Original Article

A study on bacterial and fungal isolates and their antimicrobial susceptibility pattern in patients with chronic osteomyelitis in a tertiary care hospital

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How to cite this article:
Devi.C, Banu S.T. A study on bacterial and fungal isolates and their antimicrobial susceptibility pattern in patients with chronic osteomyelitis in a tertiary care hospital. Innov Pharm Pharmacother 2017;5(4):195-203.

Source of Support: Nil,
Conflict of Interest: None declared.

ABSTRACT

Chronic osteomyelitis is a major challenging problem in our country, and it is a persistent disease difficult to treat and eradicate completely. The aim of the study is to analyze the predisposing factors associated with chronic osteomyelitis and to study the causative organisms and their antimicrobial susceptibility pattern and check the resistance pattern in common isolates. It is a cross-sectional study done during a time between October 2011 and September 2012 and was included in the analysis of the data. Total of 120 patients were included prospectively. In 120 patients detailed history were recorded. Collection of samples was done under strict aseptic precautions. Pus, swabs from sinus tract and sequestrum were the samples collected. Processing of samples was done by culture both bacterial and fungal, catalase test, oxidase test, biochemical reactions, and antimicrobial susceptibility was done by kir by-Bauer disc diffusion method according to CLSI guidelines. All the tests were done as per protocol. ATCC strains *Staphylococcus aureus* - ATCC 25923, *Escherichia coli* - ATCC 25922, and *Pseudomonas aeruginosa* - ATCC 27853 were used as controls. Detection of β-lactamase enzymes in Gram-negative bacilli (GNB), detection of methicillin resistance in *S. aureus* were also done phenotypically. Minimum inhibitory concentration (MIC) of vancomycin was done to detect vancomycin resistance against *S. aureus.* Fungal cultures were identified by macroscopic appearance, microscopy analysis (Gram staining, LCB), germ tube test, CHROM agar media, and sugar fermentation. MIC determination by microbroth dilution method was also done. ATCC *Candida albicans* 90028 was used as quality control. In this study, 97 (80.83%) were males and 23 (19%) were females. 40% of the patients had a duration of the illness from 7 to 12 months. 35.8% of patients had illness ranged from 13 to 24 months. 50.8% of patients had compound fracture leading to infection. Among the samples collected, 63 (52.5%) were sequestrum/per-operative collections of pus and tissue fluids, and 57% (47.5%) were swabs. Culture positivity was 83.3%, an increased number of polymicrobial (12.2%) infections were noted in swabs, though monomicrobial infection was the most common type even in swabs (57.8%). The common organism isolated was *S. aureus* (36.7%), *S. aureus* which was the most common bacteria isolated in this study showed 100% sensitivity to Rifampin, 97.4% sensitivity to vancomycin, 64% sensitivity to amikacin, chloramphenicol and Erythromycin, 51.2% to Penicillin, and 51.2% were sensitive to cefoxitin. Among Gram-negative isolates, *P. aeruginosa* was the most common isolate, which showed 100% sensitivity to imipenem, 76.4% to cefoperazone sulbactam, 52.9% to amikacin, and 35.2% to cefotaxime and ceftazidime. One isolate of *Mycobacterium tuberculosis* was sensitive to all first and second line drugs. The one fungal isolate *Candida tropicalis* was sensitive to fluconazole, amphotericin B, itraconazole, and voriconazole. 40.5% of aerobic bacteria were multidrug-resistant. 56.6% were aerobic Gram-positive cocci (GPC) and 43.3% were aerobic GNB, one acid-fast bacillus *M. tuberculosis*, and one yeast *C. tropicalis* were isolated in the study. Among GPC, *S. aureus* (36.7%) was the most common pathogen isolated followed closely by *Staphylococcus epidermidis* (10.5%). All GPC except one were sensitive to vancomycin and rifampin. Among GNB, all were sensitive to imipenem and 90% to cefoperazone sulbactam. Pseudomonas had lower sensitivity (76.4%) to cefoperazone. Various factors in open fracture leading to chronic osteomyelitis

Access this article online	
Website: www.innpharmacotherapy.com	**e-ISSN:** 2321-323X **p-ISSN:** 2395-0781

each patient has to be routinely monitored after trauma and treatment for developing osteomyelitis. Treatment given in the early stage will prevent dreadful complications and sequelae.

