



BACTERIOLOGICAL PROFILE AND THEIR ANTIBIOGRAM IN PATIENT WITH CHRONIC OSTEOMYELITIS

Orthopaedics

Dr. Sourabh Kumar

PGT, Department Of Orthopaedics, Assam Medical College & Hospital

Dr. Munin Borgohain

Professor & HOD, Assam Medical College & Hospital

Dr. Mandar Deb

Registrar, Assam Medical College & Hospital

Dr. Jyoti Prakash Roy

PGT, Department Of Orthopaedics, Assam Medical College & Hospital

Dr. Kumar Madhurya Pratim

PGT, Department Of Orthopaedics, Assam Medical College & Hospital

Dr. Abhishek Kayal

PGT, Department Of Orthopaedics, Assam Medical College & Hospital

Dr. Zakaria Ul Akman

PGT, Department Of Orthopaedics, Assam Medical College & Hospital

ABSTRACT

Introduction: Osteomyelitis is a progressive inflammatory process which destroys the bone, forms sequestrum and discharging sinuses. Complete eradication of infection is one of the main issues faced in the treatment of osteomyelitis often leading to chronicity. Complete eradication of infection is one of the main issues faced in the treatment of osteomyelitis often leading to chronicity. **Material And Methods:** A hospital based observational study was performed for 23 patients admitted for treatment of chronic osteomyelitis between June 2020 and May 2021 at Assam medical college and hospital Dibrugarh. The reports of the sinus tract cultures and intraoperative cultures of each patient were collected and recorded. **Result:** Out of 23 patients, 22 (95.65%) showed positive culture in both sinus tract and intraoperative cultures, and 1 patient showed negative culture in both. Staphylococcus aureus was the most commonly isolated single bacteria from both sinus tract cultures (27.27%) and intraoperative culture (40.91%). **Conclusion:** combination therapy comprising of Ciprofloxacin or Cotrimoxazole (for Gram positive coverage) plus Levofloxacin or Amikacin (for Gram negative coverage) is effective in our centre as empirical therapy.

KEYWORDS

Antibiotics, empirical therapy, osteomyelitis, sinus tract culture

INTRODUCTION

Osteomyelitis treatment is still a challenge in the field of orthopaedics with increase in the prevalence of cases even in some developed countries. Complete eradication of infection is one of the main issues faced in the treatment of osteomyelitis often leading to chronicity.¹

The identification of the causative organism and its antibiotic susceptibility is crucial in the treatment of osteomyelitis. Culture of infected bone under anaesthesia is the gold standard for identification. Some workers found that deep sinus tract cultures can guide us towards the causative organism and its antibiotic susceptibility pattern.² However some others stated that deep tissue culture was more sensitive than superficial swabbing.³

We have undertaken this study to identify the common causative bacteria and their antibiotic susceptibility patterns in cases of chronic osteomyelitis in our centre and we hypothesized that Sinus Tract Cultures can help in identifying the causative bacteria.

OBJECTIVES

1. To isolate and identify the bacteria causing chronic osteomyelitis
2. To study the antibiotic sensitivity of the isolated bacteria to commonly used antibiotics
3. To assess the concordance of Sinus Tract Cultures with that of Intraoperative Bone Cultures to guide antibiotic therapy in chronic osteomyelitis

METHOD

A hospital based observational study was performed for all patients who were admitted for treatment of chronic osteomyelitis between June 2020 and May 2021 at Assam medical college and hospital Dibrugarh. Acute infections, Patients not giving informed consent or Patients without any of sinus, sequestrum, bone destruction or new bone formation were excluded from study.

patients of all age groups) or Patients presenting with chronic discharging sinus along with radiological evidence of sequestrum, new bone formation or destruction of bone including those having orthopaedic implants in situ were included in our study.

After admission of patient, a detailed history was taken and a thorough clinical examination was done. The sinus orifice was cleaned with 0.9% sodium chloride and a sterile cotton swab was inserted deep into the sinus tract and was labelled and sent for culture and sensitivity. All patients were taken up for surgical debridement after being declared fit for surgery and anaesthesia. During surgical debridement, implants were removed (if present) and infected material like pus, infected bone, sequestrum, infected tissues, etc were collected in sterile containers and were labelled and sent for culture and sensitivity.

