Indian Journal of Microbiology Research 2019;6(4):350–354

Content available at: iponlinejournal.com

Indian Journal of Microbiology Research

Journal homepage: www.innovativepublication.com

Original Research Article

A study of aerobic bacterial profile and their antibiogram in patients with chronic osteomyelitis with special references to staphylococcus aureus

B Neeharika[1,*], R Vinod[1], Gerard Rakesh[1]

[1] *Dept. of Microbiology, Sri Venkateshwaraa Medical College and Research Center, Pondicherry, India*

ARTICLE INFO

Article history:
Received 13-03-2019
Accepted 14-11-2019
Available online 21-11-2019

Keywords:
Chronic osteomyelitis
MRSA
Staphylococcus aureus

ABSTRACT

Introduction: Chronic osteomyelitis is a important medical illness among developing countries, it is a very expensive disease for the patient and society mostly associated with trauma and surgery as risk factors. There is a constant change in the trend of organisms involved and resistance pattern seen with advent of newer antibiotics, So early and specific therapy is needed. With this background present study aims to look for the varying trends of microorganisms involved in osteomyelitis and their antimicrobial susceptibility pattern.

Materials and Methods: A total of 100 cases studied over a period of one year, samples processed by following standard laboratory protocols and along with routine antimicrobial testing screening for MRSA done.

Results: Among 100 cases, 76 were males and 24 were females, between age group of 11 –60 years. Long bones are most involved with trauma (45%) as risk factor. *Staphylococcus aureus* (51%) predominant pathogen isolated with 27(53%) were MRSA, followed by *Pseudomonas aeruginosa* (16%). Antibiotic sensitivity testing of gram positive organisms showed hundred percent sensitivity to Linezolid and Vancomycin with poor sensitivity to Pencillin (10/15.6%). Among gram negative organisms, majority showed highest sensitivity to Amikacin (73.3%) & Imipenem (71.1%) with poor sensitivity to Ciprofloxacin (20.0%) and Ampicillin (0%).

Conclusion: Chronic osteomyelitis is a prime challenging problem in many countries with severe morbidity. Mostly due to neglect, delayed or inadequate treatment and emerging drug resistant pathogens involved. Culture based antibiotic therapy helps in effectively treating the disease also prevents the drug resistance.

1. Introduction

Chronic osteomyelitis is a persistent and relapsing disease, characterized by progressive destruction of bone and formation of sequestra.[1] The cavities formed will act as locus for persistent infection in the bone showing the chronic nature of the disease. Most of the cases factors contributing to chronic osteomyelitis is due to trauma with compound fracture, crush injury, surgical procedures, diabetic foot and sickle cell haemoglobinopathies.[2] This condition may be unifocal limited to single region of bone or multifocal may involve various regions of bone and surrounding soft tissue. Chronic osteomyelitis most commonly affects tibia and femur.[3]

The commonest causative agents of Chronic osteomyelitis are *Staphylococcus aureus, Coagulase negative staphylococci, Enterococci among gram positive organisms followed by Pseudomonas, Enterobacteriaceae spp* and rarely *Salmonella spp.*and *Actinomycetes.* Among anaerobic organisms *Peptostreptococcus spp., Bacteroides spp., Clostridium spp were involved.*[4] Staphylococcus aureus constitutes 50%-75% cases of chronic osteomyelitis. In most of the cases infection is mono microbial, infection with multiple organisms are usually seen in diabetes mellitus patients with ulcer in foot.[5]

* *Corresponding author.*
E-mail address: drneehu@gmail.com (B. Neeharika).

