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To Study the Microbiological Profiling and its Antibiotics Pattern of Osteomyelitis in Patient Attending a Tertiary Care Centre

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ABSTRACT

Background: When patients with bone infections, osteomyelitis continues to be the leading cause of morbidity. Osteomyelitis case management has become challenging due to the ongoing shift in the resistance pattern and the trend of organisms involved. In light of this, the current investigation sought to identify potential shifts in the patterns of antibiotic susceptibility and the bacteria implicated in osteomyelitis.
Aim and Objective: To study the microbiological profiling and its antibiotics pattern of osteomyelitis in patient attending a tertiary care centre.
Material and Methods: This was a Cross sectional study carried out in the Department of Orthopaedics and Department of Microbiology for a period of 12 months i.e, February 2024 to February 2025 at a tertiary care centre. A total of 100 cases were studied. Standard microbiological procedures were used to treat the samples, and antimicrobial testing was performed in accordance to the CLSI guidelines 2023.
Results: There were 36 female patients and 64 male patients out of 100 cases; the average age of all the patients was 51.6±12.32 years. Among the patients, 64% had diabetes. Trauma is the most common risk factor for long bones (45%). Pseudomonas aeruginosa (21.2%) and Acinetobacter baumannii (16.7%) were the next most common pathogens found, after Staphylococcus aureus (24.2%). Gram-positive organisms tested completely sensitive to vancomycin, whereas Gram-negative bacteria were most sensitive to cefoperazone+sulbactam, piperacillin+tazobactam, meropenem, and imipenem.
Conclusions: Methicillin-resistant Staphylococcus aureus and carbapenem-resistant gram-negative bacteria are major causes of osteomyelitis. Given the emergence of multidrug-resistant bacteria in osteomyelitis cases, cleanliness and tailored antibiotherapy should be prioritized.

Keywords: Osteomyelitis,Microbiological profile, Antibiogram, MRSA, AST

1. INTRODUCTION

Osteomyelitis has been continuing as the most important cause of morbidity among patients with bone infections. Constant change in the trend of organisms involved and resistance pattern has made management of osteomyelitis cases difficult. Osteomyelitis can be classified as acute, subacute, or chronic, depending on the duration of symptoms. The mechanism of infection can be exogenous or hematogenous. Exogenous osteomyelitis is caused by open fractures, surgery (iatrogenic), or contiguous spread from infected local tissue.

An infection-induced inflammation of the bone is known as osteomyelitis. Although polymicrobial infections can happen, particularly in the diabetic foot, the infection is usually caused by a single organism [1,2]. Low-grade inflammation, the presence of dead bone (sequestrum), new bone apposition, and fistulous tracts are the hallmarks of chronic osteomyelitis, a persistent and recurrent infection [3]. Anaerobes such as Pepto streptococcus spp., Bacteroides spp., Clostridium spp., and Salmonella spp. are seldom found in chronic osteomyelitis, which is typically caused by Staphylococcus, Pseudomonas spp., E. coli, Proteus spp., Klebsiella spp., Enterococcus spp., and Enterobacter spp [4,5].

It was established that *Staphylococcus aureus* still plays a dominating role, but that gram-negative bacteria are becoming more prevalent. Antibiotic overuse and inappropriate usage are thought to be the primary causes of drug resistance. The correct dose of antibiotics and precise microorganism isolation are essential for the effective treatment of chronic osteomyelitis. In order to find out how the microbes that cause osteomyelitis are evolving and how susceptible they are to antibiotics, the current investigation was carried out especially the tibia and femur, which are lengthy bones [6]. Infection of the bone by microorganisms can happen as a result of trauma, prosthesis implantation, or fracture stabilization.

Blood flow from skin wounds and other infectious areas allows microorganisms to reach the metaphysis of bone. Congestion, oedema, exudates, leucocytosis, necrosis, and abscess are all consequences of microbial growth in the metaphysic [4]. Coagulase-negative biochemical reactions and S. aureus are the bacteria that cause chronic osteomyelitis the most frequently [7]. In accordance with CLSI standards, the Kirby Bauer disc diffusion method was used to assess antibiotic sensitivities on Mueller Hinton agar [8].