Keywords: Antimicrobial susceptibility, osteomyelitis, cross-sectional study, gram-positive cocci

Introduction

The word osteomyelitis is a combination of Greek word "osteon" meaning bone and "my elos" meaning marrow plus the suffix. "It is" meaning inflammation. Osteomyelitis is acquired in three ways. They are direct seeding of microorganisms into bone due to trauma or surgery, hematogenous spread of microorganisms from the focus of infection elsewhere in the body and spread from surrounding infected soft tissue and joints.

Commonly the infection is monomicrobial. Infection due to multiple organisms[1] is usually seen in patients with diabetes mellitus with an ulcer in the foot. The following six components characterize chronic osteomyelitis: Sequestrum formation or sclerosis, radiological changes seen in bone due to infection for 6 weeks or longer, relapse or persistence of infection after initial treatment, osteomyelitis due to foreign bodies, osteomyelitis in association with peripheral vascular disease, and organisms that produce chronic disease (e.g., *Mycobacterium tuberculosis*).

The most common presenting symptoms are persistent pain and chronic intermittent discharge through sinuses. Bone debris and sequestra find an exit through multiple openings in an involucrum, go through the sinus tracts and present to the surface. In children, after discharge of sequestrum, the sinus is closed, and the cavity is filled with new bone. 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 usual complications of chronic osteomyelitis are reduced rate of growth, pathological fracture, septic arthritis, lengthening of bone, and contracture of muscles. Other rare complications are the formation of epithelioma, secondary amyloidosis,[2] and squamous cell carcinoma in scar tissue (<1%).

Chronic osteomyelitis is a disease, which is difficult to eradicate completely. There may be subsidence of systemic symptoms, but the cavities containing purulent material, infected granulation tissue or sequestrum act as foci of infection. There may be recurrent acute flare-ups occurring at indefinite intervals over months and years. To achieve eradication of the disease, aggressive surgical debridement with curettage of cavities, filling of cavities with soft tissues and effective antimicrobial treatment is required.[3]

The pattern and behavior of organisms are constantly changing under the pressure of newer antibiotics.[4] As a result, the wonder drugs of fifties have been relegated to a position of limited usefulness today. With this background, it is felt worthwhile to study the spectrum of organisms causing osteomyelitis and their antimicrobial susceptibility pattern.

Aim

The aim of the study was to study the predisposing factors associated with chronic osteomyelitis, study the causative organisms and their antimicrobial susceptibility pattern and check the resistance pattern in common isolates.

Materials and Methods

It is a cross-sectional study done during a time between October 2011 and September 2012 was included in the analysis of the data. Total of 120 patients were included prospectively. The study was conducted in the Institute of Microbiology, Madras Medical College in association with Institute of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai - 600 003.

Ethical consideration

The necessary Ethical Committee approval was obtained before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

Inclusion criteria

1. Patients are older than 12 years.
2. Patients admitted to orthopedic wards and those attending outpatient departments who satisfy one of the following six components of chronic osteomyelitis.
3. Osteomyelitis in association with trauma only.
4. Osteomyelitis in association with diabetes and peripheral vascular compromise.
5. Clinical evidence of chronic disease (e.g., M. tuberculosis).
6. Radiological changes suggestive of infection for 6 weeks or more.
7. Formation of sequestrum or sclerosis
8. Even after treatment, persistence or relapse of infection.