https://doi.org/10.18231/j.ijmr.2019.074

The adhesion to local tissue is the initial event to takes place by *Staphylococcus aureus* strains which possess receptors for collagen, fibrinogen, fibronectin, bone sialoprotein, and heparin sulphate.[6] Infection is predisposed by trauma or injury thus exposing the binding sites for the organism. For *staphylococcus aureus*, the polysacch aride pseudocapsule also acts as an important virulence factor forming a strong bond between cell also involved with biofilm formation. Thus microcolonies are formed by the organism will be attached to each other and the adjacent tissue in the biofilm. The highly virulence factor glycocalayx also acts to protect the pathogen from host protective mechanism and antibiotics by forming mechanical barrier.[7] Thus glycocalyx prevents phagocytosis by forming the teichoic acid moiety and opsonization and also by altering the configuration of complement. Protein A an important virulence factor of *staphylococcus aureus* cell wall acts by interfering with opsonization and by polymorpho nuclear cells thus activating complement and inducing hypersensitivity reactions. Gram negative organisms are also evolving as emerging pathogens in chronic osteomyelitis cases, mostly associated with injury or spread from adjacent foci of infection and due to prolonged duration of stay in hospital.

Chronic osteomyelitis is a complicated disease due to its diverse nature of pathophysiology, clinical manifestations and management involved. Pathogenesis mainly involves the necrosis of bone, with sourrounding granulation tissue, followed by absorption of necrotic bone, with chronic reactive new bone and cicatrix formation with adjacent soft tissue destruction. The disease is characterized by progressive destruction of bone and the sequestration of bone.[8]

Clinical features of chronic osteomyelitis vary according to severity of the bone involved, mostly presents with persistent pain and chronic discharging sinuses. Diagnosis of osteomyelitis based on clinical features, x-ray findings include bone lucency mixed with patchy sclerosis and adjacent new bone formation. MRI and confirmation of diagnosis by blood culture or culture of bone aspirate or biopsy.

Radiodiagnosis by CT scan and MRI are less often used to diagnose osteomyelitis, but they are frequently used to know the extent of disease involved and to know whether these are collections of pus that are amenable to drainage. The 'gold standard' specimen for diagnosing osteomyelitis is bone biopsy specimen.[9] In most of the cases antimicrobial therapy is based on culture obtained from deep bone biopsy sites or debrided tissues collected during surgery and their antimicrobial susceptibility pattern seen. Radio nucleotide scanning also helps in early diagnosis of osteomyelitis.

In children the predominant pathogen isolated is staphylococcus aureus contributing more than 90%. In case of adults, staphylococcus aureus commonly isolated which contributes around 50% to 75%.[10] While antimicrobial therapy is desirable in the control of osteomyelitis, surgery remains the therapeutic and diagnostic procedure.[11]

In most of the cases staphylococcus aureus is the dominant pathogen isolated. Vancomycin is used as the prime drug for strains that are resistant to ampicillin and methicillin both. Linezolid a recently evolved drug which acts against MRSA cases due to its better oral bioavailability and good bone penetration seen. But the disadvantage is on prolonged therapy with linezolid is development of significant pancytopenia, peripheral neuropathy, optic neuritis.[12] Daptomycin a recently developed drug which has better bactericidal activity. Its usage in the vancomycin resistant Enterococcus cases has yet to be defined. The duration of therapy with antibiotics is around 4 to 6 weeks period.[13]

The most common surgical intervention done for chronic osteomyelitis is sequestrectomy which includes removal of the diseased bone and adjacent soft tissue. The main idea behind surgery is to completely remove every bit of an infection and thus maintaining a viable and vascular environment. Hyperbaric oxygen therapy can also been used frequently as an additional therapy for chronic osteomyelitis. The most encountered sequelae of persistent chronic osteomyelitis are lengthening of bone, pathologic fracture, contracture of muscles, epithelioma, reduced growth rate, scar tissue carcinoma and seconadary amyloidosis.

Difficulty arises to correctly establish the etiological agent and proper treatment to cure the patient. Proper selection of treatment always be made on basis of accurate diagnosis of the causative organisms and knowledge of pattern of susceptibility. Our study is done to emphasize the prime need of culture based antibiotic therapy and thus helps the clinicians in choosing appropriate antibiotics. Proper antibiotic coverage will also help in preventing emergence of resistance to the drug which are still sensitive.