Specimen Were Processed As Follows

Gram's Staining: Smear was prepared on clean glass slide with normal saline, air dried and fixed. The slide was flooded with crystal violet for about 1 minute and then washed off. Then it was flooded with Gram's iodine solution for about a minute and then tipped off, following which it was flooded with Acetone for 2 to 3 seconds and then washed off with tap water. Then the slide was flooded with Safranin for 1 minute and then washed with tap water. The smear was examined for the presence of inflammatory cells and bacterial morphology.

Culture: The swab was inoculated onto Nutrient agar, Blood agar and MacConkey agar which were aerobically incubated at 37°C and observed for growth at 24 and 48 hours. The bacteria isolated were identified based on the colony characteristics, morphology in Grams staining of culture smear, biochemical properties, etc.

Antimicrobial Susceptibility: The bacteria isolated were tested for sensitivity to antibiotics by Kirby Bauer disc diffusion method⁴ following the Clinical and Laboratory Standards Institute guidelines.

The reports of the sinus tract cultures and intraoperative cultures of

Patients with osteomyelitis for more than 6 weeks (male and female

each patient were collected and recorded. The bacteria isolated from the intraoperative cultures (which were considered to be the gold standard) were studied to identify the common bacteria causing chronic osteomyelitis and also their antibiotic susceptibility patterns. The reports of the sinus tract cultures were compared to the reports of the intraoperative cultures to assess the ability of the sinus tract cultures to isolate the causative bacteria as in the intraoperative cultures. In our study the intraoperative culture was taken to be the gold standard and sinus tract culture was taken to be the screening test.

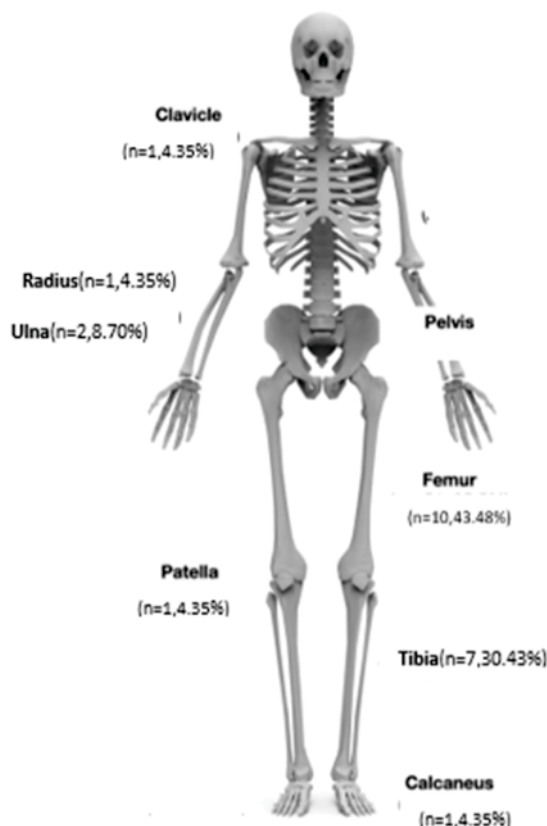
RESULTS

Between June 2020 to May 2021, 23 patients with chronic osteomyelitis were admitted in our hospital and underwent surgical treatment. 19 were males and 4 were females. The age of the patients ranged from 6 years to 65 years with the mean age being 36.65 ± 14.80 years. 39.13% of the patients were in the age group of 31 to 40 years followed by 21.74% in 41 to 50 age group and 13.04% in 21 to 30 years age group.

Table-1 : Distribution Of Patients According To Age.

AGE GROUP (in years)	NUMBER	PERCENTAGE
0-10	2	8.70
11-20	1	4.35
21-30	3	13.04
31-40	9	39.13
41-50	5	21.74
51-60	1	4.35
61-70	2	8.70
>70	0	0.00
TOTAL	23	100.00
Mean $\pm$ S.D.	36.65 ± 14.80	

Most common affected bone in our study was femur. The distribution of affected anatomical sites is shown in Fig. 1



It has been observed that most of the patients had trauma as a predisposing factor (56.52%) followed by orthopaedic implants in situ (26.09%). 5 patients had diabetes mellitus which accounted for 21.74% of the total. 2 (8.70%) patients had chronic soft tissue infection as the predisposing factor and a single patient had hematogenous infection (4.35%).