Exclusion criteria

1. Patients with prosthetic orthopedic implant devices.
2. Pediatric age group (<12 years).

History

Name, age, sex, date of admission, physical examination findings, history of trauma, associated predisposing factor (diabetes mellitus, intravenous drug abuse, immunosuppression, and tuberculosis) duration of illness, smoking, and alcoholism were also recorded.

Collection, transport and processing of samples[5]

Under strict aseptic precautions, samples were collected from the patients and transported immediately to the laboratory and sample

processing was done. Samples collected were - sequestrum and fragments of excised tissue removed during surgery or curetting from infected sinuses, three swabs from the sinus tract - one for direct Gram stain, acid-fast stain, and KOH mount, second for aerobic bacterial and fungal culture and third for bedside inoculation into Robertson's cooked meat broth, pus.

Processing of samples

Direct smear examination

Using standard laboratory techniques, pus, exudates, and swabs were subjected to the following microscopic examination, Gram stain, 10% potassium hydroxide mount,[6] acid-fast stain by Ziehl–Neelsen method as per protocol.

Culture

The samples were plated onto the following media. 5% sheep blood agar, chocolate agar, Macconkey agar, Cooked-meat broth, and sabouraud dextrose agar. All the inoculated plates except cooked meat broth were incubated at 37°C under aerobic condition and in a carbon dioxide-enriched atmosphere. Plates were evaluated for growth at 24 and 48 h and discarded after 5 days except for sabouraud dextrose agar which was kept for 4 weeks.

Interpretation

Interpretation of bacterial cultures[5]

After 24 h of incubation, identification of bacteria was done by studying the morphology of colony, Gram stain, motility, catalase, and oxidase tests. Single colony was taken and subjected to a battery of tests along with the controls. Test include in bacterial cultures are oxidase, catalase, coagulase, slide coagulase, tube coagulase, indole, methyl red, Voges-Proskauer, citrate utilization test, nitrate reduction, urease, sugar fermentation, O-F test, triple sugar iron, phenylalanine deaminase, phosphate test, bile esculin, hydrolysis, LAO decarboxylases, antimicrobial susceptibility test, and Kirby-Bauer Disc Diffusion. Test was done as per protocol.

The following standard strains were used:

1. *Staphylococcus aureus* - ATCC 25923.
2. *Escherichia coli* - ATCC 25922.
3. *Pseudomonas aeruginosa* - ATCC 27853.

Detection of β lactamase enzymes in Gram-negative bacilli (GNB)

Extended spectrum lactamases (ESBL's)

ESBL's are classified under in Bush Class A β-lactamases which are capable of hydrolyzing penicillins - oxyiminocephalosporins and monobactams (Aztreonam) and inhibited by β lactamase inhibitors (clavulanic acid, sulbactam and Tazobactam) but have no detectable activity against cephamycins or carbapenems (Imipenem, Meropenem).

Double disk diffusion synergy test

24-h young culture was used for this test. 3–4 colonies from 24 h culture were inoculated into 5 ml of nutrient broth to match 0.5 Macfarland turbidity standard. Lawn culture of the test organism should be made on MHA plate. Two discs Ceftazidime and Ceftazidime in combination with clavulanic acid were placed. The plate is incubated at 35°C for 16–18 h.

Interpretation

A >5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.[7,8]

Phenotypic confirmatory double-disk test (PCDDT), ESBL detection by E test strip, determination of MIC, and detection of methicillin resistance in *S. aureus* by disc diffusion method tests were also performed.

MIC for detecting vancomycin resistance

1. Cation-adjusted Mueller Hinton Broth (PH 7.2–7.4) was used.
2. Preparation of stock antibiotic solution.

Antibiotic stock solution was prepared using the formula.

1000/P X V X C= W

Where, P = Potency of the antibiotic in relation to the base (for vancomycin, P = 950/1000 mg HiMedia).
V = Volume of the stock solution to be prepared (10 ml).
C = Final concentration of the antibiotic solution (1024 µg/ml).
W = Weight of the antibiotic to be dissolved in volume V.

