

## A Study of The Bacteriological Profile of Chronic Osteomyelitis in A Tertiary Care Referral Centre: A Contemporary Study

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Article History	Abstract
<p>Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 01 Nov 2023</p>	<p><b>Background:</b> Chronic osteomyelitis is a huge burden on healthcare, especially in developing and poorer countries. With emerging antibiotic resistance patterns and increase in the incidence of Multi drug resistant (MDR) organisms, this challenge has gotten bigger. The notion that previous patterns would remain is being challenged now. We wanted to examine the trends in chronic osteomyelitis causing organisms in the contemporary era. <b>Materials and Methods:</b> The study included 76 chronic osteomyelitis patients who were admitted to the hospital during the course of the study period. During surgery, a deep culture was obtained intra-operatively then submitted for bacteriological examination. <b>Results:</b> Males made up 81% of the instances examined in our study, while females made up 19%. With 84.21% of the cases, the lower limb was more often affected. In 44.73% of the cases, the femur was the most commonly impacted bone; the tibia (35.52%), humerus (11.29%), and radius (3.94%) were the next most common affected bones. In 71.05% of the cases, the culture was affirmative with mono-microbial proliferation; in 5.26% of the cases, polymicrobial growth was seen; while in 23.68% of the cases, the culture was sterile. In haematogenous osteomyelitis (HOM), which accounted for 65.21% of cases, <i>Staphylococcus aureus</i> was more prevalent than in implant- or trauma-related infections (26.41%). Younger age groups have higher rates of HOM. 40.32% of isolates were Gram-negative bacteria, while 59.67% were Gram-positive bacteria. <i>Escherichia coli</i> dominated the group of isolates that were Gram-negative (9.67%). Gram-negative organisms were uncommon in HOM cases and abundant in those with a history of trauma or implant in situ. The most frequent predisposing factor was a history of prior orthopaedic surgery with implant in situ (74.19%), and in 37.09% of the cases, hemorrhagic infection was discovered. <b>Conclusion:</b> The most frequent predisposing factor (74.19%) overall in our analysis of chronic osteomyelitis was a history of previous orthopaedic surgery with implant in situ, while 37.09% of the cases had a haematogenous infection. Gram-negative organisms were frequently linked to cases with a history of trauma. But <i>Staphylococcus aureus</i> (46.77%) continues to be the most prevalent agent, followed by <i>Pseudomonas aeruginosa</i> (8.06%), <i>Escherichia coli</i> (9.67%), and <i>Enterobacter cloacae</i> complex (8.06%). 58.62% of the isolates of <i>Staphylococcus aureus</i> were resistant to methicillin. <i>Staphylococcus aureus</i> was the most frequent cause of HOM, accounting for 65% of cases. Gram-positive bacteria remain more prevalent than Gram-negative bacteria, despite the rise in the prevalence of the latter. Of the cultures, 59.67% showed Gram positivity, whereas 40.32% contained Gram-negative organisms.</p>
<p>CC License CC-BY-NC-SA 4.0</p>	<p><b>Keywords:</b> Chronic osteomyelitis, Microbiological profile, Multidrug resistance, Antimicrobial susceptibility, Methicillin-Resistant <i>Staphylococcus aureus</i>, Infection.</p>

### 1. Introduction

We must implement more stringent infection control procedures and carry out additional extensive research to determine the best empirical antibiotic option in light of the rising percentage of isolates

that are resistant to several drugs. Choosing the right antibiotics can help decrease morbidity and mortality, successfully treat the illness, and prevent resistant strains from emerging in the community and in hospital settings. Relapsing and persistent infection is the typical presentation of chronic osteomyelitis (COM), which can last for months or even years. Contiguous spread, a persistent open incision covering the bone, or direct bacterial injection at the location are the possible causes (1, 2).

Low-grade inflammation that lasts for a long time, sequestrum—a sclerotic dead bone—new reactive bone apposition, along with fistula formation accompanying purulent discharge are all characteristics of COM (1).

The bacteria usually involved in COM are aerobes such Coagulase negative *Staphylococcus* (CONS); *E. coli*; *Klebsiella spp.*; *Staphylococcus aureus*; *Pseudomonas spp.*; *Enterobacter spp.*; *Proteus spp.*; *Enterococcus spp.*; Among anaerobes there are bacteria like *Bacteroides spp.*; *Peptostreptococcus spp.*; *Clostridium spp.* While, rare organisms include *Actinomyces* and *Salmonella spp.* (3).

