Original Research Article

Microbiological and antibiotic profile of osteomyelitis in tertiary care hospital

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ABSTRACT

Background: 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. With this background present study aimed to look for the changing trends of microorganisms involved in osteomyelitis and their antimicrobial susceptibility pattern.
Methods: A total of 100 cases studied over a period of two years. Samples collected were processed by standard microbiological techniques and antimicrobial testing was done as per the clinical and laboratory Standards Institute guidelines.
Results: Among 100 cases, 62 were males and 38 were females, with the mean age of all the patient was 51.6 ± 12.32 years. 64% of the patient were diabetic patients. Long bones are most involved with trauma (45%) as risk factor. *Staphylococcus aureus* (24.2%) predominant pathogen isolated followed by *Pseudomonas aeruginosa* (21.2%) and *Acinetobacter baumannii* (16.7%). Antibiotic sensitivity testing of gram positive organisms showed hundred percent sensitivity to vancomycin and gram negative bacteria showed highest sensitivity to cefoperazone+sulbactam, piperacillin+tazobactam, meropenem and imipenem.
Conclusions: Osteomyelitis caused by methicillin resistant staphylococcus aureus and carbapenem resistance gram negative bacteria is a serious concern. Since multidrug resistant strains have emerged in osteomyelitis cases, emphasis should be given for hygiene and targeted antibiotherapy.

Keywords: Osteomyelitis, Microbiological profile, Antibiogram

INTRODUCTION

Osteomyelitis is defined as an inflammation of the bone caused by an infecting organism. The infection generally is due to a single organism, but polymicrobial infections can occur, especially in the diabetic foot.[1] Chronic osteomyelitis is a relapsing and persistent infection and is characterized by low-grade inflammation, presence of dead bone (sequestrum), new bone apposition, and fistulous tracts.[2] Chronic osteomyelitis commonly involves long bones; especially tibia and femur.[3] Introduction of microorganisms into the bone may occur during stabilization of the fracture, implanting prosthesis or due to trauma. Microorganisms reach to the metaphysis of bone through blood flow from skin wounds and other infectious regions. Multiplication of microorganisms in metaphysis will cause congestion, oedema, exudates, leucocytosis, necrosis and abscess.[4] The bacteria most commonly causing chronic osteomyelitis are *S. 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] The still dominant role of Staphylococcus aureus could be confirmed, but also the increasing number of gram-negative bacteria. Inappropriate and excessive use of antibiotics is considered as the main cause of development of drug resistance. Proper management of chronic osteomyelitis requires accurate microbial isolation and appropriate antibiotic administration. The present study was conducted to look for the changing trends of microorganisms involved in osteomyelitis and their antimicrobial susceptibility pattern.

METHODS

Study design

This is a prospective cross sectional study. Patients clinically diagnosed with osteomyelitis were included in the study.

Number of samples

Total 100 cases of osteomyelitis were included over the period of 2 years (January 2017 to December 2019).

Ethical clearance: The research protocol was approved by institutional ethics review board of S. S. Institute of Medical Sciences and Research Centre, Davanagere, Karnataka

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

Pus samples were collected in the surgical unit after extensive debridement. The sinus orifice and surrounding skin was first cleaned with iodine solution. The superficial discharge of the sinus was squeezed out gently and discarded and the deeper material was then collected aseptically in two separate swabs. In cases of multiple sinuses, the most active one was considered. The first swab was used for microscopy and the second swab was used for isolation of aerobic bacteria. For anaerobic culture, specimen was collected in a syringe and if specimen was less, specimen was collected in a swab and added immediately to appropriate media and incubated anaerobically6. The culture isolates were identified by Gram stain morphology, colony characters and

biochemical reactions.[7] Antibiotic sensitivities was done on Mueller Hinton agar by Kirby Bauer disc diffusion method as per CLSI guidelines.[8]

RESULTS

The mean age of all the patients was 51.6 years (SD 12.32). Males (62%) were more affected than females (35%). 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 9.6 years (SD 2.1). 68% of the diabetic patients had HbA1C level>than 8 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

Microbial profile of osteomyelitis is depicted in Table 1. Multiple aetiology was seen in 32 cases and single organism in 68 cases. Aerobic bacteria were isolated in 98% cases and anaerobic bacteria in 2% cases. Fungus was grown in 2 cases. Gram negative bacteria were isolated predominantly compared to Gram positive organisms.

Table 1: Aerobic and anaerobic bacteria isolated from Osteomyelitis cases.

Organisms	Number	Percentage
Gram positive bacteria		
Staphylococcus aureus	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
Morganella morganii	02	1.5
Anaerobic bacteria		
Clostridium spp	03	2.3
Bacteriodes spp	03	2.3
Yeast		
Candida spp		
Total	02	1.5

* More than one organism was isolated in 32 patients.

