

Original Research Article

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## Microbiological Profile of Chronic Osteomyelitis in a Tertiary Care Hospital

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### ABSTRACT

Osteomyelitis is the infection and inflammation of the bone. Inappropriate use of antibiotics and multidrug resistance has raised the morbidity and mortality rate in chronic osteomyelitis. This study aims to determine the bacterial profile and antimicrobial susceptibility patterns of chronic osteomyelitis with special mention to various resistant mechanisms. Samples from osteomyelitis cases were aerobically cultured and isolates from culture positives were identified by standard procedures. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method. Staphylococcal isolates were screened for methicillin resistance. The aerobic bacteriological study of chronic osteomyelitis showed *Staphylococcus aureus* is being continued to be major etiological agent followed by *Pseudomonas aeruginosa* and CoNS. Gram-positive isolates were sensitive to linezolid, Vancomycin while gram-negative isolates were sensitive to Meropenem and Piperacillin with Tazobactam in the majority. In present study prevalence of methicillin-resistant *Staphylococcus aureus* was found to be on the higher side. Antibiotic therapy on the basis of antibiotic susceptibility pattern helps the clinician to choose appropriate drugs leading to successful treatment and prevention of emergence and dissemination of drug resistant isolates.

#### Keywords

Poor blood supply, diabetic wounds, loss of protective sensation and altered immune defenses

#### Article Info

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### Introduction

Osteomyelitis is an inflammatory process of bone and bone marrow caused by infecting microorganisms which results in local bone destruction, necrosis and bone neoformation (3). Osteomyelitis can infect all bones

especially long bones and attacks all ages. Many classification systems for osteomyelitis have been presented based on the following (16).

Duration of the disease - Acute Osteomyelitis or Chronic Osteomyelitis.

Origin of the infection - Hematogenous spread, contiguous contamination and vascular or neurologic insufficiency associated infection.

Primary hematogenous spread is bone infection that has been seeded through the bloodstream of bacteria (2) Contiguous infection is usually spread from a contaminated site, most commonly seen with direct contamination of bacteria in open fractures or joint replacement surgery with an orthopedic implant (3). Vascular or neurologic insufficiency associated osteomyelitis results from poor blood supply, diabetic wounds, loss of protective sensation and altered immune defenses, commonly affecting the lower extremity (17).

Acute osteomyelitis can be further subdivided into suppurative and nonsuppurative forms as well as progressive or hematogenous forms. Chronic osteomyelitis may be classified by the causative agent as suppurative or nonsuppurative forms or sclerosing with sub classifications of diffuse or focal disease. Chronic osteomyelitis is a relapsing and persistent infection that evolves over months to years and is characterized by low-grade inflammation, presence of dead bone (sequestrum), new bone apposition, and fistulous tracts. The treatment requiring surgical interventions and long-term broad spectrum antibiotics Chronic osteomyelitis commonly involves long bones; especially tibia and femur. Chronic infections do occur in children, generally as a consequence of failed antimicrobial therapy or the presence of an orthopaedic implant (16, 17).

The microorganisms may gain access to the bones during stabilization of fracture or implanting prosthesis. Prosthetic implants create an environment which favours microbial colonization and establishment of infection successfully in the bone. The

infective agents adhere to foreign material in the body and secrete glycocalyx (biofilm formation) that inhibits the host defence mechanism and action of antibiotics so that infection can be established which would be difficult to eradicate (1,10). In adults, the sinus is not closed and the persistence of viable pathogens in cavities for a longer period leads to reactivation of infection at any time.

The infection is commonly caused by pyogenic bacteria or mycobacteria and rarely by fungus which occurs especially in immunocompromised patients.

