osteomyelitis were documented during the study period. Clinical specimens like pus, pus swabs, sequestrum of bone and synovial fluid were taken and cultured aerobically .The samples were processed using standard microbiological techniques. Identification and antimicrobial susceptibility pattern of the bacterial isolates were done using the Vitek 2 (bioMerieux, France) system.

Results: A total of 208 samples were received out of which, 91 (43.75%) were positive by culture. Out of the 91 positive samples, *Staphylococcus aureus* 65 (64.2%) was the most commonly isolated pathogen and 67. 6 % of *Staphylococcus aureus* were MRSA. Other important organisms isolated included *Acinetobacter sp* (10.98%), *E. coli* (6.59%) ,*Proteus mirabilis* (4.3%) ,*Pseudomonas aeruginosa* (2.1%) and Klebsiella pneumonia (2.1%) . All the isolates of *Staphylococcus aureus* were resistant to Penicillin. However, Vancomycin resistance was not detected in any of the patients with MRSA. All Gram negative bacilli were sensitive to Colistin.

Conclusion: The wide range of causative organisms and degree of resistance to commonly used anti-microbials supports the importance of pus culture and provides important information to guide clinician's choice of empirical antibiotics. Appropriate selection of antibiotic would help to treat the disease successfully and limit the emergence of drug resistant strains to prevent morbidity & mortality.

Keywords: Osteomyelitis, Staphylococcus.aureus, MRSA, Antibiotic resistance

1. INTRODUCTION

Osteomyelitis is a bone marrow inflammation, usually caused by an infectious agent. It has a heterogeneous patho-physiology, and is one of the most difficult infections to cure. Osteomyelitis can be classified into acute, sub- acute or

chronic, according to the time of evolution. The source of the infection may be haematogenous, acquired from a contiguous infectious focus or by direct inoculation into the bone. [1, 2, 3]

Acute osteomyelitis presents with inflammatory bone changes caused by pathogenic bacteria and symptoms are seen within two weeks after infection. Chronic osteomyelitis usually occurs by contiguous spread or direct inoculation of bacteria into bone from contiguous soft tissue infection or a chronic overlying open wound and has been present for several weeks .[4]

The bacteria most commonly causing chronic osteomyelitis are *Staphylococcus aureus, Coagulase negative Staphylococcus, Pseudomonas spp., E. coli, Proteus spp., Klebsiella spp., Enterococcus spp., Enterobacter spp.* and anaerobes like *Peptostreptococcus spp., Bacteroides spp., Clostridium spp.* and rarely *Salmonella spp.* and *Actinomycetes*.[5]

Chronic osteomyelitis commonly involves long bones; especially tibia and femur.[6]The most important risk factors of osteomyelitis are trauma (primarily open fractures and severe soft tissues injury), vascular insufficiency, diabetes, elderly, children, obesity, surgical wound infection and haemoglobinopathies such as sickle cell diseases. [6,7]

Chronic osteomyelitis is a difficult entity to eradicate completely. There may be subsidence of systemic symptoms, but the cavities containing purulent material, infected granulation tissue or sequestrum act as foci of infection. In order to achieve eradication of the disease, aggressive surgical debridement with curettage of cavities, filling of cavities with soft tissues and effective antimicrobial treatment is needed.[8]

Proper management of chronic osteomyelitis requires accurate microbial isolation and appropriate antibiotic administration. The incidence of osteomyelitis has been lowered to a certain extent due to the rapid diagnosis and the availability of multiple antibiotics along with modern treatment facilities [9]. But osteomyelitis, still, is an ongoing problem due to emergence of multi drug resistant strains.

Diagnosing the etiological agent and appropriate use of antibiotics are crucial in the treatment of infection preventing further complications [10]. This study was undertaken to emphasize the need of culture based antibiotic therapy to help the clinicians in choosing appropriate antibiotics. Proper antibiotic coverage will also help in preventing emergence of resistance to the drug to which they are still sensitive.

2. MATERIAL AND METHODS

Study Design

The present study was conducted in the Department of Microbiology, Government Medical College Srinagar over a period of 1 year from January 2019 to December 2019.

Inclusion Criteria: Consecutive samples of all clinically diagnosed patients of chronic osteomyelitis of all age groups and both sexes received in the department of Microbiology , Government Medical College Srinagar during the study period were included .

Exclusion Criteria: 1. Patients other than chronic osteomyelitis were excluded from study.

2.Causes other than aerobic bacterial organisms were excluded from study.

