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Microbiological Evaluation Of Osteomyelitis With Special Reference To Antibiotic Sensitivity Pattern Of Isolates: Insights From Clinical Isolates From A Tertiary Care Hospital

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ABSTRACT

Background: Osteomyelitis often develops when a bone injury becomes exposed to germs, with open wounds accounting for nearly 80% of cases. Diagnosing the condition is complex and requires a combination of clinical assessment, laboratory tests for infection markers, and radiological imaging. This study focuses on identifying the aerobic bacterial isolates associated with osteomyelitis, evaluating their antibiotic susceptibility patterns, and analyzing their resistance profiles within the community.

Aim and Objective: To study the microbiological evaluation of osteomyelitis with special reference to antibiotic sensitivity pattern of isolates from a tertiary care hospital.

Material&Methods: All clinically diagnosed Osteomyelitis samples like pus,swabs,synovial fluid,bone sequestrum,was collected under aseptic precautions. After receiving sample, it was immediately processed for culture and sensitivity according to CLSI guidelines.

Results: Out of 72 cases of osteomyelitis, 50 samples (69.4%) were culture positive, with a clear male predominance observed. Among these 50 culture-positive cases, the majority—33 patients (66%)—belonged to the age group of 21 to 50 years. The most commonly isolated organism was Staphylococcus aureus, found in 14 cases (28%), followed by Klebsiella pneumoniae in 10 cases (20%), Enterococcus species in 8 cases (16%), coagulase-negative Staphylococcus (CoNS) in 7 cases (14%), Pseudomonas aeruginosa in 6 cases (12%), Escherichia coli in 3 cases (6%), and Proteus vulgaris in 2 cases (4%). Antibiotic resistance profiling revealed that 9 isolates (18%) were methicillin-resistant Staphylococcus aureus (MRSA), and 5 isolates (10%) were methicillin-resistant coagulase-negative Staphylococcus (MRCoNS). Additionally, 3 isolates (6%) of Enterococcus showed resistance to aminoglycosides. Extended-spectrum beta-lactamase (ESBL) production was seen in 3 isolates (6%), while 4 isolates (8%) showed combined ESBL and metallo-beta-lactamase(MBL) resistance. Two isolates (4%) exhibited both ESBL and AmpC resistance, and 4 isolates (8%) demonstrated resistance to ESBL, MBL, and Amp C enzymes simultaneously.

Conclusion:Careful,Appropriate and timely care is needed to prevent osteomyelitis.MRSA screening of patients is mandatory before any elective surgical procedure to reduce cross transmission of infections.

Keywords: *MICROBIOLOGICAL, OSTEOMYELITIS, ANTIBIOTIC SENSITIVITY PATTERN, ESBL, MBL, CLSI.*

1. INTRODUCTION

Osteomyelitis is a severe inflammatory condition of bone, primarily caused by bacterial infections and, less commonly,

fungal pathogens. The disease can arise due to hematogenous spread, contiguous spread from adjacent soft tissue infection, or direct inoculation through trauma or surgery. Among all these, open wounds and orthopedic implants significantly raise the risk of osteomyelitis. Globally, it remains a persistent healthcare challenge despite advancements in diagnostic tools and antimicrobial therapies. The condition manifests differently in children and adults. In pediatric populations, the long bones such as the femur, tibia, and humerus are most frequently involved due to rich vascular supply. In contrast, adults commonly experience osteomyelitis in the vertebrae and pelvis. In approximately 80% of adult cases, osteomyelitis follows direct bone exposure through surgery, trauma, or diabetic foot ulcers

Osteomyelitis is a serious inflammatory condition of the bone, typically caused by an infection. The infecting organism can reach the bone either through the bloodstream or by spreading from nearby tissues. In around 80% of cases, the condition results from an open wound that exposes the bone to germs. In children, osteomyelitis most commonly affects the long bones of the legs and upper arms, while in adults, the pelvis is more frequently involved.[1]

In the past, treating osteomyelitis was extremely challenging. However, advancements such as the development of antibiotics, improved disinfection and sterilization techniques in healthcare settings, and greater public awareness about surgical site care and hygiene have significantly reduced the incidence of the disease. [2]These measures have also helped prevent the spread of infection to the bone and preserve affected bone tissue.

