

# Bacterial Isolates of Osteomyelitis Patients and Their Antibiotic Sensitivity Pattern

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## ABSTRACT

**Background:** Osteomyelitis usually occurs when an injured bone exposes to germs, about 80% of cases are due to open wound. Diagnosis of osteomyelitis is a challenging aspect, as it needs a combinatorial approach of clinical findings, laboratory infectious markers and radiological investigations. The study is mainly focused on determining aerobic bacterial profile of Osteomyelitis isolates, their antibiotic susceptibility pattern and resistotyping of these isolates in this community.

**Methods:** All clinically diagnosed Osteomyelitis samples like pus, swabs, synovial fluid, bone sequestrum, was collected under aseptic precautions. After receiving sample, it was immediately processed for culture and sensitivity according to CLSI guidelines. **Results:** Out of 72 samples of Osteomyelitis, 50 (69.4%) has shown culture positive. Male predominance noted. 33 (66%) out of 50 samples were in the age group of 21-50 years. Out of 50 isolates, 14 (28%) were Staphylococcus aureus, 10 (20%) were Klebsiella pneumoniae, 8 (16%) Enterococcus, 7 (14%) Coagulase Negative Staphylococcus, 6 (12%) Pseudomonas aeruginosa, 3 (6%) Escherichia coli and 2 (4%) Proteus vulgaris. On resistotyping of isolates, 9 (18%) isolates of 50 culture positive cases were MRSA, 5 (10%) were MRCoNS, 3 (6%) were Aminoglycoside resistant Enterococcus, 3 (6%) were pure ESBL, 4 (8%) were Combination of ESBL and MBL, 2 (4%) were both ESBL and AmpC, and 4 (8%) were ESBL+MBL+AmpC combination resistant strains. **Conclusion:** Careful, Appropriate and timely care is needed to prevent osteomyelitis. MRSA screening of patients is mandatory before any elective surgical procedure to reduce cross transmission of infections.

**Keywords:** Bacteria, Antibiotic Sensitivity pattern, Osteomyelitis.

## INTRODUCTION

Osteomyelitis is a serious inflammatory condition, caused by an infecting organism which reaches bone by travelling through the blood stream in to the bone or spreading from nearby tissue.

Osteomyelitis usually occurs when an injured bone exposes to germs, about 80% of cases are due to open wound. Most commonly affect the long bones in the leg and upper arm in children; and the pelvis involved in adults.<sup>[1]</sup>

In the past years, treating osteomyelitis was a challenging aspect. After invention of antibiotics, usage of chemical disinfection and sterilization practices at hospitals and clinics; as well as an increase in awareness of in population about surgical site case and hygienic routine practices help to

reduce osteomyelitis incidence, stop spread of infection to bone and to save the infected bone.

Predisposing factors for osteomyelitis are deep puncture wounds, bone surgeries. People with weekend immune system like chemo or radiotherapy, HIV, Malnutrition, dialysis and circulatory problems such as diabetes, peripheral arterial disease.

Osteomyelitis can occur in all age groups; younger and older population groups are commonly affected. Males are predominantly affected by Osteomyelitis than females, due to an increase in the prevalence of co morbid factors such as Diabetes Mellitus & Peripheral vascular disease.<sup>[2]</sup>

The most common organism isolated from all forms of Osteomyelitis is Staphylococcus aureus.<sup>[3]</sup> In Infants, Staphylococcus aureus, Group B Streptococci and Escherichia coli are most commonly isolated.<sup>[4]</sup> In 1-16 years age children Staphylococcus aureus, Streptococcus pyogenes and Haemophilus influenza are common and in adults, Staphylococcus aureus, Escherichia coli,

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*Pseudomonas aeruginosa*, *Serratia marcescens* and anaerobes are also common.<sup>[5]</sup>

Diagnosis of osteomyelitis is a challenging aspect, as it needs a combinatorial approach of clinical findings, laboratory infectious markers and radiological investigations.<sup>[6]</sup> Management of Osteomyelitis is another challenging aspect which requires mandatory adherence to Infection control policies, prolonged antibiotic therapy taking decision of surgical debridement or amputation in seven cases.<sup>[7]</sup>

The study is mainly focused on determining aerobic bacterial profile of Osteomyelitis isolates, their antibiotic susceptibility pattern and resistotyping of these isolates in this community.

