



RESEARCH ARTICLE

OPEN ACCESS

Microbiological Evaluation of Osteomyelitis with A Special Reference to Antibiotic Sensitivity Pattern of Isolates from A Tertiary Care Hospital

Barnini Banerjee¹ , Smitha S. Bhat¹, Chiranjay Mukhopadhyay^{1*} 
and Anil K. Bhat² 

¹Department of Microbiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, India. ²Department of Orthopedics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, India.

Abstract

Epidemiological change in osteomyelitis had been observed for the past few years with a significant rise of chronic osteomyelitis by multidrug-resistant organisms (MDROs). It complicates antibiotic selection, and if not managed adequately, it results in amputation, sepsis, or death. This study was conducted for one year in a tertiary care hospital to identify the etiological agents and their response to antibiotics for the development of adequate management policy of osteomyelitis. Fifty seven patients were analysed, & the variables examined include epidemiological characteristics, risk factors, radiographic changes, histopathological features. Etiological agents were identified, and antimicrobial susceptibility testing was performed by an automated system. About 73.7% of the cases presented with chronic osteomyelitis in the age group of 31-40 years (29.3%). In 53 patients, it resulted from contagious microbial seeding. The most common site of infection was the metatarsal bones of the foot (38.6%). The common predisposing factor was Diabetes (36.8%). The most common causative organism was *Staphylococcus aureus* (46.9%). Methicillin resistance was 41.9%. Among Gram-negative isolates, Enterobacterales group predominated (22.7%), 42.9% of Gram-negatives were multidrug -resistant (MDR). In our study, we found an increased prevalence of chronic osteomyelitis of the extremities of the lower limb instead of acute hematogenous infection of the long bones. Most of them resulted from trauma or after fracture fixation device implantation or as a sequela of diabetic foot. Forty percent of the etiological agents in our study were either methicillin resistant *S. aureus* (MRSA) or MDR Gram-negatives. This finding is challenging for the clinician to select the appropriate treatment option, reduce the risk of recurrence and improve the quality of life of the patients.

Keywords: Chronic, Osteomyelitis, Multi drug resistance, Implant

*Correspondence: chiranjay@gmail.com; + 91 820 2933529

(Received: July 22, 2019; accepted: January 27, 2020)

Citation: Barnini Banerjee, Smitha S. Bhat, Chiranjay Mukhopadhyay and Anil K. Bhat, Microbiological Evaluation of Osteomyelitis with A Special Reference to Antibiotic Sensitivity Pattern of Isolates from A Tertiary Care Hospital, *J. Pure Appl. Microbiol.*, 2020; **14**(1):485-490. <https://doi.org/10.22207/JPAM.14.1.50>

© The Author(s) 2020. **Open Access.** This article is distributed under the terms of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, sharing, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

INTRODUCTION

Osteomyelitis (OM) is a one of the most challenging bone and joint infection till the present day due to its heterogeneity. Though *Staphylococcus* is the classical culprit of OM, Gram-negative bacilli are also gaining recognition as the etiological agents. In the modern era, increase in Gram-negatives probably due to increase use of fracture fixation devices in orthopaedic surgeries, especially in open fracture cases after high energy trauma. Their contribution to diabetic foot osteomyelitis also not less than Gram-positives. The real matter of concern nowadays is multidrug resistance among both Gram-negative & Gram-positive organisms. Among Gram-negative bacilli, resistance to carbapenems is yet another concern. Carbapenem resistant not even unusual in the present day. The reason for the prevalence of multidrug resistance organisms is believed to be the virulence factors retained by this microorganism, which enable it to adhere and invade host tissues and evade immune defences and antimicrobials. Biofilm formation is the primary pathogenic mechanism leading to the chronicity and irreducibility of infections. Their presence delays the drug penetration to the bone, promote the antimicrobial resistance and increase the chronicity.

The frequency of isolation of organisms and antimicrobial resistance pattern of the different pathogens always vary in a different geographical region. Hence this study will help to develop the antimicrobial policy for osteomyelitis, which can ultimately help the clinician in the management of osteomyelitis. Present study is done to explore clinical characteristics, bacterial aetiology, and antimicrobial susceptibility pattern of the aerobic bacteria causing OM.

MATERIAL AND METHODS

Study design

A total of 57 adult patients who were diagnosed and treated for osteomyelitis in our hospital during one year were included in this study. Clinical and other laboratory data of the patients were reviewed further. We excluded the cases of tubercular or anaerobic bacterial etiology. Infections caused by more than two bacteria were also excluded.