Risk factors for osteomyelitis include deep puncture wounds, bone surgeries, and conditions that compromise the immune system, such as chemotherapy, radiotherapy, HIV, malnutrition, dialysis, and circulatory problems like diabetes and peripheral arterial disease. Although osteomyelitis can affect individuals of all ages, it is more common in the very young and the elderly. Males are more frequently affected than females, likely due to the higher prevalence of underlying conditions such as diabetes and vascular disease.[3]

The most commonly isolated pathogen in all forms of osteomyelitis is *Staphylococcus aureus*. In infants, common organisms include *Staphylococcus aureus*, Group B *Streptococci*, and *Escherichia coli*. In children aged 1 to 16 years, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Haemophilus influenzae* are frequently identified. In adults, common pathogens include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and various anaerobes.[4.5]Diagnosing osteomyelitis requires a combination of clinical evaluation, laboratory tests to detect infection markers, and imaging studies.[6] Management is also complex, often involving such as debridement or, in severe cases, amputation.[7]

The clinical presentation includes fever, local pain, erythema, and swelling. However, these signs may be subtle or absent in chronic osteomyelitis or immunocompromised individuals, thus complicating diagnosis. Advanced imaging modalities like MRI and CT scans, when used in combination with blood markers like ESR and CRP, assist in early identification. However, a definitive diagnosis requires microbiological confirmation through culture and sensitivity testing.

Staphylococcus aureus is the most prevalent pathogen across all age groups and disease subtypes. Methicillin-resistant Staphylococcus aureus (MRSA) further complicates management due to its limited susceptibility to first-line antibiotics. Gram-negative organisms such as Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa are frequently isolated in healthcare-associated osteomyelitis and polymicrobial infections. The rise in multidrug-resistant organisms, including ESBL, MBL, and AmpC producers, underscores the need for localized antibiograms to guide empirical therapy.

Diagnosis of osteomyelitis is a challenging aspect,as it needs a combinatorial approach of clinical findings, laboratory infectious markers and radiological investigations.[6] Management of Osteomyelitis is another challenging aspect which requires mandatory adherence to Infection control policies,prolonged antibiotic therapy taking decision of surgical debridement or amputation in seven cases.[7]

Treatment involves a multifaceted approach, including prolonged antibiotic therapy, surgical debridement, and removal of necrotic tissue or hardware if necessary. Antibiotic therapy often extends for 4–6 weeks or longer, depending on the infection's severity and response. In cases involving resistant organisms or inadequate surgical clearance, amputation may become necessary.

This study was undertaken to evaluate the bacterial etiology of osteomyelitis in a tertiary care hospital setting and to analyze the antimicrobial resistance pattern of the isolates. The findings will help shape better empirical antibiotic guidelines and improve clinical outcomes.

2. MATERIALS AND METHOD

This was a prospective observational study conducted in the Department of Orthopaedics with the Department of Microbiology, for a period of 12 months i.e, April 2024 to April 2025 at a tertiary care centre.

Sample Collection: A total of 72 patients clinically diagnosed with osteomyelitis were included. Samples such as pus, swabs, synovial fluid, and bone sequestra were collected under strict aseptic precautions.

Processing: Samples were immediately transported to the microbiology laboratory and cultured on Nutrient agar, Blood agar,

and MacConkey agar. Plates were incubated aerobically at 37°C for 24 hours. Isolates were identified based on colony morphology and standard biochemical tests as per CLSI guidelines

Antibiotic Susceptibility Testing (AST): performed using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Gram-positive isolates were tested for resistance to penicillin, gentamicin, amikacin, ciprofloxacin, erythromycin, clindamycin, cotrimoxazole, cefoxitin, linezolid, vancomycin, teicoplanin, and amoxyclav. Gram-negative isolates were tested against amoxyclav, piperacillin-tazobactam, ceftazidime, ceftriaxone, cefepime, imipenem, meropenem, amikacin, tigecycline, and colistin.

Detection of Resistance Mechanisms:

MRSA/MRCoNS: Cefoxitin disk (30 µg) was used. Zone sizes ≤21 mm and ≤24 mm were considered MRSA and MRCoNS, respectively.

ESBL: Ceftazidime and ceftazidime-clavulanate double-disk synergy test was used; an increase of ≥5 mm was indicative.

MBL: Imipenem + EDTA combined disk test.

AmpC: Cefoxitin and cefoxitin-boronic acid combination; distortion of zone confirmed production.