## MATERIALS AND METHODS

The Present study is a prospective observational study conducted at Department of Microbiology, Government Medical College, Anantapuram. Materials for this study were collected from osteomyelitis patients of Orthopaedics department.

**Study Period:** July 2018 to May 2019.

**Sample Size:** 72

**Inclusion Criteria:** All ages of both sexes and confirmed cases of Osteomyelitis.

All clinically diagnosed Osteomyelitis samples like pus, swabs, synovial fluid, bone sequestrum, was collected under aseptic precautions. After receiving sample, it was immediately processed for culture and sensitivity according to CLSI guidelines. Hi Media Laboratories products were being used at our lab.

### Culture & Sensitivity:

The samples were inoculated on Nutrient Agar, Blood Agar, Macconkey agar plates and inoculated aerobically at 37°C for 24 hours. After 24 hours of inoculation, colonies were examined and identified by Biochemical reactions and other tests according to CLSI guidelines.

### Antibiotic susceptibility testing:

AST was done on Muller Hinton Agar by modified Kirby bauer disk diffusion method using clinical and laboratory standard institute guidelines (CLSI) [20]. Antibiotic disks used for Gram positive organisms testing were Penicillin (10U), Gentamicin (10µg), amikacin (30µg), ciprofloxacin (5 µg), erythromycin (5µg), clindamycin (2µg), cotrimoxazole (1.25 µg/23.75 µg), cefoxitin (30 µg), linezolid (30 µg), vancomycin (30µg) and teicoplanin (30µg), amoxycylav (30 µg). Gram negative isolates antibiotics were: amoxycylav (30 µg), piperacillin+tazobactam (100/10 µg), ceftazidime (30 µg), ceftazidime (30 µg), cefipime (30 µg), imipenem (10 µg), Ceftazidime+clavulanic acid (30/10 µg), meropenem (10 µg), amikacin (30 µg), tigecycline (15 µg). Standard Quality Control strains were used as a part of testing.

### Detection of MRSA and MRCoNS:

Cefoxitin disk of 30 µg was used for determining Methicillin resistance and results were read after 24 hours of incubation. *Staphylococcus aureus* with Zone size ≤21 mm were considered as MRSA and zone size of ≤24 mm were considered as MRCoNS.

### Detection of ESBL producers:

Gram Negative Bacilli isolates showed reduced susceptibility to 3rd generation cephalosporins with zone diameter of ≤22mm for ceftazidime, ≤25 mm for ceftriaxone, ≤27mm cefotaxime were considered as potential ESBL producers and these isolates were subjected to phenotypic confirmation by double disk diffusion test using ceftazidime & ceftazidime+clavulanic acid by placing 30mm apart from centre to centre. Increase of ≥5mm in zone of inhibition of the combination disk is considered to be ESBL producer.

### Detection of MBL producers:

Gram Negative bacilli isolates were found to be resistant to Imipenem and Meropenem were considered to be screening positive and were confirmed by putting Imipenem+EDTA (IE) combined disk diffusion test. Imipenem and IE were placed at a distance of 20 mm from centre to centre, increase of ≥5 mm in Inhibition zone size of IE is considered to be MBL producer.

### Detection of AmpC Producer:

AmpC production was detected using cefoxitin and cefoxitin boronic acid combined disc. Distortion of zone indicates AmpC production.

## RESULTS

A total of 72 osteomyelitis samples were assessed. Out of 72 samples of Osteomyelitis, 50 (69.4%) has shown culture positive. Male predominance noted; 72% were male and 28% were female. Majority were adults; 33 (66%) out of 50 samples were in the age group of 21-50 years [Table 1].

**Table 1: Age and Sex wise distribution of Osteomyelitis patients**

Age in years	Male	%	Female	%	Total	%
11-20	3	6	3	6	6	12
21-30	8	16	4	8	12	24
31-40	8	16	2	4	10	20
41-50	9	18	2	4	11	22
51-60	4	8	2	4	6	12
61-70	2	4	1	2	3	6
71-80	2	4	0	0	2	4
Total	36	72	14	28	50	100

On assessment of Bacteriological study, *Staphylococcus aureus* and *Klebsiella pneumoniae* were predominant pathogens. Out of 50 isolates, 14 (28%) were *Staphylococcus aureus*, 10 (20%) were *Klebsiella pneumoniae*, 8 (16%) *Enterococcus*, 7 (14%) Coagulase Negative *Staphylococcus*, 6 (12%) *Pseudomonas aeruginosa*, 3 (6%) *Escherichia coli* and 2 (4%) *Proteus vulgaris* [Figure 1].