Definitions

a. Co-infections: When more than one organism isolated from the clinical specimen of the same patient at the same time.

b. Multidrug resistant organisms (MDROs): Microorganisms which were non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories.

c. Osteomyelitis: All the osteomyelitis cases were diagnosed based on clinical presentation, histopathological findings and radiological picture. According to the time period between diagnosis and symptom onset, osteomyelitis was classified as acute (<2 weeks), sub-acute (2 weeks–3 months), or chronic (>3 months).

Microbiological workup

Clinical specimens including deep wound swab, tissue or pus were collected and cultured on 5% sheep blood agar and MacConkey agar. The growth in the culture media was identified by the Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) VITEK[®]MS. Antimicrobial susceptibility testing was done by Vitek[®]2 system (BioMerieux, Inc, Durham, NC).

RESULTS

Demographic & clinical characteristics

Of the total 57 patients included in the study, 42 (73.7%) presented with chronic osteomyelitis having pain (54/68, 79.4%) & discharging sinus (52/68, 76.5%). In acute cases, swelling was there along with pain instead of discharging sinuses. The median length of their hospital stay was 12.5 days (range-3-30days). The mean (SD) age of the patients was 46.4 years (± 14.12), and 78.9% (45/57) of the patients were male. Maximum cases of chronic osteomyelitis were observed in the age group of 31-40 years (29.3%, 12/41). The significant correlation observed with the distribution of different types of osteomyelitis at various age group (p value-.025). OM was universal in the lower limb, and the most common site of infection was the metatarsal bones of the foot (22/57, 38.6%) followed by tibia (13/57, 22.8%). Out of 57 cases, 53 (92.9%) patients had contagious osteomyelitis resulted from direct spread from nearby ulceration (18/57, 31.6%), post-traumatic (16/57, 28%) and postoperatively after fixation of the fracture

with devices (19/57, 33.3%) (Table 1). The major underlying predisposing factor was diabetes (36.8%) and presence of fracture fixation devices (33.3%). 34.1% (14/41) of patients developed chronic OM followed by fracture fixation devices.

Microbiological Characteristics

A total of 66 microbial agents isolated from 57 patients. 45 (68.1%) of them were Gram positive (GP), and 21 of them were Gram negative (31.8%) (GN) bacteria. Out of 45 GP bacteria, 9 of them caused acute OM (20%) and 36 of them caused chronic OM (80%). Among 21 GN bacteria, 8 (38.1%) contributed to acute OM and 13 (61.9%)

of them led to chronic OM. 24.4% Of Gram positive bacteria and 23.8% of GNB caused OM post-fracture fixation with devices. Most common causative organisms were *Staphylococcus aureus* (31/66, 46.9%) followed by Enterobacteriaceae group (15/66, 22.7%) (Table 2). Out of 31 isolates of *S. aureus*, 13 (41.9%) were Methicillin-resistant (MRSA). They showed maximum no. of resistance to ciprofloxacin (24/31, 77.1%). 100 % of MRSA strains were sensitive to vancomycin. Large number of susceptibility was also observed for co-trimoxazole (80.6%) & tetracycline (30/31, 96.8%) (Figure 5). All beta-hemolytic *Streptococci* (BHS) were sensitive to a penicillin (100%) & ceftriaxone (100%) and the *Enterococci* were susceptible to penicillin & high-level gentamicin. Out of 21

Table 1. Demographic & Clinical characteristics

Age group	Acute OM n=15 (%)	Chronic OM n=42 (%)
20-40 years 19 (45.2)	03 (20)	
41-60 years	09 (60)	18 (42.9)
61-80 years	03 (20)	05 (11.9)
Gender		
Male	11 (73.3)	34 (80.9)
Female	04 (26.7)	08 (19.1)
Affected segment		
Upper limb	02 (13.3)	02 (4.7)
Lower Limb	13 (86.7)	40 (95.3)
Mechanism of OM		
Contagious OM	15 (100)	38 (90.4)
Hematogenous OM	-	04 (9.6)

Table 2. Etiological agents of OM

Etiological agents	Total Number (%)
<i>S. aureus</i>	31 (46.9%)
Beta-haemolytic <i>Streptococcus</i> spp	11 (16.7)
<i>Enterococcus</i> spp	03 (4.5)
<i>E. coli</i>	03 (4.5)
<i>Klebsiella pneumoniae</i>	04 (6.1)
<i>Citrobacter freundii</i>	03 (4.5)
<i>Enterobacter cloacae</i>	03 (4.5)
<i>Proteus vulgaris</i>	01 (1.5)
<i>Serratia marcesens</i>	01 (1.5)
<i>Pseudomonas aeruginosa</i>	06 (9.1)