One of the main causes of morbidity for orthopaedic patients, particularly in underdeveloped nations, is COM. Growth failure, septic arthritis, epithelioma development, pathological fracture, muscle contractions, and secondary amyloidosis are among the complications associated with chronic osteomyelitis (4).

Osteomyelitis is a significant issue for the medical sector due to treatment failures and antibiotic resistance. This study aimed to improve our understanding of the causative organisms and factors that help guide treatment decisions by examining the bacteriological profile as well as antibiotic sensitivity in cases of chronic OM, as well as the commonly involved bones and their related past implant and surgical history.

## 2. Materials And Methods

This research was carried out at Swami Rama Himalayan University in Dehradun's Department of Orthopaedics at the Himalayan Institute of Medical Sciences. A total of 76 individuals were included in the research who had been clinically diagnosed with chronic osteomyelitis throughout the study period. Clinical characteristics including fever, discomfort, malaise, and the presence of sinus or purulent discharge, as well as radiographic characteristics like the presence of sequestrum, were used to make the diagnosis. Patients with immunosuppressive conditions like HIV and long-term steroid usage were excluded.

Age, gender, weight, height, chief complaint, associated signs and symptoms, length of the disease, bone involved, relevant past history predisposing to chronic osteomyelitis, including previous surgeries, past trauma, on affected bone or previous implants, social and economic history, as well as laboratory investigations, like ESR, Hb, TLC, DLC, and CRP, had been taken in detail in accordance with the case recording format.

Oral and injectable antibiotics were ceased at least 24 to 48 hours before debridement. In the operating room, a deep culture of the diseased bone was obtained for microbiologic diagnosis, accepting samples from the cortical bone, bone marrow, debridement fragments and sequestra. In this investigation, no sinus specimens or superficial cultures were obtained. The organisms were cultivated by culturing them on nutrient agar, blood agar, and MacConkey agar, followed by an aerobic 37°C incubation period.

Using the Vitek-II automated system for biochemical property analysis and the morphology of the organisms isolated using Gram's staining of the culture smear, conventional procedures were used to identify the isolates. The Vitek-II automated system was used to determine the bacterial isolates' antimicrobial susceptibility to the widely used antibiotics.

## 3. Results and Discussion

A total of seventy-six cases were included in the investigation of the bacteriological profile of chronic osteomyelitis patients. With 62 men and 14 females, there were significantly more males than females. The age range of 11 to 20 years old was the most frequent one for presentations.

58 of the 76 samples that were taken contained different organisms that had been isolated, while the other 18 samples were sterile. Of the 58 positive cultures, 54 had unimicrobial growth (71.05%) and one pathogenic organism was detected in them, whereas 4 culture samples were polymicrobial (5.26%) and the deep culture isolate included two causal organisms. As a result, throughout the investigation, 58 organisms in all were isolated. Of the 58 bacteria that were isolated and identified, 37 represented 59.67% of Gram-positive organisms, while the remaining 25 bacteria made up 40.32%.

The lower limb was affected much more often than the upper limb in the cases of chronic osteomyelitis that came to the hospital, with the tibia (35.52%) and femur (44.73%) being the most frequently impacted bones. The humerus was the most frequently affected bone in the upper limb (11.29% of cases). In all, 82.87% of the cases involved the lower extremities.

Among all the organisms identified, *Staphylococcus aureus* was the most prevalent Gram-positive bacterium, accounting for 29 positive cultures (46.77%). Of the 29 poly-microbial cultures, two (50%) had isolated *Staphylococcus aureus* strains, which were found to be methicillin-resistant strains (MRSA) in 17 of the cultures. These strains were linked to both *Citrobacter freundii* and *Klebsiella pneumoniae*. As a result, 58.62% of MRSA was identified throughout the investigation.

With six positive cultures (9.67%), *E. Coli* was the second most prevalent organism and the most often detected Gram-negative bacterium. *Enterobacter cloacae* complex and *Pseudomonas aeruginosa* were next, with five cultures (8.06%) positive for each.