A single pathogenic organism is almost always recovered from the bone in haematogenous osteomyelitis, whereas, multiple organisms are usually isolated in contiguous focus osteomyelitis, especially in the diabetic foot (18). The bacteria most commonly isolated from chronic osteomyelitis are *Staphylococcus aureus*, coagulase negative staphylococci (especially in implant-associated infections), *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Enterococcus* spp., *Enterobacter* spp. and anaerobes like *Peptostreptococcus* spp., *Bacteroides* spp., *Clostridium* spp. and rarely *Salmonella* spp. (in individuals with sickle cell disease) and *Actinomycetes* (1,7).

Osteomyelitis is an ongoing problem due to emergence of the pattern and behaviour of organisms are constantly changing under the pressure of newer antibiotics. With this background, the present study was conducted to study the spectrum of organisms causing chronic osteomyelitis and their antimicrobial susceptibility pattern for ensuring proper treatment of the patients.

## **Materials and Methods**

This is a prospective observational study done at Microbiology Department in Coimbatore

Medical College and hospital for a period of 1 year. A total number of 23 clinically diagnosed cases of chronic osteomyelitis were included in the study.

### **Inclusion Criteria**

Patients admitted to orthopaedic wards and those attending outpatient departments who satisfy one of the following components of chronic osteomyelitis.

Presence of sinus/sequestrum or sclerosis /Intra operative pus.

Patients older than 12 years.

Radiological changes suggestive of infection for 6 weeks or more.

Even after treatment, persistence or relapse of infection.

### **Exclusion Criteria**

Pediatric age group (<12 years).

Patients who had shorter than 4-week history.

### **Collection, transport and processing of samples**

Samples like pus, sinus discharge or exudates were collected. Two swabs for each specimen was collected aseptically. After the collection of specimen, they were immediately processed in microbiology laboratory according to the standard operative procedure (11, 12). The first swab was used for direct gram staining and was examined under oil immersion objective for the presence of inflammatory cells and bacteria while second swab was inoculated on Blood agar, Mac-conkey agar and nutrient agar(13). All plates were aerobically incubated overnight at 37°C. The isolated organisms were identified according to morphology in gram staining, colony

characteristics and biochemical properties (13, 12). Antimicrobial susceptibility of bacterial isolates to the commonly used antibiotics was done by using modified Kirby Bauer disc diffusion method on Mueller Hinton agar equivalent to 0.5 McFarland standard as per CLSI guidelines.

### **Results and Discussion**

A total of 23 clinically diagnosed cases of osteomyelitis were included in the study of which 20 (87%) were culture positive and 3(13%) had no growth in culture.

In this study, Out of 20 patients with Osteomyelitis, 19 were males and 1 was female.

In the present study, the predominantly affected age group was between 31-40 followed years by 21-30 yrs.

Of the 20 isolates 60% were gram positive cocci followed by Gram negative bacilli (40%). Among the gram positive isolates *Staphylococcus aureus* (40%) was the predominant organism followed by CoNS (20%). Among the Gram negative bacilli maximum were *Pseudomonas aeruginosa* (30%) followed by *Proteus* (10%).

In the present study *Staphylococcus aureus* showed 100% sensitivity to Vancomycin and Linezolid, 60-75% sensitivity to Erythromycin and Doxycycline.

The next common organism was CoNS with 100% sensitivity to Vancomycin and Linezolid. The sensitivity to Doxycycline was 75%.

Out of 8 *Staphylococcus aureus* isolates 6 (75%) were Methicillin resistant. Two out of 4 CoNS isolates (50%) were Methicillin resistant.

In this present study, *Pseudomonas aeruginosa* showed 100% sensitivity to Meropenem, Piperacillin tazobactam, Ceftazidime and Amikacin. The sensitivity to Gentamicin, Ciprofloxacin and Cefotaxime were 80%, 60% and 40% respectively. *Proteus* was 100% sensitive to Amikacin, Gentamicin, Ciprofloxacin, Meropenem, Piperacillin tazobactam and Ceftazidime. The sensitivity to Cefotaxime was 50%

In present study, maximum osteomyelitis cases belonged to younger age groups of 31-40 years (60%) followed by 21-30 years (25%). This coincides with the study by Khatoon *et al.*, (1). This may reflect the fact that the usage of motor vehicles is common among young adults and working community.