Table 1 gives the distribution of aerobic organisms isolated from the study. Gram negative bacteria were isolated predominantly compared to Gram positive organisms. Among gram positive bacteria, Staphylococcus aureus was isolated in 32 (24.2%) cases. *Enterococcus faecalis* in 18 (13.6%) cases and *Streptococcus pyogenes* was isolated in 04 (3.0%) cases.

Among the gram negative aerobic bacteria, Pseudomonas aeruginosa (28) and *Acinetobacter baumannii* (22) were the predominant bacteria isolated followed by *Klebsiella pneumoniae* (8) *Proteus mirabilis* (6), *Citrobacter freundii* (4) and *Morganella morganii* (2) cases. Candida species has been isolated in 2 cases.

Among anaerobic bacteria, bacteriodes species and clostridium species were isolated in three cases each.

Antibiotic susceptibility pattern

The antimicrobial resistance pattern of the gram-positive cocci is shown in table 2. In 32 Staphylococcus aureus, 30 (93.8%) were resistant to penicillin and 29 (90.6%) to ampicillin. In aminoglycosides group, 24 (75%) were resistant to gentamicin and 15 (46.9%) to amikacin. In quinolones, maximum resistance was observed to ciprofloxacin 26 (81.3%) followed by Ofloxacin 17 (53.1%) and Sparfloxacin 16 (50%). Among the cephalosporins, 19 (59.4%) were resistant to cefotaxime and 18 (56.3%) to ceftazadime. Thirteen (40.6%) isolates were resistant to clindamycin, 17 (53.1%) to linezolid and 15 (46.9%) to netilmycin. None of the isolates were resistant to vancomycin.

All *Enterococcus Faecalis* (18) were all resistant to Penicillin, 94% were resistant to ampicillin and 94.4% were resistant to gentamicin, 94.4% to cephalexin and 83.3% to gentamicin. None of the species of enterococci were resistant to Vancomycin

Among *Streptococcus pyogenes* 2 (40.0%) were resistant penicillin, ampicillin, and one isolate was resistant to cephalexin.

Resistance pattern of aerobic gram negative bacteria isolated from osteomyelitis cases is depicted in Table 3. Out of 28 *Pseudomonas isolates*, 28 (100%) were resistant to ampicillin, 13 (46.4%) to amikacin. In fluoroquinolones, 16 (57.1%) of Pseudomonas isolates were resistant to ciprofloxacin and 15 (53.6%) to ofloxacin. Among cephalosporins, 18 (64.3%) were resistant to cefotaxime, 16 (57.1%) to ceftazidime and 18 (64.3%) were resistant to piperacillin. Among the carbapenems, 15 (53.6%) to imipenem and 12 (42.9%) to meropenem. 13 (46.4%) are resistant to cefoperazone+sulbactam and 11 (39.3%) to piperacillin+tazobactam.

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

Table 2: Aerobic and anaerobic bacteria isolated from osteomyelitis cases.

Antibiotics	*Staphylococcus aureus* N (%)	*Enterococci faecalis* N (%)	*Streptococcus pyogenes* 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 (By cefoxitin)	16 (50.0)	--	--
Amikacin	15 (46.9)	10 (55.6)	00
Netilmycin	15 (46.9)	11 (61.1)	00
Vancomycin	00	00	00

Eight *Klebsiella pneumoniae* were isolated from osteomyelitis cases. Out of 8 Klebsiella isolates, 6 (75%) were resistant to ampicillin, 3 (38%) to amikacin. In

fluoroquinolones, 6 (75.0%) of Klebsiella isolates were resistant to ciprofloxacin and 5 (63%) to Ofloxacin. Among cephalosporins, 6 (75%) were resistant to cefotaxime, 5 (63%) to ceftazidime and 4 (50%) were

resistant to piperacillin. Among the carbapenems, 3 (38%) to imipenem and 2 (25%) to meropenem. 2 (25%) are resistant to cefoperazone+sulbactam and 02 (25%) to piperacillin+tazobactam.

Table 3: Resistance pattern of aerobic gram negative bacterial isolates osteomyelitis cases.

Antibiotics	Pseudomonas aeruginosa N (%)	Acinetobacter baumanni N (%)	Klebsiella pneumoniae N (%)	Proteus mirabilis N (%)	Citrobacter freundii 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 6 *Proteus mirabilis*, 5 (83%) were resistant to ampicillin, 3 (50%) to amikacin. In fluoroquinolones, 4 (67%) of *Proteus mirabilis* were resistant to ciprofloxacin and 3 (50%) to ofloxacin. Among cephalosporins, 4 (67%) were resistant to cefotaxime, 5 (83%) to ceftazidime and 4 (67%) were resistant to piperacillin. Among the carbapenems, 3 (50%) to imipenem and 03 (50%) to meropenem. 2 (33%) are resistant to cefoperazone+sulbactam and 02 (33%) to piperacillin+tazobactam.

