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Original Research Article

MICROBIOLOGICAL AND ANTIBIOTIC PROFILE OF OSTEOMYELITIS IN TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Osteomyelitis is a persistent bone infection caused by a wide spectrum of bacteria, with changing microbiological profiles and increasing antimicrobial resistance posing challenges to therapy. **Aim:** To identify the evolving trends in microbial isolates causing osteomyelitis and to assess their antibiotic susceptibility patterns in a tertiary care hospital.
Materials and Methods: A prospective cross-sectional study was conducted on 120 culture-positive osteomyelitis cases. Isolates were identified using standard microbiological techniques, and antimicrobial susceptibility was determined by the Kirby–Bauer disc diffusion method following CLSI 2024 guidelines.
Results: Staphylococcus aureus was the predominant isolate (24.2%), followed by Pseudomonas aeruginosa (21.2%) and Acinetobacter baumannii (16.7%). High resistance to cephalosporins and fluoroquinolones was observed, whereas carbapenems and β-lactamase inhibitor combinations retained good efficacy.
Conclusion: Gram-negative pathogens are increasingly emerging in osteomyelitis, showing multidrug resistance, while MRSA continues to remain prevalent. Continuous microbial surveillance and rational antibiotic use are essential to optimize management outcomes. **Keywords:** Osteomyelitis, Antibiotic Resistance, Microbiological Profile, Multidrug Resistance.

INTRODUCTION

Osteomyelitis remains a formidable clinical challenge due to its complex pathogenesis, protracted course, and high morbidity, particularly in tertiary-care settings where comorbidities and resistant organisms prevail. The disease arises when microorganisms invade bone tissue—via direct inoculation, contiguous spread, or hematogenous routes—leading to persistent inflammation, bone necrosis and development of sequestra that serve as reservoirs for infection. Recent decades have seen evolving microbial profiles and rising antimicrobial resistance, complicating management and outcomes. Historically, Staphylococcus aureus was the dominant pathogen in osteomyelitis, accounting for the majority of culture-positive cases. However, more recent investigations demonstrate an increasing prevalence of Gram-negative rods (such as Pseudomonas aeruginosa and Acinetobacter baumannii) and polymicrobial infections, particularly in patients with prior trauma, surgery or implants.[1] A large single-centre 10-year UK study found that not only did S. aureus proportions remain stable but also methicillin-resistant S. aureus (MRSA) among isolates decreased — but multiple-drug-resistant (MDR) organisms persisted at similar levels, indicating changing trends in microbiology rather than simple shifts in species.[2]

In contemporary tertiary-care settings, evidence suggests that no single pathogen dominates and that resistance patterns vary widely by geography, population and prior antibiotic exposure. A recent epidemiological study of orthopaedic infections noted that pathogen distributions and antimicrobial-susceptibility profiles differ across regions and over time, making empirical therapy more challenging.[3] In the context of osteomyelitis, timely identification of the causative organism and its susceptibility is essential, because empirical regimens may no longer cover emerging pathogens or resistance mechanisms.[4]

Moreover, antibiotic-susceptibility patterns are evolving. Many studies report high rates of resistance among bone-infection isolates—MRSA, extended-spectrum β-lactamase (ESBL) producing Enterobacterales, carbapenem-resistant Pseudomonas and Acinetobacter—necessitating review of empirical-therapy guidelines and local antibiograms.[5] One recent investigation in a tertiary-care hospital found that 47% of S. aureus isolates from osteomyelitis were MRSA and Gram-negative isolates frequently harboured ESBL or metallo-β-lactamase (MBL) phenotypes.[6] These findings highlight the imperative of updating local microbiological surveillance and antibiotic policy.

Further complicating management are risk factors such as diabetes mellitus, vascular insufficiency, prior trauma or orthopaedic implant presence, which not only predispose to osteomyelitis but also increase likelihood of resistant and atypical organisms.[7] The interplay of host factors, prior surgery, implant biofilm formation and antibiotic exposure underlies the shift towards more complex microbial-profiles in bone infections.