Chronic osteomyelitis was defined as a bone infection that was worse or had not improved clinically or microbiologically after 1 month of evolution, independent of the presence or absence of surgical and/or antimicrobial therapy. Information about socio demographic characteristics like age, sex, educational background, occupation, residence, and patient setting werealso collected using structured questioner.

Specimens like pus, pus swabs, sequestrum of bone, synovial fluid were collected under aseptic precautions. The samples were processed aerobically using routine standard operative procedures. The culture isolates were identified by

Gram stain morphology, colony characters and biochemical reactions [11, 12]. Identification and antimicrobial susceptibility pattern of the bacterial isolates was confirmed using the Vitek 2 (bioMerieux, France) system.

Statistical analysis

Data were collected and transferred to computer and statistically analyzed using SPSS Data Editor Software, Chicago, version 20. Frequency and percentage were used for the categorical and ordinal variables. Mean, range (minimum and maximum values) and standard deviation (SD) were used for the continuous variables. Chi-square test was performed and p value ≤0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

Results

In all, 208 patients with chronic osteomyelitis were documented during the study period out of which 91 cases (43.7%) were positive after aerobic culture.

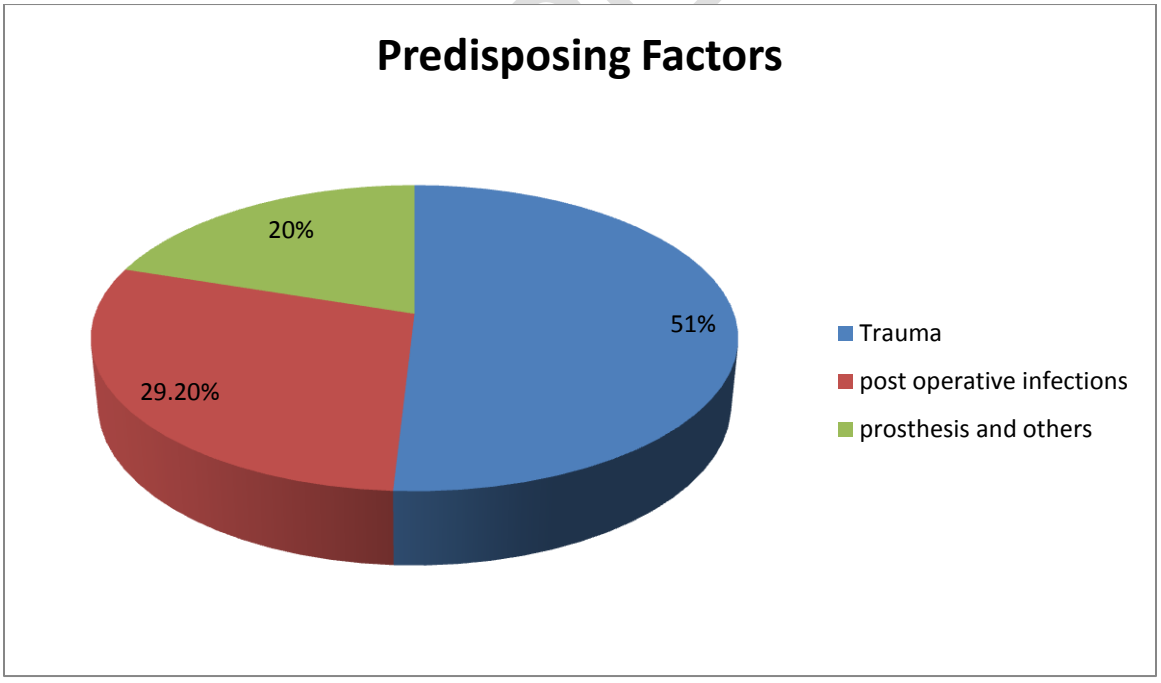
There was a male preponderance (82%) with majority, in the age group of 1-20 years (55.76%) followed by 21-40 years (17.3 %) , 41-60 (17.3 %) , 61-80 (7.6 %) and above 80 (1.9%) as shown in Table 1.

Table 1: Distribution of osteomyelitis cases according to their age groups

Age Group(years)	Number of cases of osteomyelitis(%) N=208
1-20	116 (55.8%)
21-40	36 (17.3%)
41-60	36 (17.3%)
61-80	16 (7.7%)
>80	04(1.92%)

The major predisposing factor identified was trauma (51 %) , followed by post surgical wounds (29 %) , prosthesis & others (20 %) as shown in Figure1 .

Figure 1: Distribution of osteomyelitis cases according to its various predisposing factors.



The bones of the lower limb were affected the most of which femur (25,27.4%) was the commonest bone involved followed by tibia(16,17.5%), refer (Table 2).