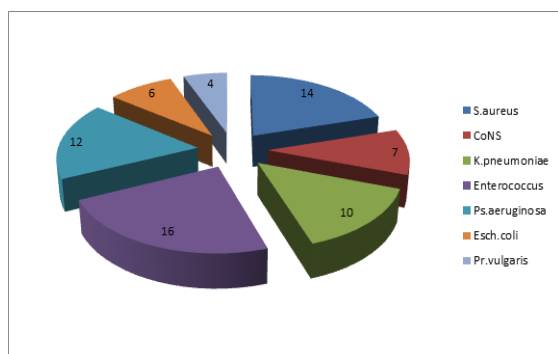


Figure 1: Bacteria responsible for Osteomyelitis

On resistotyping of isolates, 9 (18%) isolates of 50 culture positive cases were MRSA, 5 (10%) were MRCoNS, 3 (6%) were Aminoglycoside resistant Enterococcus, 3 (6%) were pure ESBL, 4 (8%) were Combination of ESBL and MBL, 2 (4%) were both ESBL and AmpC, and 4 (8%) were ESBL+MBL+AmpC combination resistant strains [Figure 2].

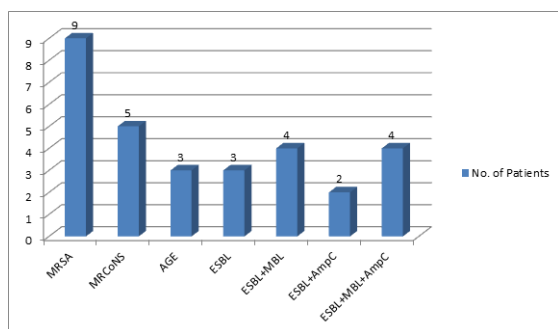


Figure 2: Resistance patterns of bacterial isolates

\*MRSA – Methicillin Resistant Staphylococcus aureus, MRCoNS – Methicillin Resistant Coagulase Negative Staphylococci, AGE – Aminoglycoside resistant Enterococci, ESBL – Extended Spectrum Beta Lactamases, MBL – Metallobetactamse.

Out of 50 isolates 26% were ESBL, 4% were AmpC and 16% were MBL Producers. 21 Gram negative bacilli were isolated from 50 Osteomyelitis samples, among them 13 (61.9%) were ESBL, 2 (9.5%) were Amp C and 8 (38.09%) were MBL producers. Pseudomonas showed MBL production predominantly. Whereas Klebsiella and Escherichia

coli showed ESBL production in majority of isolates [Table 2].

Table 2: Resistance pattern distribution among various bacterial isolates

Organism	Total no. of isolates	ESBL (n=13)	AmpC (n=2)	MBL (n=8)
Esch. Coli	3	3	0	1
K.pneumoniae	10	8	1	4
Pr. vulgaris	2	0	0	0
Ps.aeruginosa	6	2	1	3

Out of 21 Staphylococcus species, 14 (66.6%) were Methicillin resistant and 7 (33.3%) were Methicillin sensitive. Out of 14 MRS, 100% were sensitive to teicoplanin and vancomycin, 85.7% were sensitive to Linezolid, 78.5% were sensitive to amikacin, clindamycin, cotrimoxazole and 64.7% were sensitive to ciprofloxacin, erythromycin. Out of 7 MSS isolates, 100% were sensitive to Amikacin, ciprofloxacin, cefoxitin, linezolid, vancomycin, teicoplanin, amoxycylav, 85.7% were sensitive to clindamycin, cotrimoxazole, 71.4% were sensitive to erythromycin and 28.5% were sensitive to penicillin [Table 3].

Table 3: Sensitivity pattern of MRS and MSS isolates.