The most common cause of chronic osteomyelitis was a history of trauma or other diseases involving orthopaedic implants in situ, when osteomyelitis developed as a result of implant infection. Orthopaedic implants were found in 74.19% of the patients that presented, with plates being more prevalent than nails. 23 instances (37.09%) had no history of any prior trauma or surgery, with or without implants; these were cases of chronic HOM. Seven cases (11.29%) had a history of trauma but were treated conservatively without implants.

In HOM instances, *Staphylococcus aureus* was much more prevalent than other bacteria, such as Gram-negative bacterias, in cases involving trauma or implants. *Staphylococcus aureus* growth was detected in 15 out of 23 patients with HOM (65.21%), however it was only observed in 26.41% of implant/trauma associated cases (14 out of 53). The majority of gram-negative organisms were linked to trauma and a history of procedures related to orthopaedic implants.

Only one incidence of HOM in our investigation was brought on by Gram-negative bacteria; in all other cases, trauma or implants were the culprit. Thus, Gram-negative bacteria were responsible for over half (45.28%) of trauma/implant related cases of chronic osteomyelitis, but only 4.34% of haematogenous cases.

As chronic osteomyelitis is a chronic illness, curing or eliminating the infection is often difficult. Prolonged osteomyelitis continues to be a noteworthy contributor to elevated morbidity rates, particularly in cases where timely diagnosis and treatment are lacking. Treatment for Chronic OM is still challenging in part because of the prevalent medication resistance that makes antibiotic treatments fail. As a result, orthopedic surgeons continue to face an increasing challenge from chronic osteomyelitis, particularly in light of the growing antibiotic resistance of bacteria. Therefore, for the treatment of Chronic OM to be carried out effectively, precise microbiological separation and suitable antibiotic dosage are essential. We investigated this issue in one of the very few research that we could find in the literature. An effort was made to understand the bacteriology of Chronic OM and their pattern of antibiotic susceptibility in the current research. Enhancing treatment choices and results may be substantially aided by this knowledge.

According to this research, individuals between the ages of 11 and 20 make up the largest age group of those who arrive with chronic osteomyelitis—21 cases, or 27.6%—of the total. This was comparable to the research conducted by Gupta, Gupta, and Gupta (2), whereby 27.4% of the cases were individuals aged 16 to 20. Other studies generally found that the age group most commonly affected was older; Banarjee et al. (4) reported that the most common age group was between 31 and 40, Shenoy et al. (5) reported that the most common age group was between 31 and 60, and Pandey et al.'s study found that the most commonly presenting age group was between 41 and 60 (7). A higher proportion than in earlier research indicates a greater number of individuals with chronic HOM.

There were fourteen females (19%) and sixty-two men (81%) in the study group. Accordingly, our analysis revealed a 4.4:1 male to female ratio, indicating a majority of men. These findings were consistent with previous research of this kind that found a preponderance of male participants. For example, Shenoy et al. (5) reported 78.8% of their study's participants were men, Gupta, Gupta, and Gupta (2) reported 73.2%, and Pandey et al. (7) found 79.0% of the study's participants were men.

Penicillin did not work on any of the *Staphylococcus aureus* samples. Nevertheless, none of the MRSA patients had vancomycin resistance. Imipenem and piperacillin-tazobactam were effective against all gram-negative bacteria. 50–75% of instances of chronic osteomyelitis have been linked to *Staphylococcus aureus*, which has been shown to be the most prevalent isolate in all forms of bone infections (1).

A total of 76 individuals were included in our research, and a deep culture from OT was submitted for sensitivity analysis and culture. 58 (76.32%) of the 76 samples exhibited microbial growth, whereas the remaining 18 (23.68%) cultures were sterile. This and the Pandey et al. (7) research, which had an 85% culture positive rate, were comparable.

A total of 62 organisms were identified from the 58 positive cultures in our investigation, of which 54 had mono-microbial growth (93.10%) and 4 cultures were poly-microbial (6.89%). These results were comparable to those of the Singh et al. (6) investigation, which revealed 5.2% poly-microbial cultures. Other research, such as Pandey et al. (7), which found poly-microbial growth in 18.9% of the samples, and Neeharika et al. (4), reported a larger percentage of poly-microbial flora.