Dilution of antibiotics and inoculum preparation for the test and ATCC control were performed as per the protocol.

Interpretation of fungal culture

Samples were inoculated onto two SDA slants and were incubated at two different temperatures, 25°C and 35°C. These slants were inspected daily during the 1st week and twice weekly during the next 3 weeks for growth. In the fungal culture macroscopic appearance, microscopy analysis by Gram staining, LCB test and germ tube test were also performed as per the protocol.

Chromagar media[9]

It is a rapid, plate-based test for the simultaneous isolation and identification of various Candida species.

Sugar fermentation

Biochemical tests like sugar fermentation were done for identification of yeast isolate Glucose, Maltose, Sucrose, Lactose, Galactose, and Trehalose sugars (2%) were used.

Determination of MIC by microbroth dilution method[6]

As per the guidelines of CLSI, the test was performed. For water-insoluble drugs, dimethyl sulfoxide was the solvent used.

Media used - RPMI 1640. Varying concentrations of the drugs were tested.

ATCC *Candida albicans* ATCC 90028 was used for quality control of the test.

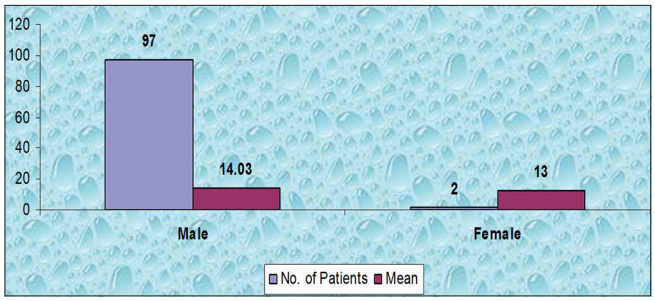

Figure 1: Correlation of sex and duration of illness

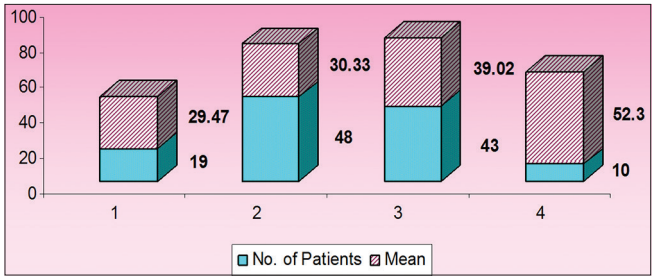

Figure 2: Correlation of age and duration of illness

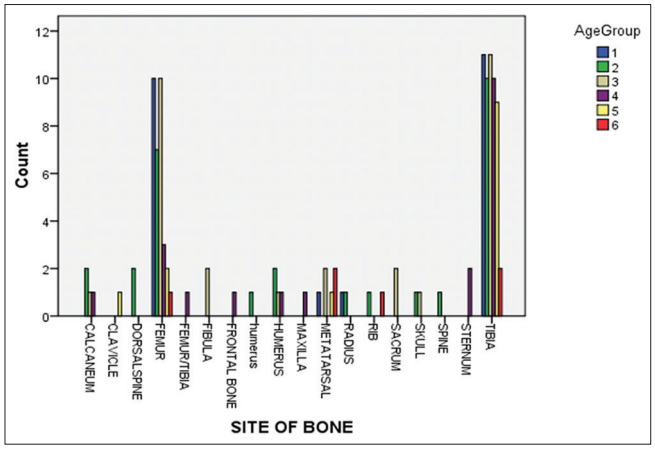

Figure 3: Site of bone*Age group cross tabulation. $P = 0.140$. There is no statistical significance exists among different age groups with respect to the bone site

Culture for M. tuberculosis

All the samples were screened for the presence of acid-fast bacilli by Ziehl Neelsen method of acid-fast staining. Few samples were sent to tuberculosis Research Centre, Chetpet for culture and sensitivity.