2. Materials and Methods

The present study was a laboratory investigation based study, Study was done after getting permission from the institutional scientific and ethical committee approval, informed consent was obtained. Study was done for a period of one year, from Oct 2016 to Nov 2017, clinically and radiologically diagnosed cases of chronic osteomyelitis were included in this study. Cases apart from chronic osteomyelitis and those who underwent surgery with prosthetic joints were not included in the study. All the necessary data was collected from patient case sheets.

Under stringent aseptic measures followed, all samples - pus, pus swabs and sequestrum were collected from cases with chronic osteo myelitis from deep wound site and sinus tract, for those cases who were attending the outpatient and inpatient department of orthopaedics, then they were

subjected to bacteriological study over a period of 1 year. Two pus swabs were collected from cases subjected to-direct gram stain and other for aerobic bacterial culture. Then the smear is stained by Gram's staining and viewed under microscope to look for the presence of pus cells and any microorganisms. The specimen is plated on Nutrient agar, Mac Conkey agar and Blood agar and incubated for 18 hrs at 37° C. Culture isolates thus identified were further processed by observing the colony characteristics, culture smear and preliminary tests were done along with the controls by following a series of standard laboratory methods. After identification antibiogram was performed by lawn culture of organism on Muller Hinton agar plate by following standard Kirby-Bauer disc diffusion method and breakpoints interpretated according to CLSI guidelines.[13] Along with routine sensitivity testing MRSA detection was done by placing 30μg cefoxitin disc and interpretation was done by following CLSI guidelines. Clinical isolates showing zone diameters more than or equal to 22mm considered as susceptible to oxacillin (MSSA), minimal inhibitory concentration of vancomycin was performed by macro broth dilution method.

3. Results

Study group comprised, hundred chronic osteomyelitis cases. Among hundred cases studied, Males were commonly affected compared to females, 76 males > 24 females. Majority of the cases were in the age group of 11–60 years. Positive cultures were 90% and negative culture 10%. Femur is the commonest bone involved in chronic osteomyelitis followed by Tibia, refer (Table 2). There was no statistical significance found between male and female with respect to site of bone involved p=0.709. The predominant risk factor found among 100 cases of chronic osteomyelitis, compound fracture due to trauma (45%), followed by postoperative infections 22(22%), 19 patients had diabetes with vascular insufficiency as predisposing factor, 10 patients(10%) had smoking and alcohol as predisposing factor and 4(4%) patients acquired infection following hematogenous spread. (Figure 1).

Among hundred cases of chronic osteomyelitis studied, monomicrobial flora was seen in 71(71%) cases, poly microbial flora in 19 cases and no growth in 10 cases. The commonest organisms isolated were *Staphylococcus aureus* (51%) and *Pseudomonas aeruginosa* (16%) followed by *Staphylococcus epidermidis* (13%), *Escherichia coli* (11%), *Klebsiella pneumoniae* (9%), *Enterobacter* (5%) and *Proteus mirabilis* (4%), refer (Table 3).

Among 51 culture isolaes of *staphylococcus aureus* obtained 24 (47%) were Methicillin Sensitive, *followed by* 27(53%) showed Methicillin Resistance refer. Antibiogram of Gram positive organisms showed hundred percent sensitivity to two drugs, Linezolid and Vancomycin with less sensitivity to Pencillin (15.6%). Antibiogram of gram negative organisms showed maximum sensitivity to Amikacin (73.3%) & Imipenem (71.1%) with poor sensitivity to Ciprofloxacin (20.0%) and Ampicillin (0%).