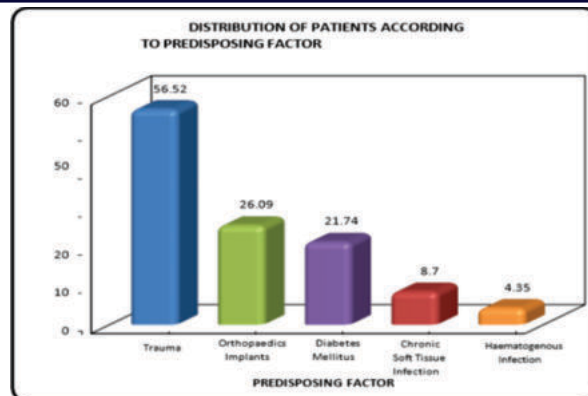


Figure 2. Microbial Isolates

Out of 23 patients, 22 (95.65%) showed positive culture in both sinus tract and intraoperative cultures, and 1 patient showed negative culture in both. It was observed that 14 (63.63%) out of 22 positive sinus tract cultures showed polymicrobial flora and 8 (36.36%) of them showed monomicrobial flora. However in case of intraoperative cultures all 22 positive cultures (100%) showed monomicrobial flora.

Staphylococcus aureus was the most commonly isolated single bacteria from both sinus tract cultures (27.27%) and intraoperative culture (40.91%). Other bacteria isolated from sinus tract cultures were Klebsiella pneumonia (4.55%) and Escherichia coli (4.55%). In intraoperative cultures, the second most commonly isolated bacteria was Pseudomonas aeruginosa (22.73%) followed by Enterococcus (13.64%).

Table-3 : Distribution Based On Bacteria Isolated

BACTERIA ISOLATED	SINUS TRACT CULTURE		INTRAOPERATIVE CULTURE	
	N	%	n	%
Staphylococcus aureus	6	27.27	9	40.91
Enterococcus	0	0.00	3	13.64
Pseudomonas aeruginosa	0	0.00	5	22.73
Escherichia coli	1	4.55	2	9.09
Klebsiella pneumoniae	1	4.55	2	9.09
Enterobacter cloacae	0	0.00	1	4.55
Mixed Bacterial Flora	14	63.64	0	0.00
TOTAL	22	100.00	22	100.00

It was observed that the gram positive bacteria showed 100% sensitivity for Linezolid, 83.33% sensitivity for both Ciprofloxacin and Cotrimoxazole. The gram negative bacteria were maximally susceptible to Amikacin (80%) and Imipenem (80%) followed by Levofloxacin (70%).

Table 3 shows the pattern of antibiotic sensitivity of the individual bacteria isolated from the intraoperative cultures to the commonly tested antibiotics. As observed the bacteria were susceptible mainly to Linezolid, Cotrimoxazole, Ciprofloxacin, Imipenem, Amikacin and Levofloxacin.

Table-4 Pattern Of Antibiotic Sensitivity Of Isolated Bacteria From Intraoperative Cultures

Anti-biogram	Staphylococcus aureus	Enterococcus	Pseudomonas aeruginosa	Klebsiella pneumoniae	Escherichia coli	Enterobacter cloacae
Ampicillin	11.11%	0	-	-	-	-
Azithromycin	33.33%	33.33%	-	-	-	-
Amoxycillin	-	-	40%	50%	0	100%
Gentamicin	-	-	40%	100%	0	0
Amikacin	-	-	80%	100%	50%	100%
Ciprofloxacin	88.89%	66.67%	-	-	-	-
Erythromycin	44.44%	33.33%	-	-	-	-
Clindamycin	66.67%	33.33%	-	-	-	-
Linezolid	100%	100%	-	-	-	-
Cotrimoxazole	88.89%	66.67%	-	-	-	-
Cefotaxime	-	-	40%	0	0	0

Ceftazidime	-	-	20%	50%	0	0
Imipenem	-	-	100%	100%	0	100%
Levofloxacin	-	-	80%	50%	100%	0
Doxycycline	-	-	60%	0	50%	100%

DISCUSSION

In our study the age of the patients ranged from 6 years to 65 years with the mean age being 36.65 ± 14.80 years. 39.13% of the patients were in the age group of 31 to 40 years followed by 21.74% in 41 to 50 age group and 13.04% in 21 to 30 years age group. Similarly, in a study conducted by Neeharika B et al. on 100 patients of chronic osteomyelitis, the cases were found to be between 11 years to 60 years.⁵

In our study, 19 (82.61%) of the patients were male and 4 (17.39%) of the patients were female. In another study conducted by Jerzy K et al., they studied 30 consecutive cases of chronic osteomyelitis and found that 24 (87%) of them were males and 6 (13%) were females.⁶