Antibiotics	Methicillin Resistant Staphylococcus (MRS)	%	Methicillin Sensitive Staphylococcus (MSS)	%
Penicillin	0	0	2	28.5
Amikacin	11	78.5	7	100
Ciprofloxacin	9	64.2	7	100
Erythromycin	9	64.2	5	71.4
Clindamycin	11	78.5	6	85.7
Cotrimoxazole	11	78.5	6	85.7
Cefoxitin	0	0	7	100
Linezolid	12	85.7	7	100
Vancomycin	14	100	7	100
Teicoplanin	14	100	7	100
Amoxycylav	0	0	7	100

Table 4: Sensitivity pattern of Gram Negative bacilli isolates

Antibiotics	Esch.coli (n=3)	%	K.pneumoniae (n=10)	%	Pr.vulgaris (n=2)	%	Ps.aeruginosa (n=6)	%
Amoxycylav	0	0	2	20	2	100	-	-
Piperacillin+tazobactam	0	0	2	20	2	100	4	66.6
Ceftazidime	0	0	2	20	2	100	4	66.6
Ceftriaxone	0	0	2	20	1	50	-	-
Cefipime	3	100	9	90	2	100	-	-
Ceftazidime+clavulanic acid	0	0	2	20	2	100	4	66.6
Imipenem	3	100	6	60	2	100	3	50
Meropenem	3	100	6	60	2	100	3	50
Colistin	3	100	10	100	2	100	6	100
Ciprofloxacin	2	66.6	5	50	2	100	6	100
Amikacin	2	66.6	6	60	2	100	4	66.6
Tigecycline	3	100	10	100	2	100	-	-

All *Escherichia coli* isolates have shown 100% sensitivity to cefipime, imipenem, meropenem, tigecycline, colistin and 66.6% were sensitive to ciprofloxacin and amikacin. All isolates of *Proteus vulgaris* were sensitive to all tested antibiotics except ceftriaxone.

100% of *Klebsiella pneumoniae* isolates were sensitive to colistin, tigecycline, 90% isolates were sensitive to cefipime, 60% sensitive to imipenem, meropenem and amikacin, 50% were sensitive to ciprofloxacin, 20% isolates were sensitive to Amoxycylav, piperacillin+tazobactam, ceftazidime, ceftriaxone, ceftazidime+clavulanic acid.

Out of 6 *Pseudomonas* isolates, all 6 (100%) were sensitive to colistin, ciprofloxacin, 66.6% were sensitive to piperacillin+tazobactam, ceftazidime, ceftazidime+clavulanic acid, amikacin, 50% isolates were imipenem and meropenem [Table 4].

## DISCUSSION

Osteomyelitis is a rare but fulminant condition of bone caused by a bacterial or fungal infection.<sup>[8]</sup>

Osteomyelitis can present as Acute Osteomyelitis - infection develops within 2 weeks of an injury, initial infection or the start of an underlying disease. It is life threatening condition; common in children. Sub Acute Osteomyelitis - Infection develops within 1-2 months. Chronic Osteomyelitis - Infection starts at least 2 months after injury, initial infection or the start of an underlying disease and Non Suppurative Osteomyelitis.

Clinical symptoms are directly proportionate to infection severity, damaged region and patient condition. Chronic osteomyelitis can lead to complications such as pathological fractures, epithelioma, bone growth interference, amyloid disease.<sup>[9]</sup>

In similar to the present study, most of the other studies documented majority of the patients got affected in the age group of 16-50 years.<sup>[6-8]</sup> Male predominance noted in Osteomyelitis by Sameulo BA et al,<sup>[9]</sup> Zuluaga AF et al,<sup>[10]</sup> Ako Nai Ak et al,<sup>[6]</sup> and Faria Malik et al,<sup>[7]</sup> showed prevalence of 66.4%, 72%, 70.7%, and 70% respectively.

Nisreen OM et al,<sup>[11]</sup> did a bacteriological study on 207 patients, among them 66 patients were <4 years old and 141 patients were ≥4 years. Causative pathogens were identified in 70% of patients. *Staphylococcus aureus* comprised 55% of positive results in children < 4Y and 73% in children ≥4Y. Among *S. aureus* cultures, 70-76% were methicillin sensitive (MSSA). *Kingella kingae* was exclusively identified in children <4Y (21% of positives), which was also the group with the highest rate of culture-negative infection (41%).