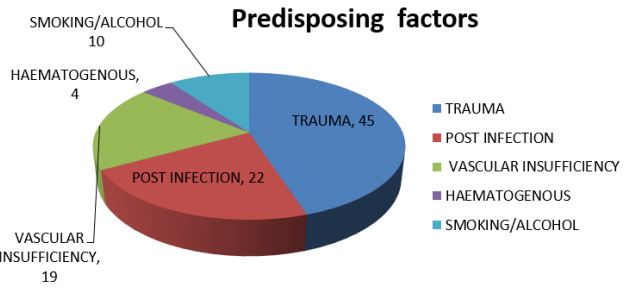

Fig. 1: Showing predisposing factors of patients with chronic osteomyelitis

Table 1: Showing bones involved in chronic osteomyelitis

Site of Bone	No of Cases	Percentage
Femur	42	42%
Tibia	26	26%
Radius	8	8%
Ulna	12	12%
Fibula	1	1%
Malleoli	2	2%
Metatarsal	8	8%
Metacarpal	1	1%
Total	**100**	**100%**

Table 2: Showing various organisms isolated

Organisms	No. of organisms	Percentage (%)
Staphylococcus aureus	51	46.8%
Staphylococcus epidermidis	13	11.9%
Pseudomonas aeruginosa	16	14.7%
Escherichia coli	11	10.0%
Klebsiella pneumoniae	9	8.3%
Enterobacter cloacae	5	4.6%
Proteus mirabilis	4	3.6%
Total	**109**	**100%**

4. Discussion

Chronic osteomyelitis is an incessant and recurring disease difficult to treat and eradicate completely. In the absence of early diagnosis, prompt treatment or failure to antibiotic therapy leads to development of drug resistance, chronic osteomyelitis still an important cause of high morbidity. Better management of chronic osteomyelitis requires

Table 3: Showing antibiotic susceptibility pattern of gram positive organisms isolated

Organisms	No of isolates	Antibiotics (percentage%) P	E	CD	LZ	VA	AK	G	CIP	DOX
S. aureus	51	5 (9.8)	35 (68.6)	40 (78)	51 (100)	51 (100)	49 (96.0)	41 (80.3)	39 (76.4)	39 (76.4)
S.epidermidis	13	5 (38.4)	10 (76.9)	10 (76.9)	13 (100)	13 (100)	12 (92.3)	10 (76.9)	10 (76.9)	10 (76.9)
Total	64	10 (15.6)	45 (70.3)	50 (78.1)	64 (100)	64 (100)	61 (95.3)	51 (79.6)	49 (76.5)	49 (76.5)

P-Pencillin, E-Erythromycin, CD- Clindamycin, LZ- Linezolid, VA- Vancomycin

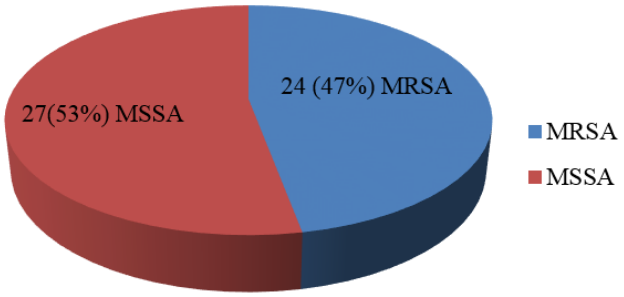

Fig. 2: Showing distribution of MRSA isolated

early and accurate microbial isolation with appropriate antimicrobial therapy. Pathogens involved in chronic osteomyelitis play a dominant role in its development along with the inappropriate administration of antibiotics has led to the development of drug resistant pathogens like MRSA not responding to betalactam antibiotics. The clinicians should ask for bacterial culture and sensitivity for chronic osteomyelitis as a routine with special requisition for anaerobes. Since gram negative organisms & anaerobes are also involved in a major proportion of chronic osteomyelitis, culture and sensitivity should be done for both the organisms.