It was observed in our study that most of the patients had trauma as a predisposing factor (56.52%) followed by orthopaedic implants in situ (26.09%). 5 patients had diabetes mellitus which accounted for 21.74% of the total. 2 (8.70%) patients had chronic soft tissue infection as the predisposing factor and a single patient had hematogenous infection (4.35%). Similarly, Neeharika B et al. stated in their study that the most common predisposing factor they encountered was trauma accounting for 45%, followed by postoperative infections accounting for 22%, 19 patients had diabetes as predisposing factor, and 4% of the patients acquired infection via hematogenous spread.⁵

In our studied cases the most common bone involved was femur in 10 patients (43.48%) followed by tibia in 7 patients (30.43%). There were 2 (8.7%) patients where ulna was involved. There was 1 case each for radius, patella, calcaneum and clavicle accounting for 4.35% each. Simpson et al. studied 50 patients and reported the most common bones to be involved in chronic osteomyelitis were femur (36%) and tibia (36%). In another study conducted by Jerzy K et al. they found that the most common bone to be affected was tibia (63%) followed by bones of the forearm accounting for 17%.⁶

In our study, out of 23 patients, 22 (95.65%) showed positive culture and 1 patient showed negative culture in our study. All 22 positive intraoperative cultures showed monomicrobial growth. Isolation of predominantly monomicrobial flora was reported in the following studies too. Mthethwa PG et al. in their study of 108 patients of chronic osteomyelitis found that 27 of them showed multiple organism growth, 4 cases showed no growth and the rest 77 showed growth of single organism.⁸ Sheehy SH et al. concluded that majority of the isolates showed monomicrobial growth followed by polymicrobial cultures in 29% of cases. Culture was negative in 28%.⁹

From the antibiotic sensitivity tests of the intraoperative culture, it was observed that the gram positive bacteria showed 100% sensitivity for Linezolid, 83.33% sensitivity for both Ciprofloxacin and Cotrimoxazole (Sulfamethoxazole – Trimethoprim). The gram negative bacteria were maximally susceptible to Amikacin (80%) and Imipenem (80%) followed by Levofloxacin (70%).

Pliska et al. reported that the sensitivity of *Pseudomonas aeruginosa* is mainly to fluoroquinolones (levofloxacin, etc), aminoglycosides & carbapenems; except the following drugs to which the sensitivity patterns show some decrease – cefepime, pefloxacin & ertapenem.¹⁰ Deconinck L et al. reported that Cotrimoxazole (SXT) appears to be efficacious in bone joint infections in gram positive as well as gram negative bacteria and polymicrobial infection.¹¹

Euba G et al. found that oral rifampin-cotrimoxazole therapy is an effective alternative in the treatment of chronic osteomyelitis caused by *Staphylococcus aureus*.¹²

Batra P et al. reported that Sulfonamides (Sulfamethoxazole) have wide antimicrobial activity and are effective against both Gram-positive and Gram-negative bacteria.¹³

Kim BN et al. also stated that fluoroquinolones (Levofloxacin, etc) are effective in bone and joint infections.¹⁴

Suggested Antibiotic Regimen

In our study, as mentioned above, it was seen that 54.55% of the causative bacteria were Gram positive and 45.45% were Gram

negative bacteria. Therefore antibiotic coverage for both Gram positive and negative bacteria is essential till the intraoperative culture reports are available. The bacteria were mainly susceptible to Linezolid/Ciprofloxacin/Cotrimoxazole (for Gram positive) and Imipenem/Amikacin/Levofloxacin (for Gram negative).

Linezolid and Imipenem are reserved for high grade infection and are not preferred for empirical therapy to preserve their efficacy and prevent resistance. Therefore it may be suggested that a combination therapy comprising of Ciprofloxacin or Cotrimoxazole (for Gram positive coverage) plus Levofloxacin or Amikacin (for Gram negative coverage) may be effective in our centre till intraoperative culture reports are available, provided there are no contraindications to the above mentioned drugs.

CONCLUSION

Our study which was aimed to find the common causative bacteria, their antibiotic sensitivity pattern and the ability of sinus tract cultures to detect the causative bacteria showed us that chronic osteomyelitis cases that presented to our hospital were majorly caused by gram positive bacteria (predominantly *Staphylococcus aureus*) who were sensitive to Linezolid, Ciprofloxacin and Cotrimoxazole.