Ako-Nai AK et al,<sup>[6]</sup> did a study on 82 patients of chronic osteomyelitis and isolated *Staphylococcus aureus* (20.5%), Coagulase Negative *Staphylococci* (12.8%), *Streptococcus* species (1.2%),

*Pseudomonas aeruginosa* (8.5%), *Escherichia coli* (5.1%), *Citrobacter freundii* (2.5%) and *Salmonella* species (2.5%).

Barbari et al,<sup>[12]</sup> documented that >50% of isolates were *Staphylococcus aureus* and >25% isolates were enterobacteriaceae and anaerobes like *Peptostreptococcus* spp, *Bacteroides fragilis* and rarely isolated organisms were *Brucella* species, *Salmonella* species and *Mycobacteria* species.

Samuel BA et al,<sup>[9]</sup> reported the incidence rate of culture positive osteomyelitis was 80.3%. 37.6% *Staphylococcus aureus*, 11.2% Coliforms, 9.3% *Klebsiella*, 9.3% *Escherichia coli*, 6.5% *Proteus* and *Pseudomonas*.

Abid A Salman et al,<sup>[13]</sup> documented that 68% of osteomyelitis were culture positive. *Staphylococcus aureus* was the major pathogen isolated (44%), followed by *Klebsiella* (8%) and *Pseudomonas* (8%).

Majda Qureshi et al,<sup>[14]</sup> reported 54% of staphylococcus isolates, 23% enterobacteriaceae, 18% *Pseudomonas aeruginosa*, 12.5% *Proteus* species, 8% *Escherichia coli*, 2.5% *Klebsiella*, and 2.5% Miscellaneous.

Wadekar Mita D,<sup>[15]</sup> did a study for one year (2011) on 100 patients of chronic osteomyelitis. Majority of Gram positive organisms were sensitive to Amikacin, Linezolid, Vancomycin and Gram negative organisms to Amikacin and Imipenem with prevalence of MRSA being 40%, ESBL 70.6% and MBL 18.9%.

In similar to our study Supriya S Tahnkiwale et al,<sup>[16]</sup> and Sachin Sharma et al,<sup>[17]</sup> documented 19.5% and 23.7% respectively. Other studies noted higher rate of MRSA prevalence of 69% by Vidya Pai et al,<sup>[18]</sup> and 58% by Christian H Jacobus et al.<sup>[19]</sup>

Amita Jain et al,<sup>[20]</sup> reported 63.6% of *Esch.coli* and 86.6% of *K.pneumoniae* were ESBL producers. Basavaraj C Metri et al,<sup>[21]</sup> reported 31.7% of *Esch.coli* and 46.4% of *K.pneumoniae* as ESBL producers.

Uma Chaudhary et al,<sup>[22]</sup> reported 7.5% of *Ps.aeruginosa*, 1.25% *Esch.coli* and 3.5% *K.pneumoniae* were MBL producers. Nirav PP et al,<sup>[23]</sup> reported 9.9% of *Ps.aeruginosa*, 2.8% *Esch.coli* and 7.2% *K.pneumoniae*.

According to Viudes A et al,<sup>[24]</sup> MRSA is 42.85%. All MSSA were susceptible to vancomycin, gentamicin, teicoplanin, ciprofloxacin and linezolid. 90% were erythromycin susceptible. All the MRSA were susceptible to vancomycin and linezolid, 92% to gentamicin.

Management of osteomyelitis is mainly by using appropriate antibiotics either orally or parenterally depending on clinical condition. Non surgical adjunctive modalities including usage of hyperbaric oxygen therapy, growth factor, platelet rich plasma, pulsed electromagnetic fields have not been widely used, mostly due to a lack of perceived efficacy, and have remained in a state of infancy.<sup>[25]</sup> Local

antibiotic delivery using antibiotic beads is a valuable adjuvant therapy.<sup>[26]</sup>

## CONCLUSION

Careful, Appropriate and timely care is needed to prevent osteomyelitis. MRSA screening of patients is mandatory before any elective surgical procedure to reduce cross transmission of infections. Early bacteriological investigations are necessary to determine bacterial etiology and their sensitivity to antibiotics are highly desirable so that first line antimicrobials are administered.

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