Also noteworthy is the evidence from longitudinal bone-culture studies: one retrospective analysis from South China recorded a decreasing proportion of S. aureus over 12 years and rising Gram-negative representation, with 28.1% of cases being multiple-organism and increased resistance within P. aeruginosa and Acinetobacter spp.[8] Similar broad-infection-surveillance studies reveal that bone-infection culture positive rates are falling while the diversity of causative organisms and resistance mechanisms is expanding.[9]A very recent study of recurrent bone and joint infection reported that microbial persistence or replacement with new organisms contributes heavily to treatment failure in chronic osteomyelitis, underlining the dynamic nature of bone-infection microbiology.[10]

In light of these developments, it is paramount to assess not only the current spectrum of organisms causing osteomyelitis in a tertiary-care setting, but also their antimicrobial susceptibility patterns and evolving trends. Such data guide empirical therapy, antimicrobial stewardship initiatives and outcome optimisation. Therefore, the aim of this study is to look for the changing trends of microorganisms involved in osteomyelitis and their antimicrobial susceptibility pattern in a tertiary care hospital context.

MATERIALS AND METHODS

This was a prospective, cross-sectional observational study conducted in the Department of Microbiology of a tertiary care hospital after obtaining approval from the Institutional Ethics Committee. A total of 120 patients of all age groups and both sexes, clinically diagnosed with osteomyelitis and attending either inpatient or outpatient departments, were enrolled over the study period. Written informed consent was obtained from all participants or their legal guardians before sample collection.

Samples such as pus, bone biopsy, sinus discharge, and tissue aspirates were collected aseptically from patients with suspected osteomyelitis prior to initiation of antibiotic therapy. Each specimen was transported immediately to the microbiology laboratory for culture and sensitivity testing. Gram staining and microscopic examination were performed to assess the morphology and gram reaction of the organisms. Culture was done on Blood agar, MacConkey agar, and Nutrient agar, followed by incubation at 37 °C for 24–48 hours under aerobic conditions. Anaerobic cultures were set up wherever clinically indicated. Isolates were identified based on colony characteristics, Gram staining, and a battery of standard biochemical tests including catalase, coagulase, oxidase, indole, citrate utilization, urease, triple sugar iron reaction, and motility tests.

Antimicrobial susceptibility testing (AST) was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2024. The antibiotic discs tested for Gram-positive bacteria included penicillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, linezolid, and vancomycin. For Gram-negative isolates, amikacin, gentamicin, piperacillin-tazobactam, ceftriaxone, cefepime, imipenem, meropenem, ciprofloxacin, and colistin were tested. Methicillin resistance among *Staphylococcus aureus* isolates was determined using the cefoxitin disc diffusion method, while extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) production in Gram-negative bacilli were detected using the combined disc and double-disc synergy tests. Quality control strains such as *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used to ensure accuracy.

Patient demographic details, clinical features, risk factors such as diabetes mellitus, previous trauma, orthopedic implants, and prior antibiotic use were recorded through structured proformas. Culture positivity rate, distribution of isolates, and antimicrobial resistance patterns were analyzed. Data were entered into Microsoft Excel and statistically analyzed using SPSS version 26. Descriptive statistics were expressed as frequencies and percentages. The Chi-square test was used to compare categorical variables, and $p < 0.05$ was considered statistically significant.

RESULTS

The microbiological profile of osteomyelitis cases in this study was derived from 120 culture-positive samples, revealing a diverse distribution of aerobic, anaerobic, and fungal isolates. Gram-positive organisms remained predominant, with Staphylococcus aureus being the most frequent isolate, accounting for nearly one-fourth of all

positive cultures (Table 1). Enterococcus faecalis and Streptococcus pyogenes were also significant contributors among Gram-positive bacteria. Among Gram-negative organisms, Pseudomonas aeruginosa and Acinetobacter baumannii were isolated frequently, followed by Klebsiella pneumoniae and Proteus mirabilis. Anaerobes such as Clostridium spp. and Bacteroides spp. were identified in a smaller fraction of cases, and Candida spp. accounted for rare yeast isolates. Mixed infections were observed in 32 patients, indicating polymicrobial osteomyelitis, particularly in chronic and postoperative cases.