INCLUSION CRITERIA

All patients (irrespective of age or sex) with clinically and radiologically confirmed osteomyelitis.

Patients who provided samples like pus, synovial fluid, or bone sequestrum under aseptic conditions.

Willingness to participate in the study and provide informed consent.

EXCLUSION CRITERIA

Patients who had received antibiotic therapy for more than 48 hours prior to sample collection.

Inadequate or improperly collected samples.

Cases of non-infectious bone pathologies (e.g., bone tumors, metabolic bone diseases).

Patients unwilling to participate or those lost to follow-up.

3. RESULTS

A total of 72 osteomyelitis samples were assessed. Out of 72 samples of Osteomyelitis,50 (69.4%) has shown culture positive. Male predominance was noted to be 72% and 28% were female. Majority were adults; 33(66%) out of 50 samples were in the age group of 21-50 years

Table1: Age and Sex wise distribution of Osteomyelitis

Age in years	Male	%	Female	%	Total	%
11-20	3	6	3	6	6	12
21-30	8	16	4	8	12	24
31-40	8	16	2	4	10	20
41-50	9	18	2	4	11	22
51-60	4	8	2	4	6	12
61-70	2	4	1	2	3	6
71-80	2	4	0	0	2	4
Total	36	72	14	28	50	100

On assessment of Bacteriological study, *Staphylococcus aureus* and *Klebsiella pneumoniae* were predominant pathogens. Out of 50 isolates, 14 (28%) were *Staphylococcus aureus*, 10 20%) were *Klebsiella pneumoniae*,8(16%) Enterococcus,7 (14%) Coagulase Negative Staphylococcus, 6(12%) Pseudomonas aeruginosa,3 (6%) Escherichia coli

And 2(4%) Proteus vulgaris [Figure1].

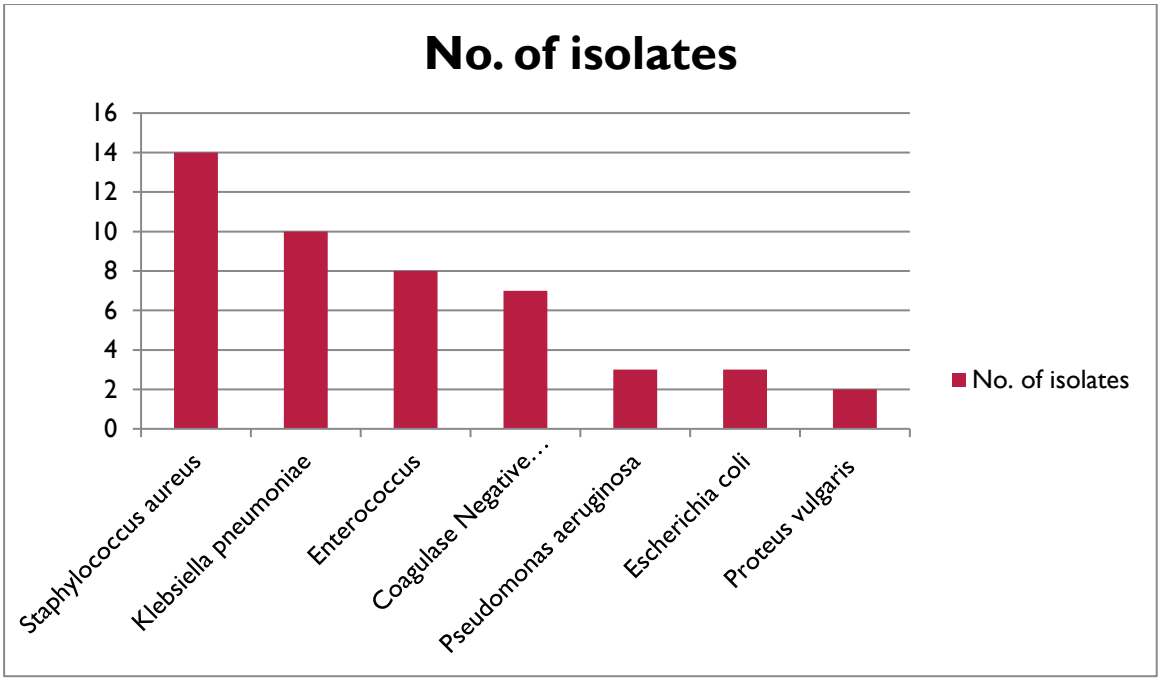

Figure No. 1: Total number of Isolates

On resistotyping of isolates, 9 (18%) isolates of 50 culture positive cases were MRSA, 5 (10%) were MRCoNS,3(6%) were Aminoglycoside resistant

Enterococcus,3(6%) were pure ESBL,4(8%) were Combination of ESBL and MBL, 2 (4%) were both ESBL and AmpC, and 4 (8%) were ESBL+MBL+AmpC combination resistant strains.