Osteomyelitis is an ongoing problem due to emergence of Multidrug Resistance (MDR) strains among bacterial pathogens. Beta lactamases are the most evolving mechanism of antibiotic resistance among the family Enterobacteriaceae due to the selective pressure imposed by inappropriate use of third generation cephalosporins, most often encountered in Intensive Care Unit (ICU) settings [9]. Extended Spectrum Beta Lactamases (ESBL) and AmpC enzymes are the most common known beta lactamases. Carbapenems represented a great advance for the treatment of serious bacterial infections caused by beta lactam resistant bacteria [10]. But extensive and unnecessary use of the carbapenems facilitated the emergence of carbapenem resistant bacteria which produced carbapenem hydrolysing enzyme Metallo Beta Lactamase (MBL), so called because they contain metal ion that works as a co-factor for enzymatic activity [11]. Methicillin-resistant Staphylococcus aureus (MRSA) is prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide [12].

The mechanism of infection can be exogenous or hematogenous. Exogenous osteomyelitis is caused by open fractures, surgery (iatrogenic), or contiguous spread from infected local tissue. Chronic osteomyelitis is defined clinically as bone infection with clinical signs persisting for more than 10 days or the relapse of a previously treated or untreated osteomyelitis and bone infection was defined as at least two bone cultures with the same organism growth, or one positive bone culture combined with the intraoperative finding of purulence, acute inflammation on histologic examination consistent with infection, or a sinus tract communicating to the bone [6]. Currently, morbidity and mortality from osteomyelitis are relatively low because of modern treatment methods, including the use of antibiotics and aggressive surgical treatment. But the treatment of chronic osteomyelitis remains a challenge because of the rapid development of antimicrobial resistance and expression of virulence factors. Pus culture yields the causative organism and this may helpin selecting the proper preoperative antibiotics.

Therefore, the present study was undertaken to study the microbiological profiling and its antibiotics pattern of osteomyelitis in patient attending a tertiary care centre.

2. MATERIAL AND METHODS

Study design

This was a prospective cross-sectional study carried out in the Department of Orthopaedics and Department of Microbiology for a period of 12 months i.e, February 2024 to February 2025 at a tertiary care centre. Patients clinically diagnosed with osteomyelitis were included in the study. A total of 100 cases were studied. Standard microbiological procedures were used to treat the samples, and antimicrobial testing was performed in accordance to the CLSI guidelines 2023.

Inclusion criteria

All the deep tissue and bone tissue received from osteomyelitis patients (Non repetitive).

Exclusion criteria

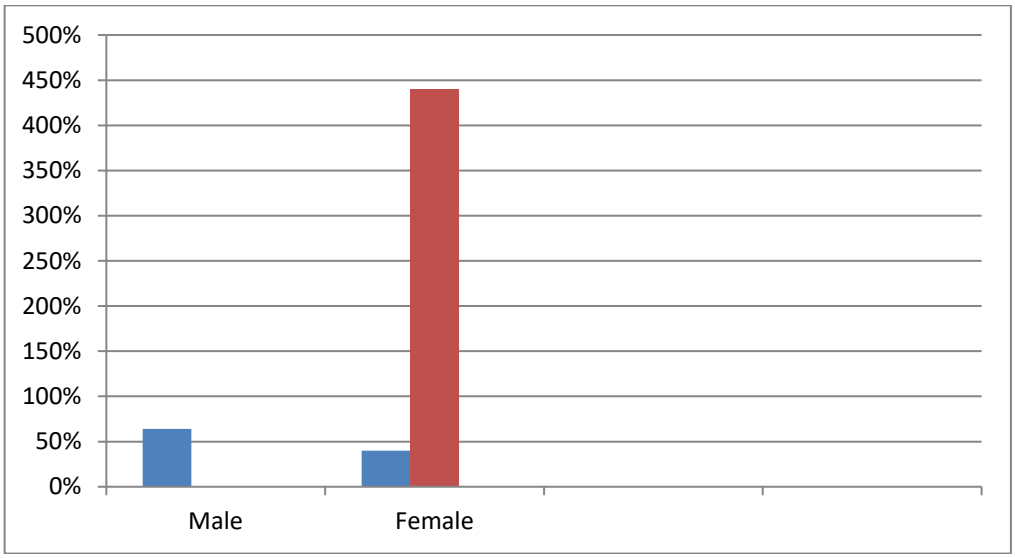
Superficial wound infections and patients on antibiotic treatments.

Specimen collection

Following extensive debridement, pus samples were obtained in the surgical unit. First, iodine solution was used to clean the sinus entrance and the skin around it. The deeper material was then aseptically collected in two different swabs after the surface sinus discharge was carefully squeezed out and disposed of. The most active sinus was taken into consideration when there were several. Microscopy was done on the first swab, and aerobic bacterial isolation was done on the second. A syringe was used to collect the specimen for the anaerobic culture, or a swab if there was less. The specimen was then promptly put to the proper media and incubated anaerobically. Gram stain morphology, colony characteristics, and other factors were used to identify the culture isolates.