In the present study, chronic osteomyelitis was common in the elderly age group, males are more affected than females between age group 11-60 years which is in correlation with the studies done by Mita D W et al[4] and Faria Malik et al.[14] In the present study most common predisposing factor is as a result of trauma (45%) associated with compound fracture more common followed by postoperative infections (22%), diabetes with vascular insufficiency (19%) as predisposing factor, (10%) had smoking and alcohol as predisposing factor and 4(4%) patients acquired infection following hematogenous spread.

Monomicrobial flora 71(71%) was common than polymicrobial flora 19(19%). Gram positive 64(64%) were common than gram negative organisms 44(44%) consistent with studies done by Zuluaga AF et al[1] and Mita D Wadekar et al.[15] Staphylococcus aureus was the commonest isolate 51(46.8%), followed by P.aeruginosa 16(14.7%), S.epidermidis 13(11.9), K.pneumoniae 9(8.5%), Escherichia coli 11(10.0%), Enterobacter cloacae 5(4.6%) and P.mirabilis 4(3.6%). Alonge TO et al, studied 60 cases of chronic osteomyelitis, of which 47 culture positive isolates were obtained and the organism commonly isolated both in single and polymicrobial culture isolates was *Staphylococcus aureus.*[16]

Out of 64 gram positive isolates, MRSA isolates detected is 24 (47%) it correlates with the study one by Sachin Sharma et al[17] According to a study done by S Anupurba et al, MRSA is(54.85%). MRSA resistance to penicillin, cotrimoxazole, ciprofloxacin, gentamicin, erythromycin, tetracycline is >80% and 60.5% to amikacin. No vancomycin resistance was appreciated.[18] According to a study done by S.H. Sheely et al[19] *Staphylococcus aureus* was most commonly isolated followed by gram negative organism, other organisms are *Coagulase negative Staphylococcus, Pseudomonas, Escherichia coli, Klebsiella and Proteus*. Of all the gram negative org anisms. Faria Malik et al, studied 150 patients of osteomyelitis, out of 150 isolates among gram negative organisms, *Enterobacteriaceae* (32.8%) is commonly isolated followed by *Staphylococcus aureus* (29.5%). among gram positive isolates, other isolates were *Pseudomonas* aeruginosa (15.5%), Anaerobes (2.6%) and Miscellaneous (19.3%) showing mixed infection.

Ethan Rubinstein et al, found 20%–40% of MRSA patients. All isolates are resistant to erythromycin and β-lactams, Success rate was 66% for patients on linezolid treatment and 68% for patients on vancomycin.[20]

In this study majority of gram positive isolates showed maximum sensitivity to two drugs to both Linezolid and Vancomycin each 64(100%), followed by Amikacin 61(95.3%) Among gram negative organisms majority showed maximum sensitivity to Amikacin (73.3%) & Imipenem (71.1%) with less sensitivity to Ciprofloxacin (20.0%) and Ampicillin (0%). Though antimicrobial therapy desirable in controlling the infection chronic osteomyelitis is treated surgically by sequestrectomy along with antibiotic therapy. The aim of surgical intervention done is to remove every piece of an infection complrtely and thereby attaining a viable and vascular environment.

5. Conclusion

Present study showed chronic osteomyelitis common in elderly age group as a sequel of trauma involving long bones commonly. The most commenest organism isolated was *Staphylococcus aureu*s as predominant pathogen involved in chronic osteomyelitis followed by *Pseudomonas aeruginos*a among gram negative organisms. Prevalence of MRSA among *Staphylococcus aureus* and ESBL, MBL producers among gram negative organisms being high. Since resistant organisms are highly involved in chronic osteomyelitis, culture based antibiotic therapy helps in effectively treating the condition, also helps in preventing drug resistance.

6. Source of funding

None.

7. Conflict of interest

None.