In the present study males were commonly affected when compared to females. This is in concordant with the study conducted by Devi *et al.*, (2). This could be due to more frequent exposure of males to accidents, trauma and fracture.

Even though the gram negative organisms are increasing rapidly since longer time, still staphylococcus remained the most common isolate of osteomyelitis with methicillin resistant strains aggravating the disease further. Present study reported that *Staphylococcus aureus* was the commonest

isolate from osteomyelitis cases (40%), followed by *Pseudomonas* spp. (30%), coagulase negative staphylococci (20%) *Proteus* (10%). This finding is in concordance with another study by Wadekar *et al.*,(5) Various previously done studies by Suguneswari *et al.*, Rao *et al.*, Zuluaga *et al.*, and Ellies *et al.*, (6, 7, 9) also reported staphylococcus as the major isolate from osteomyelitis cases. Present study reported the prevalence of methicillin resistant staphylococci (MRSA) to be 66%, with 75% among *Staphylococcus aureus* isolates and 50 % among coagulase negative staphylococci. All the MRSA isolates were 100% sensitive to vancomycin and linezolid followed by sensitivity to erythromycin & Doxycycline (60-75%). This is similar to a previous study done by Wadekar *et al.*, and Kaur *et al.*,(5, 8). But Raviprakash *et al.*, study showed only 47% of MRSA (4).

In present study amongst the Gram negative organisms isolated from osteomyelitis cases, maximum were *Pseudomonas* spp. (30%), followed by *Proteus* spp. (10%) and both showed 100% sensitivity to Meropenem, Piperacillin tazobactam, Ceftazidime and Amikacin. The sensitivity to Gentamicin, Ciprofloxacin and Cefotaxime were 80%, 60% and 40% respectively for *Pseudomonas* spp. *Proteus* was 50% sensitive to Cefotaxime.

**Table.1** Age-wise distribution of Osteomyelitis

Age	No. of cases
<20 yrs	1
21-30 yrs	5
31-40 yrs	12
41-50 yrs	1
>50 yrs	1

**Table.2** Bacterial Profile of Osteomyelitis

Name of the isolate	No. of isolates (n=20)
<i>S.aureus</i>	8
<i>P.aeruginosa</i>	6
CoNS	4
<i>Proteus</i>	2

**Table.3** Antibiotic sensitivity pattern of gram positive organisms

Antibiotic sensitivity tests of gram positive organisms									
Microorganism	Antibiotic sensitivity in percentage (sensitive no./total tested no.)								
	P	G	CIP	COT	E	DO	CX	Van	LZ
<i>Staphylococcus aureus</i>	-	50%	25%	38%	75%	63%	25%	100%	100%
	0/8	4/8	2/8	3/8	6/8	5/8	2/8	8/8	8/8
CoNS	-	50%	50%	25%	50%	75%	50%	100%	100%
	0/4	2/4	2/4	1/4	2/4	3/4	2/4	4/4	4/4

P – Pencillin, G-Gentamicin. CIP-Ciprofloxacin, COT-Cotrimoxazole, E-Erythromycin, DO-Doxycycline, Cx-Cefoxitin, Van-Vancomycin, LZ-Linzolid.

**Table.4** Antibiotic sensitivity pattern of gram negative organisms

Microorganism	Antibiotic sensitivity in percentage (sensitive no./total tested no.)							
	CIP	COT	AK	G	CT X	MRP	PIT	CAZ
<i>Pseudomonas aeruginosa</i>	50%	-	100%	83%	50%	100%	100%	100%
	3/6	-	6/6	5/6	3/6	6/6	6/6	6/6
<i>Proteus</i>	100%	0%	100%	100%	50%	100%	100%	100%
	2/2	0/2	2/2	2/2	1/2	2/2	2/2	2/2

CIP-Ciprofloxacin, COT-Cotrimoxazole, Ak-Amikacin, CTX=Cefataxime, MRP-Meropenam, PIT-Piperacillintazobactam, CAZ-Ceftazidime, G-Gentamicin.