In our investigation, *Staphylococcus aureus* was the most frequently identified bacterium, growing in 46.77% of the cultures. The majority of the organisms in most investigations were *Staphylococcus aureus*, and this was generally consistent across all of the research. Studies by multiple authors continue to demonstrate that Staph. aureus is the most common causative organism of COM, despite the rise in infections caused by Gram-negative organisms. Singh et al. (6) reported that *Staphylococcus aureus* was the cause of 59.5% of cases, Pandey et al. (7) reported 36.2%, Gupta, Gupta, and Gupta (2) reported 63.3%, and Salim et al. (8) reported 48.3% growth of *Staphylococcus aureus*.

MRSA was isolated from *Staphylococcus aureus* in 58.62% of the patients in our investigation, which was comparable to the MRSA percentage in previous studies of a similar nature. Lakshmi et al. (9) discovered 46% MRSA among *Staphylococcus aureus* isolates, whereas Banarjee et al. (3) identified 41.9% MRSA. Mthethwa et al. (10) discovered 69% MRSA isolates. Studies by Singh et al. (6) with 30.2% and Shenoy et al. (5) with 38.77% showed lower proportions of MRSA.

*Escherichia coli* was the most frequent causal bacterium among Gram-negative organisms, accounting for 9.67% of isolates exhibiting growth. *Escherichia coli* was the most often isolated Gram-negative bacteria in the investigations by Singh et al. (20.2%), Pandey et al. (12.9%), and Elsheikh et al. (9.3%) (6, 7, 11). This was also consistent with previous findings.

The femur (44.73%) was the most often afflicted bone in our analysis, followed by the tibia (35.52%) and humerus (11.29%). The two most often engaged in many such experiments were the femur and the tibia. According to Shenoy et al. (6), 32.4% of cases involved the tibia and 21.6% included the femur. According to Jerzy and Francis (12), the femur was implicated in 28.9% of instances, whereas the tibia was in 62.9%. According to Pandey et al. (7), the femur was affected in 38.0% of cases and the tibia in 36.0% of instances; however, Jerzy and Francis (12) found that in cases with chronic osteomyelitis, the femur was implicated in 33.0% of cases and the tibia in 34.0% of cases.

We found some errors and inadequacies in our research. Among these is our very modest sample size, which may be expanded in further research. Furthermore, since our research was conducted in a single jurisdiction, it's possible that the conclusions and results don't apply to the broader public. Furthermore, it's probable that some of the instances exhibiting sterile cultures were brought on by fungal or anaerobic cultures since we were unable to produce such cultures. It is important to remember that these illnesses are quite uncommon.

#### 4. Conclusion

The most prevalent kind of osteomyelitis (OM), particularly in adult patients, is chronic osteomyelitis, which is often the result of trauma. Studies on antibiotic sensitivity and the identification and isolation of the causal bacterium are crucial since proper therapy necessitates the right use of potent antibiotics. Due to the widespread usage of antibiotics, resistant microbes are becoming more and more isolated these days. Therefore, C/S-guided, carefully chosen antibiotic medication is needed for successful treatment. Therefore, there is likely to be less development of drug resistance and improved treatment results by reducing the indiscriminate use of antibiotics. Gram-positive bacteria are still more prevalent than Gram-negative bacteria, despite the latter's recent rise in prevalence. Of the cultures, 59.67% showed Gram positivity and 40.32% included Gram-negative organisms. *Escherichia coli* dominated the group of isolates that were Gram-negative (9.67%). *Staphylococcus aureus* (46.77%) was the most often found agent in the bacteriological research of chronic osteomyelitis, followed by *Escherichia coli* (9.67%), *Enterobacter cloacae* complex (8.06%), and *Pseudomonas aeruginosa* (8.06%). 58.62% of the isolates of *Staphylococcus aureus* were resistant to methicillin. Haematogenous infection was detected in 37.09% of the patients, and the most frequent predisposing factor overall was a history of past orthopaedic surgery with implant in situ (74.19%). *Staphylococcus aureus* was the most frequent cause of haematogenous

osteomyelitis (65% of cases), but Gram-negative organisms were more often linked to cases that included previous trauma or implant procedures.

### Conflict of interest

The authors declare that they have no conflict of interest.

### Informed Consent

Informed consent was taken from the patients participating in this study.

### Abbreviations

OM	Osteomyelitis
DLC	Differential leucocyte count
CONS	Coagulase negative Staphylococcus
CRP	C- Reactive protein
TLC	Total leucocyte count
ESR	Erythrocyte sedimentation rate

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