Table 3. Antibiotic susceptibility of Gram positive bacteria [Number (%) of sensitive strain]

Antibiotics	<i>S. aureus</i> N=31	Beta-hemolytic <i>Streptococcus</i> spp (N=11)	<i>Enterococcus</i> spp (N=3)
Cloxacillin	18 (58%)	-	-
Penicillin	11 (35.5%)	11 (100%)	3 (100%)
Ciprofloxacin	7 (22.6%)	11 (100%)	01(33.3%)
Co-trimoxazole	25 (80.6%)	9 (81.8%)	-
Erythromycin	19(61.3%)	07 (63.6%)	01(33.3%)
Gentamycin	24 (77.4%)	11(100%)	3 (100%)
Tetracycline	30 (96.8%)	3 (27.3%)	3 (100%)
Clindamycin	28 (90.3%)	9 (81.8%)	-
Vancomycin	31 (100%)	11(100%)	3 (100%)
Linezolid	31 (100%)	11 (100%)	3 (100%)
Teicoplanin	31 (100%)	11(100%)	3 (100%)
Ceftriaxone	-	11(100%)	-
Levofloxacin	-	8 (72.7%)	-

Table 4. Antibiotic susceptibility of Gram negative bacteria [number(%) of sensitive strain]

Antibiotics	<i>E.coli</i> N=3	<i>Klebsiella</i> N=4	<i>Enterobacter</i> N=3	<i>Citrobacter</i> N=3	<i>Pseudomonas</i> N=6
Ampicillin	1 (33.3%)	-	-	1(33.3%)	-
Amoxy-clav	1 (33.3%)	-	-	2(66.7%)	-
Ciprofloxacin	1 (33.3%)	1(25%)	3(100%)	2(66.7%)	4(66.7%)
Co-trimoxazole	1 (33.3%)	1(25%)	2 (66.7%)	2(66.7%)	-
Amikacin	3 (100%)	3 (75%)	3(100%)	3(100%)	4(66.7%)
Gentamycin	1 (33.3%)	3 (75%)	3(100%)	2(66.7%)	4(66.7%)
Ceftriaxone	-	-	3(100%)	2(66.7%)	-
Cefuroxime	-	-	-	1(33.3%)	-
Cef-sulbactam	3(100%)	1 (25%)	3(100%)	3(33.3%)	4(66.7%)
Pip-tazobactam	3(100%)	1(25%)	3(100%)	2(66.7%)	05(83.3%)
Imipenem	3(100%)	2 (50%)	3(100%)	3(100%)	5(83.3%)
Cefepime	3(100%)	2 (50%)	3(100%)	3(100%)	5(83.3%)
Ceftazidime	-	-	-	-	4(66.7%)

Gram negative isolates, 9 (42.9%) were multidrug-resistant (MDR). Most common MDR organism was *K. pneumoniae* (3/4, 75%). 13.3% (2/15) of Enterobacterales & 16.7% (1/6) of *Pseudomonas aeruginosa* were carbapenem-resistant. More than 80% of Enterobacterales were resistant to ampicillin, amoxy-clavulanic acid & first, second & third generation of cephalosporin (Table 3 & 4).

Clinical Outcome

Out of 57 patients, 18 of them not improved clinically and radiologically. They revisited the hospital within six months. Of 18 patients, 11 of them had infection due to MDR organisms (61.1%). Twelve of them (21%) had undergone amputation. Out of 16 patient with an implant, 6 of them had implant failure (37.5%).

DISCUSSION

Osteomyelitis is an acute or chronic inflammation of the bone caused by an infectious organism and is a challenging clinical problem. It is essential to identify the etiological agents to treat the infection.

The present study, after investigating a cohort of 57 patients, found that the mean age of patients was 46.4 years & 78.9% of patients were male. Similar results were found by Jiang N *et al.* where they found the median age to be 42 years & increased male predilection¹.

We observed out of 57 cases, 73.7 % of them had chronic osteomyelitis and a significant

number of them were in the age group of 31-60 years (30/42, 71.4%). Similarly, Prieto-Pérez *et al.*² found more number of chronic osteomyelitis cases among patients within the age group of 41- 60 years. The incidence of acute osteomyelitis was only 26.3%. Acute osteomyelitis is more common in children less than five years of age due to bacteremic seeding of bone¹⁸. As we have not included children in our study, so less number of acute osteomyelitis had been depicted. Chronic osteomyelitis is much less observed among children³.