Table 2. Distribution of osteomyelitis cases on the basis of the affected bone (n =91).

Bone Involved	Number of cases(%)
Femur	25(27.4%)
Tibia	16(17.5%)
Humerus	8(8.7)%
Radius	6(6.5%)
Metatarsals	11(12%)
Vertebrae	15(%)
Metacarpals	10(10.9%)

Socioeconomic status of the cases was analyzed and almost 63% cases affected were from the lower income group and 37 % were from the middle income group; no involvement of higher income group with osteomyelitis was observed in this study.

Among the 208 cases studied, culture positivity was obtained in 91 cases (43.7 %). The dominant organism obtained in the present study was *Staphylococcus aureus* ,65 (64.21 %), followed by *Acinetobacter sp*.10 (10.98%),*E.coli* 6 (6.59%), *Proteus mirabilis* 4 (4.39 %), *Klebsiella pneumonia* 2 (2.19%) *Pseudomonas areuginosa* 2 (2.19 %),*Enterococcus* sp 1 (1.09%) and *Serratia marcescens1*(1.09%) as depicted in Table 3.

Table: 3 Organisms isolated in chronic osteomyelitis

Organism	No. of organisms	Percentage (%)
Staphylococcus aureus	65	64.21 %
Acinetobacter sp	10	10.98%
E.coli	6	6.59%
Proteus mirabilis	4	4.39%
Klebsiella pneumonia	2	2.19%
Pseudomonas areuginosa	2	2.19%
Enterococcus sp	1	1.09%
Serratia marcescens	1	1.09%

Among Staphylococcus species, 67.69% were MRSA and 32.30 % were MSSA.

Antibiotic susceptibility patterns of *Staphylococcus* species, Gram negative bacilli / fermenter and Gram negative bacilli / non- fermenter have been presented in Table 4 , Figure 2 and Figure 3 respectively .

Table 4 :Antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from osteomyelitis cases.

Antibiotics Tested	Antibiotic Resistant Pattern
Penicillin	Sensitive: 5 (7.69%) Resistant: 60(92.3%)
Erythromycin	Sensitive:27 (41.5%) Resistant: 38 (58.4%)
Clindamycin	Sensitive: 42 (64.6%) Resistant:23 (35.3%)
Tetracycline	Sensitive: 60 (92.3%) Resistant: 04 (6.1%)
Gentamicin	Sensitive:07(10.7%) Resistant: 58(89.2%)
Ciprofloxacin	Sensitive: 28(43.0%) Resistant:37(56.9%)
Cotrimaxazole	Sensitive:40(61.5%) Resistant: 25(38.4%)
Linezolid	Sensitive :63 (96.9%) Resistant : 02(3.0%)
Vancomycin	Sensitive: 65(100%) Resistant :0(0%)
Cefoxitin	Sensitive : 21(32.3%)

	Resistant : 44 (67.6%)