3. RESULTS

The mean age of all the patients was 51.6 years. Males (64%) were more affected than females (36%). Out of 100 osteomyelitis cases 64% were diabetic patients, of which 34 were maintained on oral hypoglycemic agents and 30 were maintained on insulin. The mean duration of diabetes mellitus was 9.6years (SD 2.1). 68% of the diabetic patient shad HbA1Clevel>than8 and 48% of the subjects had leukocytosis during admission. The commonest bone affected in the study was tibia (70%) followed by femur (28%) and the other small bones.



Diabetes complications

Out of 100 cases, 64% patients presented with diabetic complications. 13% patients had peripheral neuropathy, 8% nephropathy, 4% retinopathy and 45% had hypertension.

Microbial profile

Table1. Shows the microbial profile of osteomyelitis. 32 cases had multiple etiologies, while 68 cases had a single organism. Anaerobic bacteria were recovered in 2% of cases, while aerobic bacteria were isolated in 98% of them. In two cases, fungus was grown. More Gram negative bacteria than Gram positive species were identified.

Table1:Aerobic and anaerobic bacteria isolated from Osteomyelitis cases.

Organisms	Number	Percentage
Gram positive bacteria		
Staphylococcu saureus	32	24.2
Enterococcus faecalis	18	13.6
Streptococcus pyogenes	04	3
Gram negative bacteria		
Pseudomonas aeruginosa	28	21.2
Acinetobacter baumanni	22	16.7
Klebsiella pneumoniae	08	6.1
Proteus mirabilis	06	4.5
Citrobacter freundii	04	3
Morganellamorganii	02	1.5
Anaerobicbacteria		

Clostridiumspp	03	2.3
Bacteriodesspp	03	2.3
Yeast		
Candidaspp		
Total	02	1.5

***More than one organism was isolated in 32 patients.**

Table 1 shows the distribution of the study's isolated aerobic microbes. Compared to Gram positive species, gram negative bacteria were found more frequently. *Staphylococcus aureus* was recovered in 32 (24.2%) of the gram-positive bacterial infections. 18 (13.6%) of the cases had *Enterococcus faecalis*, while 4 (3.0%) had *Streptococcus pyogenes*. The most common gram-negative aerobic bacteria that were isolated were *Pseudomonas aeruginosa* (28) and *Acinetobacter baumannii* (22). *Klebsiella pneumoniae* (8), *Proteus mirabilis* (6), *Citrobacter freundii* (4), and *Morganella morganii* (2) were the next most common bacteria. In two instances, Candida species have been isolated. Bacteriodes and Clostridium species were isolated in three instances each among anaerobic bacteria.

Antibiotic susceptibility pattern

Table 2. Shows the pattern of antimicrobial resistance in the gram-positive cocci. Thirty (93.8%) and 29 (90.6%) of the 32 *Staphylococcus aureus* strains were ampicillin and penicillin resistant, respectively. Of the aminoglycosides group, 15 (46.9%) were resistant to amikacin and 24 (75%) to gentamicin. The highest resistance to quinolones was found to be to ciprofloxacin 26 (81.3%), which was followed by Ofloxacin 17 (53.1%) and Sparfloxacin 16 (50%). Out of the cephalosporins, 18 (56.3%) and 19 (59.4%) were resistant to ceftazadime and cefotaxime, respectively. Isolates that were resistant to clindamycin were 13 (40.6%), linezolid was 17 (53.1%), and netilmycin was 15 (46.9%). There was no vancomycin resistance among the isolates.

All 18 Enterococcus faecalis strains were resistant to penicillin, 94% to ampicillin, 94.4% to cephalexin, and 83.3% to gentamicin. Vancomycin was not a barrier for any of the enterococci species.

Two (40.0%) isolates of Streptococcus pyogenes were ampicillin and penicillin resistant, while one isolate was cephalexin resistant.

Table 3 shows the resistance pattern of aerobic gram-negative bacteria that were isolated from instances of osteomyelitis. Thirteen (46.4%) and 28 (100%) of the 28 Pseudomonas isolates were resistant to amikacin and ampicillin, respectively. Of the Pseudomonas isolates treated with fluoroquinolones, 15 (53.6%) were resistant to ofloxacin and 16 (57.1%) to ciprofloxacin. Out of all the cephalosporins, 18 (64.3%) were resistant to piperacillin, 16 (57.1%) to ceftazidime, and 18 (64.3%) to cefotaxime. Of the carbapenems, 12 (42.9%) were meropenem and 15 (53.6%) were imipenem.11 (39.3%) are resistant to piperacillin+tazobactam, while 13 (46.4%) are resistant to cefoperazone+sulbactam.