Several etio-pathogenic mechanisms for osteomyelitis were reported in the past. Infection can occur secondary to a contiguous-focus, by direct contamination after trauma or surgery or infections originating from the bloodstream⁴. In this study, we found, most of the disease developed by direct inoculation (45/57, 78.9%) of bacteria or by seeding of them from next focus (31.6%). Jorge SL *et al.* also depicted that the incidence of osteomyelitis by direct inoculation has increased over the last decades⁵. This is due to the increased frequency of accidents followed by more use of orthopaedic fixation devices and joint prostheses. Traditional chronic hematogenous osteomyelitis is now supplemented by frequently encountered postop (33.3%), post-traumatic (28%) & implant-related infection (33.3%)⁶. The primary underlying predisposing factor was diabetes (36.8%) for acute & chronic osteomyelitis

secondary to contagious soft tissue infection. It has been reported that up to one-half of patients with diabetes develop peripheral neuropathy, which may reduce their awareness of wounds and increase the risk of unrecognized infections.

The metatarsal bones of the foot followed by the tibia and femur were the most commonly affected bones in our study (38.6%, 22.8% & 15.9% respectively). Jiang N *et al.*¹ observed the tibia was the most common site in traumatic osteomyelitis, while the femur and toes were the most common sites in hematogenous and diabetic foot osteomyelitis. We found frequent infection in metatarsal bones as a complication of diabetic foot.

Clinical symptoms of osteomyelitis can be nonspecific and difficult to recognize. Signs and symptoms change depending on the category of infection, organism & anatomical location of the disease. In our study, the majority of the patients with osteomyelitis presented with pain (79.4%) & discharging sinus (76.5%). Pain & discharging sinuses were common clinical presentations in chronic cases, whereas pain & swelling were frequent in acute cases.

During the study period, a total of 86 samples were collected from 57 patients for microbiological analysis. Out of 57 patients, monomicrobial infection observed among 55.3% of patients & co-infection was 42.6%. In our study, the majority of postoperative and post-traumatic infections were caused by two microbes (12/27=44.4%) similar to Mathewa PG *et al.*²⁶ but higher than Trampuz *et al.* (27%)⁷. Monomicrobial infection by Gram positive bacteria was commonly observed (9/16, 56.2%) in contagious osteomyelitis due to the spread from adjacent ulcer site. This is in contrast to Calhoun JH *et al.*⁸.

Staphylococcus aureus (46.9%) was the most frequently isolated bacteria overall similar to Lobati F *et al.*⁹. We also had a fair number of beta-hemolytic *Streptococcus spp* (20%) from chronic cases. Generally, they are frequent etiological agent among children, rare in adults¹⁰.

In view of chronic osteomyelitis, PG Mthethwa *et al.*¹¹ found *Enterobacteriales* as a most common organism (34%). In contrast, we found *S. aureus* as the most prevalent organism for both acute (46%) & chronic infection (50%) followed by *Enterobacteriales* (18.8% & 24%

respectively). Prevalence of *P. aeruginosa* in our study was 9.1% similar to other study (11%)¹¹.

Out of 31 isolates of *S. aureus*, 41.9% were MRSA with 100% of them being susceptible to vancomycin. Reason for the high number of MRSA could be that most of our cases were having post-traumatic or post-op infection. They showed high susceptibility for co-trimoxazole (80.6%) which have been found to achieve bone concentrations at ~50% of serum & as effective as vancomycin. Other than co-trimoxazole, clindamycin is another oral agent available against community-acquired MRSA, which reliably penetrates bone at levels of approximately 40%–70% of serum. In our study, susceptibility of clindamycin was 76.9%¹². We also found, out of 15 *Enterobacteriales*, more than 80% of them were ESBL producers, resistant to ampicillin, amoxy-clavulanic acid & first, second & third generation of cephalosporins. More than 80% of the ESBL producers were susceptible to carbapenem, piperacillin-tazobactam & cefepime similar to Mthethwa PG *et al.* 60% of Gram-negatives also showed resistance against fluoroquinolones & aminoglycosides in contrast to Spellberg *et al.*¹². So piperacillin-tazobactam along with clindamycin can be considered for empirical therapy which can be modified later based on culture findings.