The gram negative flora was sensitive mainly to Amikacin, Imipenem and Levofloxacin. Linezolid and Imipenem, being reserved drugs for higher infections, are not preferred for empirical therapy. Therefore Ciprofloxacin/Cotrimoxazole plus Levofloxacin/Amikacin may prove to be effective. However it was observed that sinus tract cultures had a sensitivity of 36.36% and were not able to appropriately demonstrate the causative bacteria. This information will guide us in deciding the antibiotic coverage to be provided to the patients of chronic osteomyelitis in our centre till intraoperative culture and sensitivity reports are obtained. This will also prevent indiscriminate use of antibiotics and development of antibiotic resistance.

Our study, however, had the limitations of being a single centre study with a small sample size. A multicentric study with a larger sample size would provide more information regarding the topic.

REFERENCES

- Walter N, Baertl S, Alt V, Rupp M. What is the burden of osteomyelitis in Germany? An analysis of inpatient data from 2008 through 2018. *BMC Infect Dis.* 2021 Jun;21(1):550.
- Bernard L, Uçkay I, Vuagnat A, Assal M, Stern R, Rohner P, et al. Two consecutive deep sinus tract cultures predict the pathogen of osteomyelitis. *Int J Infect Dis.* 2010 May;14(5):e390-3.
- Pellizzer G, Strazzabosco M, Presi S, Furlan F, Lora L, Benedetti P, et al. Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb-threatening diabetic foot infection. *Diabet Med.* 2001 Oct;18(10):822-7.
- Bauer AW, Kirby WM, Sherris JC, Tenckhoff M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966 Apr;45(4):493-6.
- BN, R V, Gerard R. A study of aerobic bacterial profile and their antibiogram in patients with chronic osteomyelitis with special references to *Staphylococcus aureus* [Internet]. Vol. 2019, *Indian Journal of Microbiology Research*. IP Innovative Publication; 2019. p. 10099. Available from: <https://www.ijmronline.org/article-details/10099>
- Jerzy K, Francis H. Chronic Osteomyelitis - Bacterial Flora, Antibiotic Sensitivity and Treatment Challenges. *Open Orthop J.* 2018;12:153-63.
- Simpson AH, Deakin M, Latham JM. Chronic osteomyelitis. The effect of the extent of surgical resection on infection-free survival. *J Bone Joint Surg Br.* 2001 Apr;83(3):403-7.
- Kehr P, Edward C, Benzel: Biomechanics of spine stabilization. *Eur J Orthop Surg Traumatol* [Internet]. 2015 Oct [cited 2022 Sep 30];25(7):1219-1219. Available from: https://www.researchgate.net/publication/283884772_Edward_C_Benzel_Biomechanics_of_spine_stabilization
- Sheehy SH, Atkins BA, Bejon P, Byren J, Wyllie D, Athanasou NA, et al. The microbiology of chronic osteomyelitis: prevalence of resistance to common empirical anti-microbial regimens. *J Infect.* 2010 May;60(5):338-43.
- Pliska NN. *Pseudomonas Aeruginosa* as the Main Causative Agent of Osteomyelitis and its Susceptibility to Antibiotics. *Drug Res (Stuttg).* 2020 Jun;70(6):280-5.
- Deconinck L, Dinh A, Nich C, Tritz T, Matt M, Senard O, et al. Efficacy of cotrimoxazole (Sulfamethoxazole-Trimethoprim) as a salvage therapy for the treatment of bone and joint infections (BJIs). *PLoS One.* 2019;14(10):e0224106.
- Euba G, Murillo O, Fernández-Sabé N, Mascará J, Cabo J, Pérez A, et al. Long-term follow-up trial of oral rifampin-cotrimoxazole combination versus intravenous cloxacillin in treatment of chronic staphylococcal osteomyelitis. *Antimicrob Agents Chemother.* 2009 Jun;53(6):2672-6.
- Batra P, Deo V, Mathur P, Gupta AK. Cotrimoxazole, a wonder drug in the era of multiresistance: Case report and review of literature. Vol. 9, *Journal of laboratory physicians*. Germany; 2017. p. 210-3.
- Shin JC, Kim DH, Yu SJ, Yang HE, Yoon SY. Epidemiologic change of patients with spinal cord injury. *Ann Rehabil Med* [Internet]. 2013 Feb [cited 2022 Sep 25];37(1):50-6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23525183>