Out of the 22 Acinetobacter baumannii, 21 (95%) were resistant to ampicillin, 16 (73%) to amikacin, 21 (95%) to ciprofloxacin, 20 (90.9%) to ofloxacin, 18 (81.8%) to cefotaxime, 17 (77.3%) to ceftazidime, and 15 (68.2%) to piperacillin. Out of the carbapenems, 12 (54.5%) were meropenem and 15 (68.2%) were imipenem.09 (40.9%) are resistant to piperacillin+tazobactam, while 10 (45.5%) are resistant to cefoperazone+sulbactam.

Table2:Aerobicandanaerobicbacteriaisolatedfromosteomyelitiscases.

Antibiotics	*Staphylococcusaureus*	*Enterococcifaecalis*	*Streptococcuspyogenes*
	N (%)	**N (%)**	**N (%)**
Penicillin-G	30(93.8)	18(100)	02(40.0)
Ampicillin	29(90.6)	17(94.4)	02(40.0)
Linezolid	17(53.1)	9(50.0)	00
Clindamycin	13(40.6)	8(44.4)	00
Gentamicin	24(75.0)	15(83.3)	00
Ciprofloxacin	26(81.3)	13(72.2)	02
Ofloxacin	17(53.1)	14(77.8)	00
Sparfloxacin	16(50.0)	13(72.2)	00
Cefotaxime	19(59.4)	15(83.3)	00
Ceftazadime	18(56.3)	16(88.9)	00
Cephalexin	29(90.6)	17(94.4)	01(20.0)
Methicillin(Bycefoxitin)	16(50.0)	--	--
Amikacin	15(46.9)	10(55.6)	00
Netilmycin	15(46.9)	11(61.1)	00
Vancomycin	00	00	00

Eight isolates of Klebsiella pneumoniae were found in cases with osteomyelitis. 6 (75%) and 3 (38%) of the eight Klebsiella isolates were ampicillin and amikacin resistant. is not susceptible to piperacillin. 2(25%) of the carbapenems are meropenem, while three (38%) are imipenem. Five (63%) of the Klebsiella isolates were resistant to Ofloxacin, 6(75.0%) to Ciprofloxacin, and 2(25%) to fluoroquinolones. 6(75%) of the cephalosporins were resistant to cefotaxime, 5 (63%) to ceftazidime, 4 (50%) to cefoperazone+sulbactam, and 2 (25%) to piperacillin+tazobactam.

Table3:Resistancepatternofaerobicgram-negativebacterialisolatesosteomyelitiscases.

Antibiotics	*Pseudomonas aeruginosa*	*Acinetobacter baumanni*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Citrobacter freundii*
	N (%)	N (%)	N (%)	N (%)	N (%)
Ampicillin	28(100)	21(95.0)	6(75.0)	05(83.0)	4(100)
Amikacin	13(46.4)	16(73.0)	3(38.0)	03(50.0)	2(50.0)
Ofloxacin	15(53.6)	20(90.9)	5(63.0)	03(50.0)	3(75.0)
Ciprofloxacin	16(57.1)	21(95.0)	6(75.0)	04(67.0)	3(75.0)
Cephotaxime	18(64.3)	18(82.0)	6(75.0)	04(67.0)	4(100)
Ceftazadime	16(57.1)	17(77.0)	5(63.0)	05(83.0)	4(100)
Cefoperazone+Sulbactam	13(46.4)	10(45.0)	2(25.0)	02(33.0)	2(50.0)
Piperacillin	18(64.3)	15(68.0)	04(50.0)	04(67.0)	2(50.0)
Piperacillin+Tazobactam	11(39.3)	9(41.0)	2(25.0)	02(33.0)	2(50.0)
Imipenem	15(53.6)	15(68.0)	03(38.0)	03(50.0)	2(50.0)
Meropenem	12(42.9)	12(55.0)	02(25.0)	03(50.0)	2(50.0)

Out of the 6 Proteus mirabilis, 3 (50%) were resistant to amikacin and 5 (83%) to ampicillin. Proteus mirabilis showed resistance to ciprofloxacin in 4 (67%) and to ofloxacin in 3 (50%) of the fluoroquinolones. Of these cephalosporins, 4 (67%) were resistant to piperacillin, 5 (83%) to ceftazidime, and 4 (67%) to cefotaxime. Of the carbapenems, 3 (50%) are imipenem and 3(50%) are meropenem.2 (33%) are resistant to piperacillin+tazobactam, and 2 (33%) are resistant to cefoperazone+sulbactam.