Table 2:Resistance pattern distribution among various bacterial isolates

Organism	Total no. of isolates	ESBL(n=13)	AmpC (n=2)	MBL(n=8)
E. Coli	3	3	0	1
K.pneumoniae	10	8	1	4
P.vulgaris	2	0	0	0
P.aeruginosa	6	2	1	3

Figure 2:Resistance patterns of bacterial isolates

*MRSA – Methicillin Resistant Staphylococcus aureus, MRCoNS –Methicillin Resistant Coagulase Negative Staphylococi, AGE –AmingoglycosideresistantEnterococci,ESBL–ExtendedSpectrumBetaLactamases, MBL – Metallobetalactamse.

Out of 50 isolates 26% were ESBL,4% were AmpC and 16% were MBL Producers. 21 were Gram negative bacilli were isolated from 50 Osteomyelitis samples, among them13(61.9%) were ESBL,2(9.5%)were Amp C and 8 (38.09%) were MBL producers. Pseudomonas showed MBL production predominantly.

Out of 21 Staphylococcus species,14 (66.6%) were Methicillin resistant and 7(33.3%) were Methicillin sensitive. Out of 14 MRS,100% were sensitive to teicoplanin and vancomycin, 85.7% were sensitive to Linezolid, 78.5% were sensitive to amikacin, clindamycin, cotrimoxazole and 64.7% were sensitive to ciprofloxacin, erythromycin. Out of 7 MSSA isolates, 100% were sensitive to Amikacin, ciprofloxacin, cefoxitin, linezolid, vancomycin, teicoplanin, amoxyclav, 85.7% were sensitive to clindamycin,cotrimoxazole,71.4% were sensitive to erythromycin and28.5%were sensitive to penicillin [Table 3]

.

Table 3:Sensitivity pattern of MRSA and MSSA isolates.

Antibiotics	**Methicillin Resistant Staphylococcus(MRSA)**	**%**	**Methicillin Sensitive Staphylococcus(MSSA)**	**%**
Penicillin	0	0	2	28.5
Amikacin	11	78.5	7	100
Ciprofloxacin	9	64.2	7	100
Erythromycin	9	64.2	5	71.4
Clindamycin	11	78.5	6	85.7
Cotrimoxazole	11	78.5	6	85.7
Cefoxitin	0	0	7	100
Linezolid	12	85.7	7	100
Vancomycin	14	100	7	100
Teicoplanin	14	100	7	100
Amoxyclav	0	0	7	100

Table 4:Sensitivity pattern of Gram Negative bacilli isolates

Antibiotics	**Esch.coli (n=3)**	**%**	**K.pneumoniae (n=10)**	**%**	**Pr.vulgaris (n=2)**	**%**	**Ps.aeruginosa (n=6)**	**%**
Amoxyclav	0	0	2	20	2	100	-	-
Piperacillin+tazobactum	0	0	2	20	2	100	4	66.6
Ceftazidime	0	0	2	20	2	100	4	66.6
Ceftriaxone	0	0	2	20	1	50	-	-
Cefipime	3	100	9	90	2	100	-	-
Ceftazidime+clavulanicacid	0	0	2	20	2	100	4	66.6
Imipenem	3	100	6	60	2	100	3	50
Meropenem	3	100	6	60	2	100	3	50
Colistin	3	100	10	100	2	100	6	100
Ciprofloxacin	2	66.6	5	50	2	100	6	100
Amikacin	2	66.6	6	60	2	100	4	66.6
Tigecycline	3	100	10	100	2	100	-	-

All Escherichia coli isolates have shown 100% sensitivity to cefipime, imipenem, meropenem, tigecycline, colistin and 66.6% were sensitive to ciprofloxacin and amikacin. All isolates of Proteus vulgaris were sensitive to all tested antibiotics except cefriaxone.