References

1. Zuluaga AF, Galvis W, Saldarriaga JG, Agudelo M, Salazar BE, Vesga O. Etiologic diagnosis of chronic osteomyelitis a prospective study. *Arch Intern Med*. 2006;166:95–100.
2. Ako-Nai AK, Ikem IC, Aziba A, Ajayi AA, Onipede OA. Bacteriological examination of chronic osteomyelitis cases in ILE-IFE, Southwestern Nigeria. *Af J Clin Exp Microbiol*. 2003;4:41–51.
3. Abid AS, Ehan AH, Yonis AR. Epidemiological and bacteriological study of chronic osteomyelitis. *Tikrit Med J*. 2008;14:59–62.
4. Wadekar MD, Anuradha K, Venkatesha D. Chronic osteomyelitis: aetiology and antibiotic susceptibility pattern. *Int J Recent Trends Sci Tech*. 2014;9:337–340.
5. Canale ST, James HB. Campbell's Operative Orthopaedics, 11th ed. . vol. 1. Mosby: St Louis Missouri ; 2008,. p. 695–709.
6. Buxton TB, Rissing JP, Horner JA. Binding of a staphylococcus aureus bone pathogen to type-I collagen. *Micro Pathol*. 1990;8:441–448.
7. Buxton T, A HJH, Rissing J, J. In vitro glycocalyx expression by staphylococcus aureus phage type 52/52A/80 in S.aureus osteomyelitis. *J Infect Dis*. 1987;156:942–946.
8. Mandell LG, Bennett JE, Mandells RD. Douglas& Bennett's principles and practices of infectious diseases, 6th edition. vol. 1. Philadelphia: Elsevier ; 2005,. p. 1322–1330. 6th edition.
9. Mackowiak PA, Jones SR, Smith JW. Diagnostic value of sinus tract culture in chronic osteomyelitis. *JAMA*. 1978;239:2772–2775.
10. Browner B, Levine A, Jupiter J, Trafton P, Krettek C. Skeletal Trauma, 4th edition. Saunders and Elsevier Publications ;. p. 21–591. 4th edition.
11. Nally MMA, Small JO, Tofighi HG, Mollan RAB. Two stage management of chronic ostomyelitis, the long bones. *J Bone Joint Surg*. 1993;758:375–380.
12. Waldrep TW, Skiest DJ. Linezolid induced anemia and thrombocytopenia. Pharmacother ; 2002,.
13. Frippiat F, Bergers C, Michel C. Severe bilateral optic neuritis associated with prolonged linezolid therapy. *J Antimicrob Chemother*. 2004;53:1114–1115.
14. Bacterial aetiology of osteomyelitis cases at four hospitals of Lahore. *J Ayub Med Coll Abbottabad*. 1999;p. 15–224. 1Faria Malik.
15. Performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement. CLSI document M100–S25 . Wayne, Pennsylvania, USA ; 2015,. CLSI document.
16. Alonge TO, Ogunlade SO, Fashina AN. Microbial isolates in chronic osteomyelitis- a guide to management. *Afr J Med Sci*. 2002;31(2):167–169.
17. Sharma S, Mall A. The prevalence, antibiogram and characterization of methicillin resistant staphylococcus aureus among the patients from the Doon Valley hospitals. *Afr J Microbiol Res*. 2011;5(21):3446–3451.
18. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Department of Microbiology, Institute of India. 2003;21(1):49–51.
19. Sheehy SH, Atkins BA, Bejon P. The Microbiology Of Chronic Osteomyelitis: prevalence of resistance to common empirical antimicrobial regimens. *J Infect*. 2010;60(5):338–343.
20. Rubinstein E, Kollef MH, Nathwani D. Pneumonia Caused by Methicillin-Resistant Staphylococcus aureus;46(5):378–385.

Author biography

B Neeharika Senior Resident

R Vinod Professor and HOD

Gerard Rakesh Associate Professor

Cite this article: Neeharika B, Vinod R, Rakesh G. A study of aerobic bacterial profile and their antibiogram in patients with chronic osteomyelitis with special references to staphylococcus aureus. *Indian J Microbiol Res* 2019;6(4):350-354.