**Fig.1** Outcome of Culture

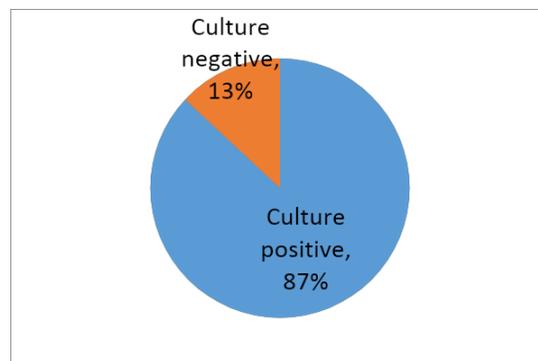


Fig.2 Gender-wise distribution of Osteomyelitis

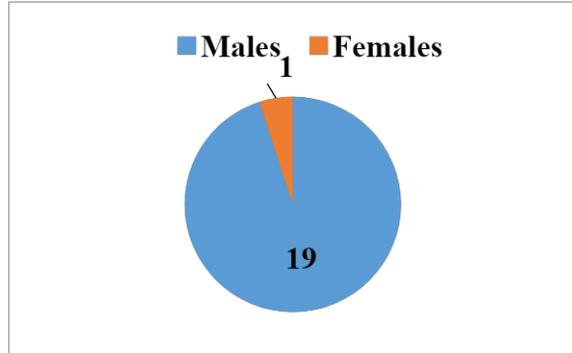