Figure 2 showing antibiogram of gram negatve organisms - Fermenters

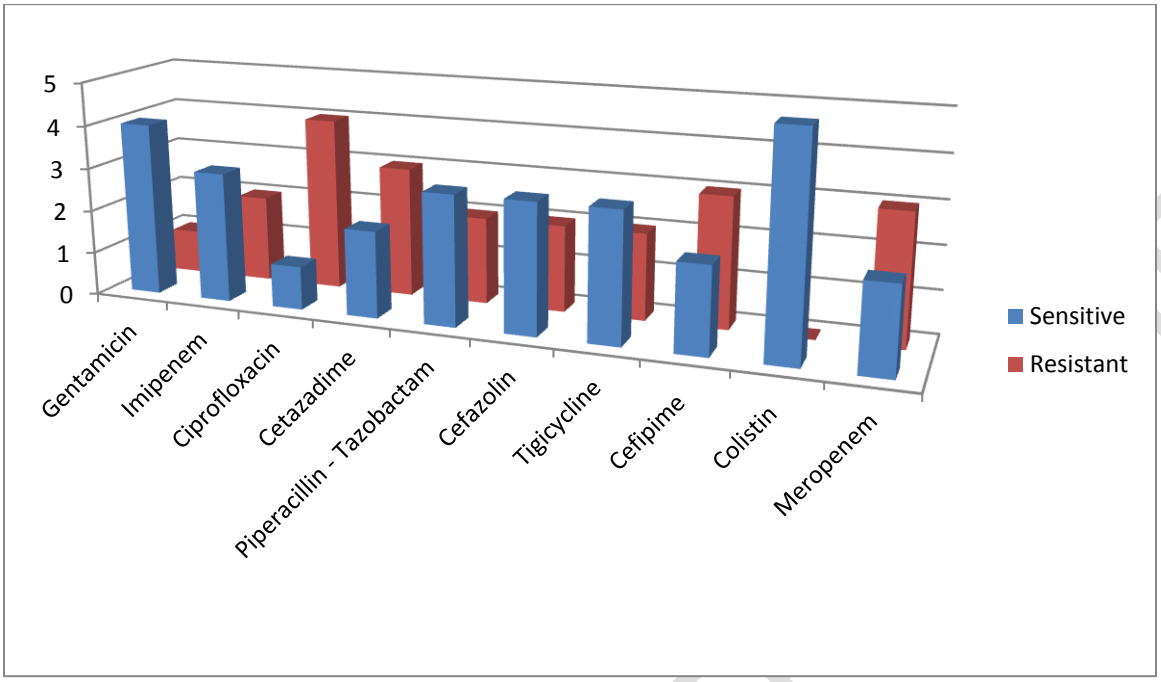
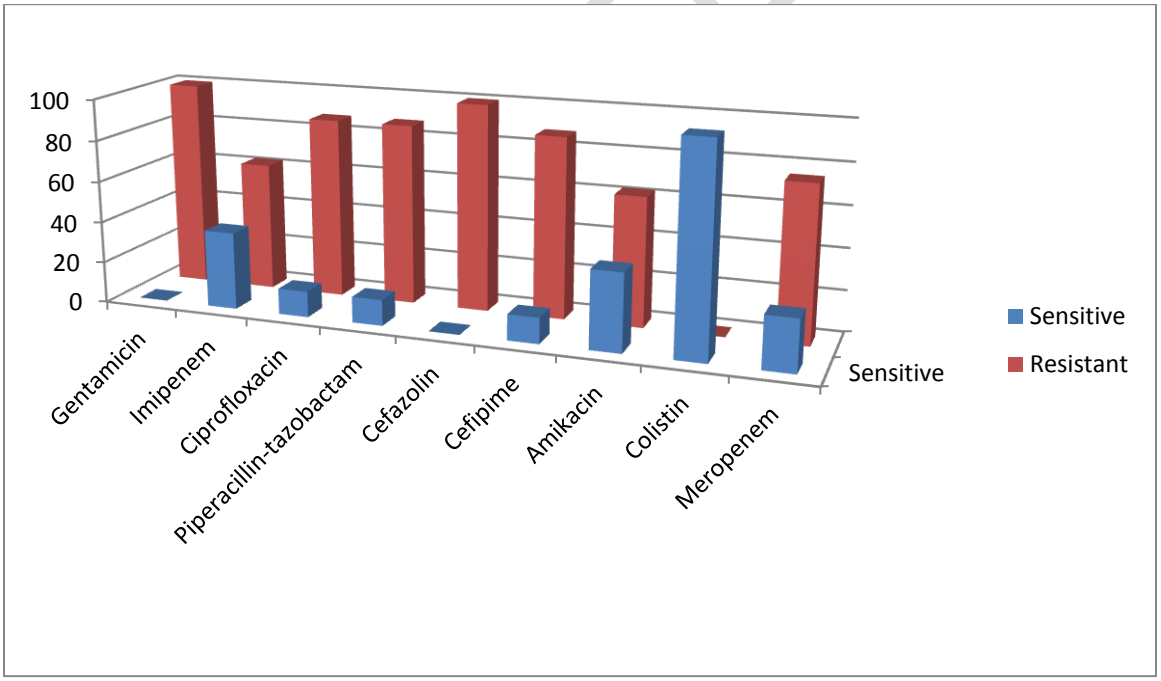


Figure 3 showing Antibiogram of gram negatve organisms -Non fermenters



Discussion:

Osteomyelitis remains a difficult entity to treat despite major advances made in surgery and antimicrobial therapy. Also the increase in the emergence of drug resistant strains makes treatment even more complicated. Hence, area-wise

continuous monitoring of bacteriological profiles and their antimicrobial susceptibility pattern are essential to guide policy on the appropriate use of antibiotics. The aim of this study was to characterise the organisms causing chronic osteomyelitis in our geographical area and to study their anti-microbial resistance and sensitivity patterns.