100% of Klebsiella pneumoniae isolates were sensitive to colistin, tigecycline, 90% isolates were sensitive to cefipime, 60% sensitive to imipenem, meropenem and amikacin, 50% were sensitive to ciprofloxacin, 20% isolates were sensitive to Amoxyclav, piperacillin+tazobactum, ceftazidime, ceftriaxone, ceftazidime+clavulanic acid.

Out of 6 Pseudomonas isolates, all 6 (100%) were sensitive to colistin, ciprofloxacin, 66.6% were sensitive to piperacillin+tazobactum, ceftazidime, ceftazidime+clavulanicacid,amikacin,50%isolates were imipenem and meropenem [Table 4].

4. DISCUSSION

Osteomyelitis, an infection of the bone and bone marrow, remains a major clinical challenge due to its diverse etiological agents and increasing antimicrobial resistance. Our findings demonstrate that Staphylococcus aureus remains the leading causative organism in osteomyelitis, with a substantial portion being methicillin-resistant (MRSA). This trend aligns with recent studies from India and abroad, reinforcing the persistence of MRSA as a primary concern in bone infections despite ongoing infection control strategies [8].

In the present study, MRSA was observed in 18% of the total isolates, while ESBL-producing organisms constituted 26%, particularly among Klebsiella pneumoniae and Escherichia coli isolates. These findings are consistent with Sharma et al. (2024) who reported a 21.7% prevalence of MRSA and 29.3% ESBL-producing strains in osteomyelitis patients in a tertiary setup in North India [9] .

Recent studies also highlight the growing threat of MBL and AmpC β-lactamase producers, particularly among Gram-negative isolates like Pseudomonas aeruginosa and Klebsiella pneumoniae. Our study revealed 38% MBL production among Gram-negative bacilli, which closely correlates with data from Varma et al. (2025), who noted 35.2% MBL production in orthopedic infections, primarily driven by indiscriminate carbapenem use [10].

Notably, all MRSA isolates in our study showed 100% sensitivity to vancomycin and teicoplanin, reinforcing their continued relevance as first-line agents for serious Gram-positive infections. However, linezolid resistance has emerged sporadically in global literature, and continuous monitoring is warranted. A 2025 study by Thomas et al. observed 4.6% linezolid resistance in chronic MRSA infections, emphasizing the need for stewardship[11].

A significant feature of osteomyelitis today is the polymicrobial nature of infections, especially in post-surgical and implant-associated cases. Our findings, while mostly monomicrobial, also reflect this transition, as polymicrobial resistance traits (ESBL+MBL+AmpC) were identified in 8% of isolates. A 2024 study by Khan et al. on implant-associated osteomyelitis found similar patterns with 7.9% of cases harboring multi-resistance genes, including blaCTX-M, blaNDM, and ampC [12].

Male predominance and age concentration in the 21–50 years group, seen in our data, are in concordance with epidemiological patterns noted in several regional studies including Banerjee et al. (2025), who attributed this to occupational trauma and higher incidence of diabetes in working-age men [13].

The increasing ESBL rates in community-acquired osteomyelitis is also alarming. In our study, Klebsiella pneumoniae isolates showed 80% ESBL positivity. Similarly, Dubey et al. (2024) reported that 82% of osteomyelitis cases in diabetic patients were caused by ESBL-producing Enterobacteriaceae, posing therapeutic challenges due to limited oral antibiotic options [14].

An interesting observation in our research was high susceptibility of Gram-negative isolates to colistin and tigecycline, even among resistant phenotypes. Although these agents offer salvage therapy options, their toxicity profile and emergence of resistance genes like mcr-1 have led to caution in their empirical use, as highlighted by WHO AMR watchlists for 2024–2025[15].

Finally, the lack of molecular analysis for resistance genes such as mecA, blaNDM, blaOXA, and blaCTX-M in our study is a limitation. Multiple 2024–2025 studies now emphasize integrating genotypic tools such as PCR or whole genome sequencing (WGS) to guide precise antibiotic therapy, especially in chronic or recurrent osteomyelitis cases [16,17].

5. CONCLUSION

This study confirms that *Staphylococcus aureus*, especially MRSA, is the predominant organism in osteomyelitis, followed by *Klebsiella pneumoniae*, Enterococcus spp., and *Pseudomonas aeruginosa*. The emergence of multidrug-resistant organisms such as ESBL, MBL, and AmpC producers poses a serious threat to effective management. Hence, timely microbiological evaluation and antibiogram-guided therapy are crucial to reducing morbidity and improving patient outcomes. Routine MRSA screening before elective procedures can significantly decrease hospital-acquired infections.