Fig.3 Age-wise distribution of Osteomyelitis

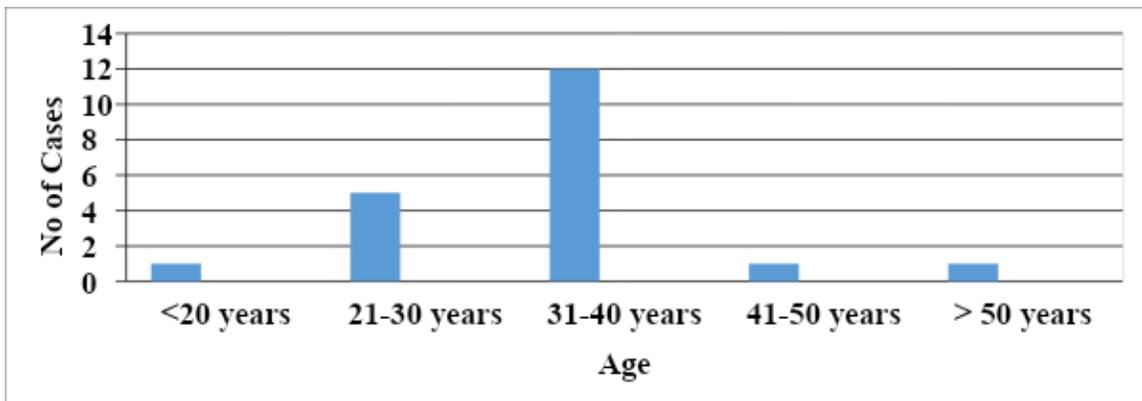
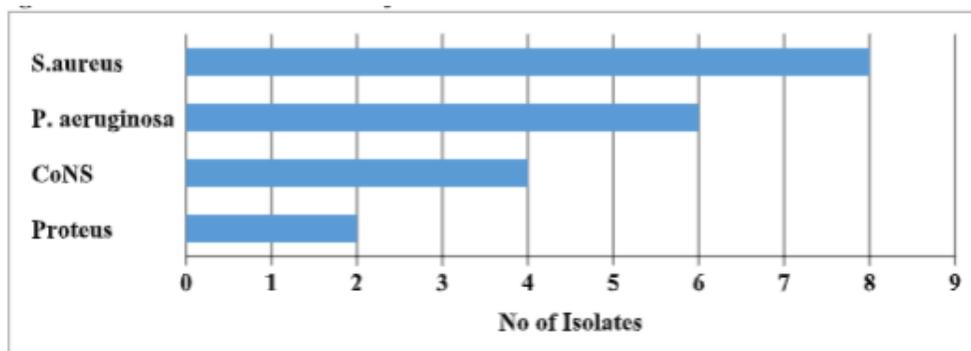
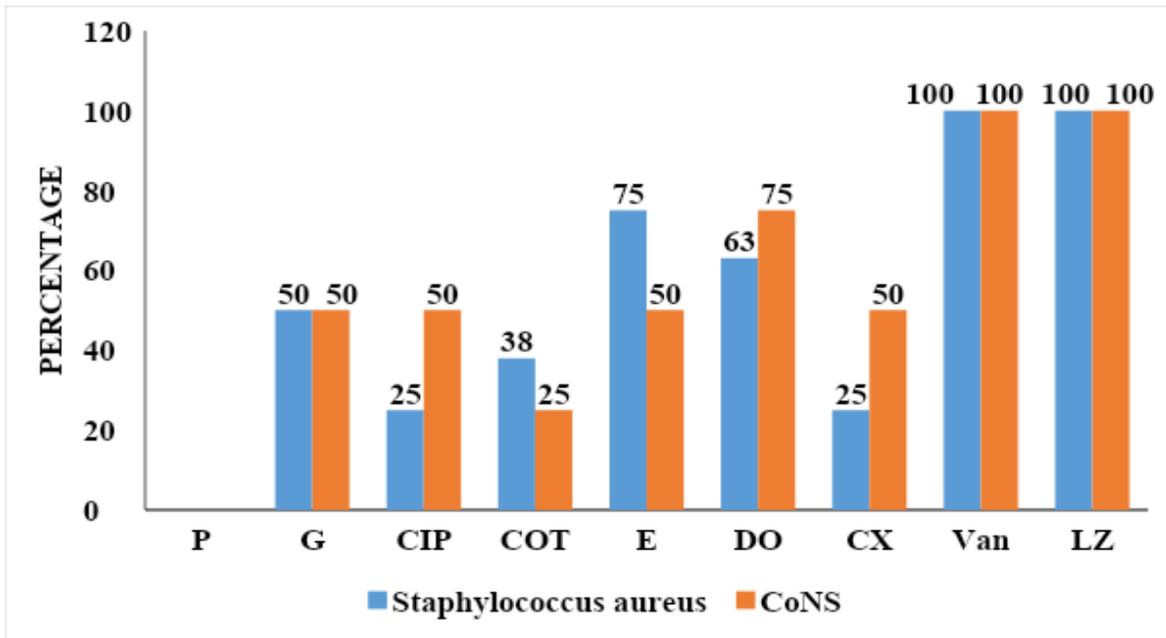