We included 208 patients in our study out of which 91 cases (43.7%) were positive after aerobic culture. In the current study the ratio of infected males to females is 4.5:1; this finding may be due to gender bias present in the society. And also the more frequent exposure of males to accidents, missile injuries, trauma, and fractures .Male preponderance was also reported in a number of studies.[13,14]

In our study the maximum incidence of osteomyelitis was observed among males and in age groups between 1-20 and 20 -40 years. In most other studies also same findings were seen. [13, 15] This may be because patients with open fractures are usually young men in their teens or twenties and also young adults and adolescents who use motor vehicles are more prone to develop post traumatic osteomyelitis following accidents.The major predisposing factor of oseteomyelitis was trauma (51%), followed by post-operative infections (29%) and orthopaedic implants and prostheses (21.0%).Similar findings were observed by Suguneswari et al and Muggeridge et al [16, 17]

The common bones involved in this study were lower extremities which are similar to the studies by, Kaur J. et al [9] and Muggeridge E. et al .[17] Commonest bone affected was femur 43.33% which was also observed in the studies by Zuluaga AF et al [18]and Perry CR et al[19]

The dominant organism obtained in the present study was *Staphylococcus aureus* ,65 (64.21 %), followed by *Acinetobacter sp*.10 (10.98%),*E.coli* 6 (6.59%), *Proteus mirabilis* 4 (4.39 %), *Klebsiella pneumonia* 2 (2.19%) *Pseudomonas areuginosa* 2 (2.19 %),*Enterococcus* sp 1 (1.09%) and *Serratia marcescens* 1(1.09%).

Gilmore et al. (2009),[20] also observed that the most common pathogen responsible for osteomyelitis in humans is *Staphylococcus* species followed by Enterobacteriaceae and *Pseudomonas* species. Chihara S et al. (2010),[21] reported that coagulase-negative staphylococci are often seen in association with foreign bodies such as prosthetic joints.Various previously done studies also reported *Staphylococcus* as the major isolate from osteomyelitis cases.[16,22,23]

However, results of study done by Agarwal AC et al. (2008),[24] showed *E. coli* was the main pathogen in 34.4% cases especially in open fractures, chronic osteomyelitis, bedsores, and patients with spinal instrumentation. This may be because *E. coli* is a commensal of the gut and as many orthopaedic patients are bedridden for prolonged periods, contamination of wounds, dressings, linen, clothes, and even hands during perineal hygiene plays a major role in increasing chances of transmission of infection. Identifying the pathogenic bacteria involved is an important step in treating chronic osteomyelitis. Based on characteristics of the pathogenic bacteria, effective antibiotics specific to the bacteria found should be used in clinical practice;

The most alarming finding was that 67.69% of *S. aureus* strains were resistant to methicillin (MRSA) (Table 1). All MSSA strains were susceptible to vancomycin, linezolid, clindamycin and tetracycline. All the MRSA strains were resistant to beta-lactam drugs and multiple antibiotics but no resistance against vancomycin was seen. This finding is similar to a previously done study which reported prevalence of MRSA to be 40 [11] Raviprakash et al. showed 46.67% of methicillin-resistant *S. aureus*.[25] Ethan Rubinstein et al, found 20%–40% of MRSA patients[26.] in their respective studies.

All the Gram negative isolates isolates showed a high degree of resistance to 3 rd generation cephalosporins with 100% sensitivity to colistin, followed by sensitivity to amikacin (60%) and piperacillin/ tazobactam (45%) among ESBL producers, 100% sensitivity to colistin among AmpC producers and 100% sensitivity to colistin among MBL producers. This is in accordance with study conducted by Wadekar et al[13]which also showed that most of the Gram negative bacilli belonging to Enterobacteriaceae and non-fermenters showed resistance against 3 rd generation cephalosporins. Multi-drug resistance among pathogenic organisms poses a major challenge in the treatment of infections and increase the morbidity and mortality associated with these infections. [27,28, 29]

4. CONCLUSION

Despite the ever growing therapeutic arsenal, chronic osteomyelitis remains a challenging and difficult-to-treat clinical entity, with frequent recurrences, the latency of infection and significant impact on patients' quality of life. Osteomyelitis is a complex infection with different causes and many associated factors that complicate its therapeutic approach. It is essential to undertake studies to see the prevalence of bacterial isolates and their antibiotic sensitivity pattern in a

particular geographical area that will enable appropriate and judicious selection of antibiotics and would limit the emerging drug-resistant strains in the future to treat the disease successfully. Our study highlights the importance of culture-directed antibiotic therapy and thus helps the clinician in choosing appropriate antibiotics which not only contribute to better treatment but there judicious use will also help in preventing emergence of resistance to the drug which are still sensitive.

ETHICAL APPROVAL.

This study was approved by the institutional ethical committee

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