The antimicrobial susceptibility profile of Gram-positive isolates showed high resistance to β-lactam antibiotics, while better sensitivity was noted to linezolid and aminoglycosides. Staphylococcus aureus isolates demonstrated 50 % methicillin resistance as determined by cefoxitin disc testing, emphasizing the persistence of MRSA in bone infections. Enterococcus faecalis exhibited 100 % sensitivity to penicillin and ampicillin, whereas resistance was higher to fluoroquinolones and clindamycin. Streptococcus pyogenes showed partial susceptibility to β-lactams and moderate resistance to macrolides. No vancomycin resistance was observed among Gram-positive isolates.

Among Gram-negative isolates, Pseudomonas aeruginosa and Acinetobacter baumannii exhibited high resistance to ampicillin and cephalosporins, with relatively better sensitivity to carbapenems and β-lactam/β-lactamase inhibitor combinations. Klebsiella pneumoniae, Proteus mirabilis, and Citrobacter freundii showed moderate susceptibility to amikacin and piperacillin-tazobactam, whereas complete resistance to ampicillin and third-generation cephalosporins was common. The highest sensitivity among all Gram-negative organisms was observed with meropenem and imipenem.

Table 1: Aerobic and Anaerobic Bacteria Isolated from Osteomyelitis Cases (n = 120)

Organisms	Number	Percentage
Gram-positive bacteria		
Staphylococcus aureus	32	24.2
Enterococcus faecalis	18	13.6
Streptococcus pyogenes	4	3.0
Gram-negative bacteria		
Pseudomonas aeruginosa	28	21.2
Acinetobacter baumannii	22	16.7
Klebsiella pneumoniae	8	6.1
Proteus mirabilis	6	4.5
Citrobacter freundii	4	3.0
Morganella morganii	2	1.5
Anaerobic bacteria		
Clostridium spp.	3	2.3
Bacteroides spp.	3	2.3
Yeast		
Candida spp.	2	1.5
Total isolates	132	—

Table 2: Antibiotic Susceptibility Pattern of Gram-Positive Isolates in Osteomyelitis Cases (n = 120)

Antibiotics	Staphylococcus aureus N (%)	Enterococcus faecalis N (%)	Streptococcus pyogenes N (%)
Penicillin-G	30 (93.8)	18 (100)	2 (40.0)
Ampicillin	29 (90.6)	17 (94.4)	2 (40.0)
Linezolid	17 (53.1)	9 (50.0)	0 (0.0)
Clindamycin	13 (40.6)	8 (44.4)	0 (0.0)
Gentamicin	24 (75.0)	15 (83.3)	0 (0.0)
Ciprofloxacin	26 (81.3)	13 (72.2)	2 (40.0)
Ofloxacin	17 (53.1)	14 (77.8)	0 (0.0)
Sparfloxacin	16 (50.0)	13 (72.2)	0 (0.0)
Cefotaxime	19 (59.4)	15 (83.3)	0 (0.0)
Ceftazidime	18 (56.3)	16 (88.9)	0 (0.0)
Cephalexin	29 (90.6)	17 (94.4)	1 (20.0)
Methicillin (by cefoxitin)	16 (50.0)	—	—
Amikacin	15 (46.9)	10 (55.6)	0 (0.0)
Netilmicin	15 (46.9)	11 (61.1)	0 (0.0)
Vancomycin	0 (0.0)	0 (0.0)	0 (0.0)

Table 3: Resistance Pattern of Aerobic Gram-Negative Bacterial Isolates in Osteomyelitis Cases (n = 120)

Antibiotics	Pseudomonas aeruginosa N (%)	Acinetobacter baumannii N (%)	Klebsiella pneumoniae N (%)	Proteus mirabilis N (%)	Citrobacter freundii N (%)
Ampicillin	28 (100)	21 (95.0)	6 (75.0)	5 (83.0)	4 (100)
Amikacin	13 (46.4)	16 (73.0)	3 (38.0)	3 (50.0)	2 (50.0)
Ofloxacin	15 (53.6)	20 (90.9)	5 (63.0)	3 (50.0)	3 (75.0)
Ciprofloxacin	16 (57.1)	21 (95.0)	6 (75.0)	4 (67.0)	3 (75.0)
Cefotaxime	18 (64.3)	18 (82.0)	6 (75.0)	4 (67.0)	4 (100)