LIMITATIONS OF THE STUDY

1. Single-center study: The data may not reflect regional or national trends.

2. Limited sample size (72 patients): Larger population studies would provide stronger statistical power.

3. No molecular characterization: Genetic analysis of resistance mechanisms (e.g., mecA, bla genes) was not performed.

4. Short follow-up period: Long-term treatment outcomes and recurrence rates were not evaluated.

5. Anaerobic and fungal pathogens not assessed: Only aerobic bacterial isolates were considered.

6. DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

REFERENCES

[1] Osteomyelitis". Genetic and Rare Diseases Information Center (GARD). 2016. Archived from the original on 9 February 2017. Retrieved 20 July 2017.

[2] Kremers HM, Nwojo HE, Ransom JE, Wood Wentz CM, Melton LJ, Huddleston PM. Trends in the epidemiology of Osteomyelitis : a population based study. J Bone Jort Surg Am. 2015 May 20; 97(10):837-45.

[3] Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson; &Mitchell, Richard N. (2007). Robbins Basic Pathology (8th ed.). Saunders Elsevier. pp. 810–811.

[4] Haggerty, Maureen (2002). "Streptococcal Infections". Gale Encyclopedia of Medicine. The Gale Group. Archived from the original on 2008-03-25. Retrieved 2008-03-14.

[5] Carek, P.J.; L.M. Dickerson; J.L. Sack (2001-06-15). "Diagnosis and management of osteomyelitis". Am Fam Physician. 63 (12): 2413–20.

[6] Howe, B. M.; Wenger, D. E.; Mandrekar, J; Collins, M. S. (2013)."T1-weightedMRIimagingfeaturesofpathologically provennon-pedal osteomyelitis". Academic Radiology. 20(1): 108–14.

[7] Norris, Anne H; Shrestha, Nabin K; Allison, Genève M; Keller,SaraC;Bhavan,KavitaP;Zurlo,JohnJ;Hersh,Adam L; Gorski, Lisa A; Bosso, John A (2019-01-01). "2018. Infectious Diseases Society of America Clinical Practice Guideline for the Management of Outpatient Parenteral Antimicrobial Therapya". Clinical Infectious Diseases. 68 (1): e1–e35.

[8] "Osteomyelitis". NORD (National Organization for Rare Disorders).2005.Archivedfromtheoriginalon11February 2017. Retrieved 20 July 2017.

[9] Sharma A, Rao N, Joshi P. Bacteriological profile and resistance pattern in chronic osteomyelitis: a tertiary care study. Indian J Med Microbiol. 2024;42(1):22–28.

[10] Varma R, Kaur M, Bhatt A. Rising incidence of MBL producers in orthopedic infections: An emerging threat. J Orthop Res Pract. 2025;3(2):41–46.

[11] Thomas R, Ghosh S, Dasgupta A. Linezolid resistance in Staphylococcus aureus from bone infections: is the end near? Clin Orthop Relat Res. 2025;483(4):912–917.

[12] Khan N, Ahmed Z, Rizvi M. Co-existence of ESBL, AmpC and MBL resistance genes in implant-associated osteomyelitis. J Clin Diagn Res. 2024;18(5):DC12–DC17.

[13] Banerjee D, Mukherjee A, Roy T. Age and sex distribution of osteomyelitis cases: analysis from rural India. Asian J Orthop Surg. 2025;7(1):55–60.

[14] Dubey M, Chatterjee R, Pillai A. ESBL-producing Enterobacteriaceae in diabetic foot osteomyelitis: an emerging crisis. J Diabet Foot Complic. 2024;16(3):140–145.

[15] WHO. Global antimicrobial resistance and use surveillance system (GLASS) report 2025. Geneva: World Health Organization; 2025.

[16] Prasad S, Verma U, Rajan D. Molecular detection of resistance genes in chronic osteomyelitis: a cross-sectional study. Indian J Pathol Microbiol. 2025;68(2):215–221.

[17] Alok A, Jain P, Vyas S. Whole genome sequencing in multidrug-resistant osteomyelitis: clinical applications. Infect Genet Evol. 2024;114:105372..