Among *Citrobacter freundii* all four were resistant to ampicillin, cephotaxime,ceftazadime,75%wereresistant to ofloxacin, ciprofloxacin and 50% of the isolates were resistant to amikacin, piperacillin, imipenem,meropenem, cefoperazone+sulbactam and to piperacillin+tazobactam

4. DISCUSSION

In the majority of underdeveloped nations, including India, osteomyelitis is one of the most bothersome illnesses. Treatment becomes increasingly more challenging when drug-resistant strains become more prevalent. The etiological pattern of illnesses and antibiotic susceptibility has been altered by the widespread use of antibiotics. 32 (32%) and 68 (68%) of the 100 samples that were analyzed had polymicrobial growth and monomicrobial growth, respectively. Bacterial infections are a frequent cause of hospitalization and particularly healthcare associated infections are more common in critical care settings. Globally the emergence of antimicrobial resistance and limited availability of treatment options present an increasing challenge for the management of bacterial infections worldwide.

Pseudomonas aeruginosa was the most common gram-negative bacterium isolated, whereas *Staphylococcus aureus* was the most common gram-positive bacterium. Similar results were reported by Wadekar et al., who found that 20% of the growth was polymicrobial and 67.0% was of mono aetiology. [13] However, in our investigation, *Pseudomonas aeruginosa*, Acinetobacter baumannii, and Staphylococcus aureus were the most common bacteria isolated, followed by Escherichia coli, although E. coli was not isolated. Our data is supported by Kaur et al.'s report that, despite a considerable increase in bone infections caused by gram-negative organisms, Staphylococcus aureus (43.0%) continued to be the most common cause of osteomyelitis, followed by Pseudomonas aeruginosa (10.0%) [14].

Wadekar et al. also found that males had a 2.7:1 greater incidence of osteomyelitis than females. Male preponderance over females was noted in our study as well (1.63:1), which may be the result of societal gender prejudice [13] The tibia (70%) and femur (28%) were the most frequently affected bones in the study, followed by the other minor bones. Diabetes was the most frequent cause of osteomyelitis (64%), followed by trauma/accidents (22%), orthopaedic implants (10%), and wounds after surgery (2%). All of the identified bacterial strains are MDR isolates since they shown resistance to two more classes of antibiotics. Half of the Staphylococcus aureus strains were methicillin-resistant, indicating that the bacteria is resistant to the antibiotic.

All MRSA strains, however, demonstrated 100% sensitivity to vancomycin, 59% sensitivity to clindamycin, 47% sensitivity to linezolid, and 51% sensitivity to netilmycin. Both the current study and previous research have made it abundantly evident that MRSA strains are becoming increasingly dangerous due to their heightened resistance to antibiotics such as amikacin, netilmicin, and to a lesser degree, vancomycin, and linezolid. This makes it more difficult for clinicians to choose the best medication to treat chronic osteomyelitis [15]. With the emergence of multidrug-

resistant microorganisms such as methicillin-resistant *S. aureus*, the treatment of the infection has become challenging in the present time.

In our study, the most resistant gram-negative bacteria were to ampicillin, cefotaxime, ceftazidime, ofloxacin, piperacillin, and imipenem. Many gram-negative bacteria were shown to be susceptible to meropenem, cefoperazone-sulbactam, and piperacillin-tazobactam.

Osteomyelitis (OM) is a one of the most challenging bone and joint infection till the present day due to its heterogeneity [16] . Osteomyelitis is a heterogeneous disease with a vivid presentation, which can lead to devastating complications if left untreated. Even if the clinical diagnosis of osteomyelitis is obvious, the microbiological workup for etiological diagnosis of cases of osteomyelitis is still not a routine practice in many hospitals, which needs to be improved [17,18]. Proper identification of the causative organism and antibiogram sensitivity testing plays a pivotal role in controlling the infection[19,20].

5. CONCLUSION

In the present study we have documented change in pattern of organisms isolated and emergence of increased drug resistance among the bacterial isolates in osteomyelitis cases. It is high time to emphasize on surveillance to monitor change in an etiology and to follow one health policy to impede the menace created by multidrug resistant bacteria.

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.

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