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

DISCUSSION

Osteomyelitis is one of the most inconvenient diseases among most of the developing countries like India. An increase in the emergence of drug resistant strains makes treatment even more complicated. Extensive use of antibiotics has changed aetiological pattern of infections and antibiotic susceptibility. Out of the 100 samples tested, 68 (68%) cases showed mono-microbial growth and 32 (32%) showed polymicrobial growth. *Staphylococcus aureus* was the predominant bacteria isolated among gram positive bacteria and *Pseudomonas aeruginosa* was the predominant gram negative bacteria isolated. Wadekar et al reported similar findings, in their study 67.0% were of mono aetiology type followed by 20% of polymicrobial growth.[9] Staphylococcus aureus followed by *Escherichia coli* were the predominant bacteria isolated, but in our study *Staphylococcus aureus* was the predominant bacteria followed by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and *E. coli* was

not isolated. Kaur et al reported that although bone infections caused by gram-negative organisms had significantly increased, but *Staphylococcus aureus* (43.0%) remained the most common cause of osteomyelitis, which was followed by *Pseudomonas aeruginosa* (10.0%) which corroborate with our study.[10] Wadekar et al also reported a higher incidence of osteomyelitis in male than in females with the ratio of 2.7:1. Even in our study male preponderance over female was observed (1.63:1) and this could be due to gender bias present in the society.[9]

The commonest bone affected in the study was tibia (70%) followed by femur (28%) and the other small bones. The most common factor leading to osteomyelitis was diabetes (64%) followed by trauma/accidents (22%) and orthopaedic implants (10%) and postsurgical wound (2%). All the bacterial strains isolated showed resistance to two more class of antibiotics, hence all the strains are MDR isolates. Among *Staphylococcus aureus*, 50% of them were resistant to methicillin, indicating methicillin resistant *staphylococcus aureus*. However, all the MRSA strains showed 100% sensitivity to vancomycin, 59% sensitivity to clindamycin, 47% linezolid, 51% to netilmycin. It is quite clear from the studies that have been conducted so far as well as from the present study that MRSA strains are becoming alarming because of their increased resistance towards antibiotics-like amikacin, netilmicin, and to a lesser extent to vancomycin and linezolid that leaves the clinicians with less choice to use the appropriate drug for treatment of chronic osteomyelitis.[11]

In our study, gram negative bacilli showed maximum resistant to ampicillin, cefotaxime, ceftazidime, ofloxacin, piperacillin, imipenem. We documented that many gram negative bacteria were sensitive to meropenem, followed by cefoperazone-sulbactam, piperacillin- tazobactam.

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 aetiology and to follow one health policy to impede the menace created by multidrug resistant bacteria.

ACKNOWLEDGEMENTS

REFERENCES

1. de Carvalho VC, de Oliveira PRD, Dal-Paz K, de Paula AP, Felix CDS, Lima LLM. Gram-negative osteomyelitis: clinical and microbiological profile. Braz J Infect Dis. 2012;16:63-7.
2. Zuluaga AF, Galvis W, Vesga O. Etiologic diagnosis of chronic osteomyelitis. Arch Intern Med 2006;166:95-100.
3. Abid AS, Ehan AH, Yonis AR. Epidemiological and bacteriological study of chronic osteomyelitis. Tikrit Medic J. 2008;14(1):59-62.
4. Reza M, Shahab F, Jalal K, Farhad. Epidemic assessment of bacterial agents in osteomyelitis and their antibiotic resistance pattern determination. J Biologic Sci. 2008;8(2):478-81.
5. Rao PS, Beena VK, Rao PS, Shivnanda PG. Bacteriological study of bone and joint infections with special reference to anaerobes. Ind J Orthopaed. 1997;31:171-4.
6. Betty A Forbes, Daniel F. Sahm , Alice S. Weissfeld; Baily & Scott's Diagnostic Microbiology; 12th edition ; Mosby Inc; 2007.
7. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Mackie & McCartney Practical Medical Microbiology. 14th edition. Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Edinburgh: Churchill Livingstone. 2006:131-49
8. Clinical Laboratory Standards Institutes. Performance Standards for antimicrobial susceptibility testing, XXI International Supplement (M100-S21). Wayne, Pennsylvania, USA: National Commit Clinic Laborat Std. 2016.
9. Wadekar MD, Anuradha K, Venkatesha D. Chronic Osteomyelitis: Aetiology and Antibiotic Suscept-ibility Pattern. Int J Rec Trends Sci Technol. 2014;9(3):337-40.
10. Kaur, J, Gulati VL. Bacteriological profile of osteomyelitis with special reference to staphylococcus aureus. Ind J Pract Doc. 2013;4(6).
11. Sheehy SH, Atkins BA, Bejon P, Byren I, Wyllie D, Athanasou NA, et al. The microbiology of chronic osteomyelitis: prevalence of resistance to common empirical anti-microbial regimens. J Infect. 2010;60:338–43.