Ceftazidime	16 (57.1)	17 (77.0)	5 (63.0)	5 (83.0)	4 (100)
Cefoperazone + Sulbactam	13 (46.4)	10 (45.0)	2 (25.0)	2 (33.0)	2 (50.0)
Piperacillin	18 (64.3)	15 (68.0)	4 (50.0)	4 (67.0)	2 (50.0)
Piperacillin + Tazobactam	11 (39.3)	9 (41.0)	2 (25.0)	2 (33.0)	2 (50.0)
Imipenem	15 (53.6)	15 (68.0)	3 (38.0)	3 (50.0)	2 (50.0)
Meropenem	12 (42.9)	12 (55.0)	2 (25.0)	3 (50.0)	2 (50.0)

DISCUSSION

The present study analyzed the microbiological and antibiotic susceptibility profile of osteomyelitis in a tertiary care setting with a sample size of 120 cases. The findings highlight the persistence of Staphylococcus aureus as the predominant causative organism, followed by a significant proportion of Gram-negative bacilli, notably Pseudomonas aeruginosa and Acinetobacter baumannii. The rise in multidrug-resistant (MDR) Gram-negative isolates underscores a concerning shift in osteomyelitis microbiology. These results align with recent global trends suggesting a gradual transition from monomicrobial to polymicrobial infections and from Gram-positive to Gram-negative dominance in certain healthcare settings.[11]

Recent literature emphasizes that hospital-acquired osteomyelitis, especially in patients with implants or prior antibiotic exposure, is frequently associated with resistant pathogens. According to Baral et al., Acinetobacter baumannii and Pseudomonas aeruginosa exhibit strong biofilm-forming capabilities, which enhance chronicity and antibiotic resistance, thereby complicating management.[12] This correlates with the present study's observation of persistent infection despite antibiotic sensitivity to carbapenems, indicating possible biofilm-associated tolerance rather than simple resistance.

A comparative multicentric study from Turkey found that nearly 60% of chronic osteomyelitis isolates were Gram-negative bacilli, reflecting changing epidemiological patterns similar to those observed here.[13] The study also emphasized the role of local antibiotic policies, surgical debridement practices, and hospital flora in influencing pathogen prevalence. Furthermore, the increased resistance to fluoroquinolones and cephalosporins among both Gram-positive and Gram-negative isolates in our study corresponds to findings by Singh et al., who noted a decline in β-lactam efficacy and recommended carbapenem or β-lactamase inhibitor combinations as more reliable empiric choices.[14]

The emergence of MRSA in nearly half of S. aureus isolates in this study underscores the need for cautious antibiotic stewardship. However, susceptibility to linezolid and aminoglycosides remains encouraging, providing viable alternatives in resource-limited settings. Similar findings by Thomas et al. demonstrated that linezolid and daptomycin remain effective for MRSA bone infections with minimal resistance over the past decade.[15] Taken together, these findings indicate that ongoing surveillance of resistance trends and individualized antimicrobial therapy are essential for optimizing outcomes in osteomyelitis management. Overall, the shift towards multidrug-resistant pathogens, polymicrobial infections, and complex host-pathogen dynamics demands continuous updating of empirical antibiotic protocols. Multidisciplinary management—combining microbiological guidance, surgical intervention, and rational antibiotic use—is essential for preventing chronicity and improving recovery.

CONCLUSION

The current study reveals that Staphylococcus aureus continues to be the leading pathogen in osteomyelitis, but Gram-negative organisms such as Pseudomonas aeruginosa and Acinetobacter baumannii are emerging as significant contributors, particularly in chronic and post-traumatic cases. High resistance rates to cephalosporins and fluoroquinolones were observed, whereas carbapenems and β-lactam/β-lactamase inhibitor combinations retained the highest efficacy. These findings highlight the importance of regular microbiological surveillance and antibiotic sensitivity testing to guide appropriate therapy and control antimicrobial resistance in tertiary care settings.

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