Fig.4 Bacterial Profile of Osteomyelitis

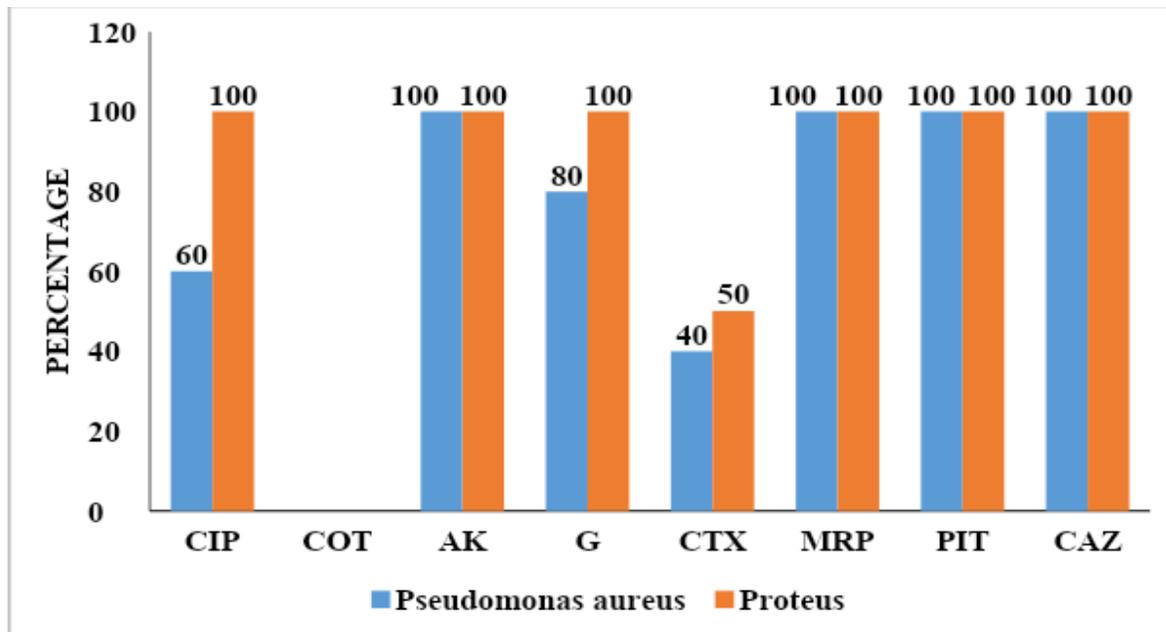


**Fig.5** Antibiotic sensitivity pattern of gram positive organisms



\*\*\* P – Pencillin, G-Gentamicin. CIP-Ciprofloxacin, COT-Cotrimoxazole, E-Erythromycin, DO-Doxycycline, CX-Cefoxitin, Van-Vancomycin, LZ-Linzolid.

**Fig.6** Antibiotic sensitivity pattern of gram negative organisms



\*\*\*CIP-Ciprofloxacin, COT-Cotrimoxazole, Ak-Amikacin, CTX=Cefataxime, MRP-Meropenam, PIT-Piperacillin tazobactam, CAZ-Ceftazidime, G-Gentamicin.

This is in accordance with a study by Suguneswari *et al.*, (9). But this is in contrast

to study by Wirbel *et al.*, study. In this study, bacterial pathogens responsible for infection

were mainly monomicrobial which is in accordance with the study Qurahi *et al.*, Osteomyelitis has been the major cause of morbidity since long.

Emerging multidrug resistant strains is of major concern as they pose challenge in the treatment of osteomyelitis. In present study the methicillin resistant *staphylococci* is found to be quite high among the organisms isolated from osteomyelitis patients.

MRSA is usually spread by direct contact with an infected wound or by contaminated items like bandages or from contaminated hands usually those of health providers resulting in life threatening infections like bloodstream infections, pneumonia, surgical site infection and sepsis. Healthcare facilities can make prevention of MRSA infections a priority, assess their relevant data, implement prevention actions like mainly contact precautions, hand hygiene with screening for colonisation of MRSA in nostrils, throat, and any skin lesions or wounds and evaluate the progress. The early detection of such drug resistant isolates may help in appropriate antimicrobial therapy since beginning and thus avoid the development and dissemination of these multidrug resistance strains in the hospital as well as in the community.

The present antibiotic resistance pattern in osteomyelitis with MRSA strains is alarming and calls for updating the antibiotic therapy guidelines in the country. Although, treatment of osteomyelitis is challenging, good results can be achieved by a single stage protocol including radical debridement in conjunction with systemic and topical antibiotic.

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