46.7% of the *Enterobacteriales* were found to be MDR. Most common MDR was *K. pneumoniae* (75%) & 50% of them were carbapenem resistance. *Pseudomonas* also showed carbapenem resistance of 16.7% in contrast to Mthethwa PG *et al.*¹¹. We also observed quite a high number of morbidity in our study due to persistence of the MDR organisms (61.1%). Among the MDROs, MDR MRSA contributed (8/11) 72.7% whereas MDR GNBS not attributed much (27.3%). But we did not find any significant correlation ($p=0.5$) when we compared clinical outcomes between patients with and without MDR organisms.

21% of our patient had undergone amputation. Even, out of the 16 patient with an implant, 6 of them had implant failure (37.5%) within six months.

CONCLUSION

Osteomyelitis is one of the challenging infections of the bone. Clinical pictures of it changed markedly in the past few decades. In our

study, we found an increased prevalence of chronic osteomyelitis of the extremities of lower limb instead of acute hematogenous infection of the long bones. Most of them resulted from trauma or post-implant with fracture fixation devices or as a sequela of diabetic foot & predominated in adult males. Monomicrobial infection was more. *S. aureus* & *P. aeruginosa* was the common Gram positive & gram negative bacteria. A total of 40% isolate were either MRSA or MDR Gram negatives. This finding is a matter of concern & challenging for the clinician in order to select the appropriate treatment option, reduce the risk of recurrence and improve the quality of life of the patients.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

AUTHORS' CONTRIBUTIONS

BB & CM conceptualized the study. SB, BB and AB contributed in data interpretation. SB & BB compiled information from the results and literature and drafted the manuscript. CM & AB helped in draft improvisation. BB, CM and AB supervised and reviewed the manuscript. BB, SB, CM, and AB read and approved the manuscript.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was conducted after taking the permission from institutional ethical committee.

REFERENCES

1. Jiang N, Ma Y, Jiang Y, Zhao X, Xie G, Hu Y *et al.* Clinical Characteristics and Treatment of Extremity Chronic Osteomyelitis in Southern China. *Medicine*, 2015; **94**(42): e1874. <https://doi.org/10.1097/MD.0000000000001874>
2. Prieto-Perez L, Perez-Tanoira R, Petkova-Saiz E, Perez-Jorge C, Lopez-Rodriguez C, Alvarez-Alvarez B *et al.* Osteomyelitis: A Descriptive Study. *Clin Orthop Surg*, 2014; **6**(1): 20. <https://doi.org/10.4055/cios.2014.6.1.20>
3. Gutierrez K. Bone and joint infections in children. *Pediatr Clin North Am*, 2005; **52**(3): 779-794. <https://doi.org/10.1016/j.pcl.2005.02.005>
4. Auh JS, Binns HJ, Katz BZ. Retrospective assessment of subacute or chronic osteomyelitis in children and young adults. *Clin Pediatr (Phila)*, 2004; **43**(6): 549-555. <https://doi.org/10.1177/000992280404300607>
5. Waldvogel FA, Medoff G, Swartz MN. Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects. *N Engl J Med*, 1970; **282**(4): 198-206. <https://doi.org/10.1056/NEJM197001222820406>
6. Jorge L, Chueire A, Baptista Rossit A. Osteomyelitis: a current challenge. *Braz J Infect Dis*, 2010; **14**(3): 310-15.
7. Trampuz A, Zimmerli W. Diagnosis and treatment of infections associated with fracture- fixation devices. *Injury*, 2006; **37**(Suppl 2): 59-66.
8. Calhoun J, Manring M, Shirliff M. Osteomyelitis of the Long Bones. *Semin Plast Surg*, 2009; **23**(02): 059-072.
9. Lobati F, Herndon B, Bamberger D. Osteomyelitis: Etiology, Diagnosis, Treatment and Outcome in a Public versus a Private Institution. *Infection*, 2001; **29**(6): 333-336. <https://doi.org/10.1007/s15010-001-1134-6>
10. Brady RA, Leid JG, Costerton JW, Shirliff ME. Osteomyelitis: Clinical overview and mechanisms of infection persistence. *Clin Microbiol Newsl*, 2006; **28**(9): 65-72. <https://doi.org/10.1016/j.clinmicnews.2006.04.001>
11. Mthethwa P, Marais L. The microbiology of chronic osteomyelitis in a developing world setting. *SA orthop*, 2017; **16**(2). <https://doi.org/10.17159/2309-8309/2017/v16n2a4>
12. Spellberg B, Lipsky A. Systemic antibiotic therapy for chronic osteomyelitis in adults clinical infectious diseases. *Clin Infect Dis*, 2012; **54**(3): 393-407. <https://doi.org/10.1093/cid/cir842>