Results

This study was conducted in the Institute of Microbiology in association with the Institute of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai-600 003.

In the present study, there were 97 males and 23 females. There was no significant difference in the mean duration of illness among males and females (14.03±0.767 vs. 13.0±9.20; P=0.574) [Figure 1]. We also found that mean age of male and female was 35.94 and 31.78 years respectively. There was no significant difference in the sex-based distribution of patients when compared between the age category [Table 1].

We also found that the majority of the patients (40%) have duration of illness of 7-12 months followed by 13-24 months (35.8%) [Tables 2 and 3].

We then compared mean age on the basis of duration of illness. We observed that mean age was significantly different among the patients had illness duration between 2-6 months (group 1), 7-12 months (group 2), 13-24 months (group 3), and 25-36 months (group 4) [Figures 2 and 3, Table 4].

[Figure 4 and Table 10] shows the organisms isolated in chronic osteomyelitis in 106 aerobic bacterial isolates. We found that 56.6% organisms were Gram positive and among them Staphylococcus aureus was the most common. While among 43.4% Gram negative organisms, Pseudomonas aeruginosa was the most common organism 57 patients (47.5%) had discharge from sinuses as the presenting symptom. The other patients had pain, low-grade fever and swelling as the presenting symptoms. Among the samples collected from them, 57 were collected as discharge from sinuses, 41 (34.1%) as sequestrum and 22 (18.3%) as an intraoperative collection of pus. Discharge from sinuses peroperatively followed by sequestrum postoperatively was collected from 7 patients [Table 7].

Among 120 cases studied, culture positivity was seen in 100 patients (83.3%) [Table 8]. Out of 100, 92 (76.6%) were grown as pure culture (monomicrobial). 9 (7.5%) showed mixed growth (polymicrobial), 20 (16.6%) showed no growth [Table 9].

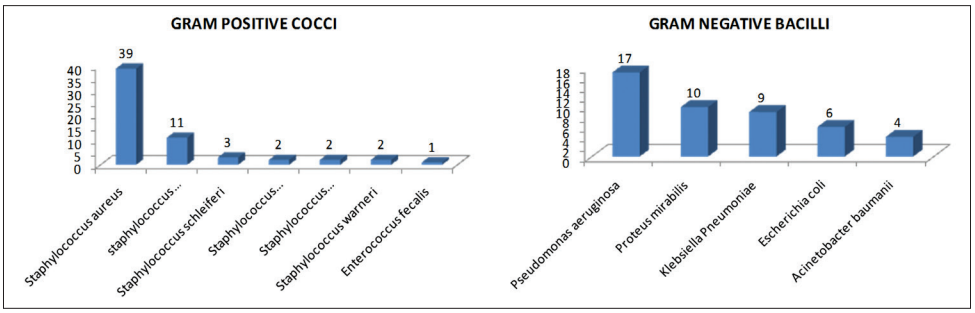

Figure 4: Organisms isolated in chronic osteomyelitis

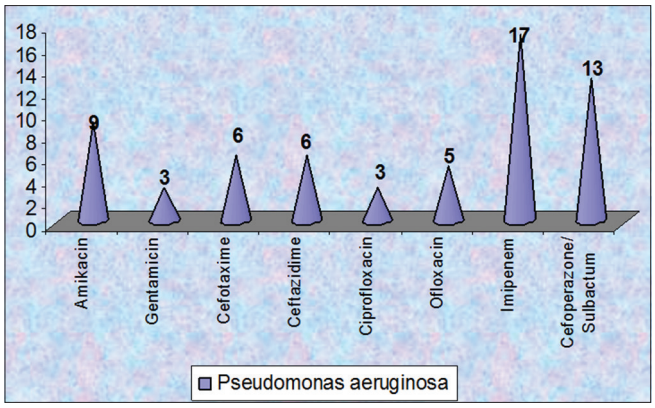

Figure 5: Antimicrobial sensitivity patterns of Gram-negative bacilli

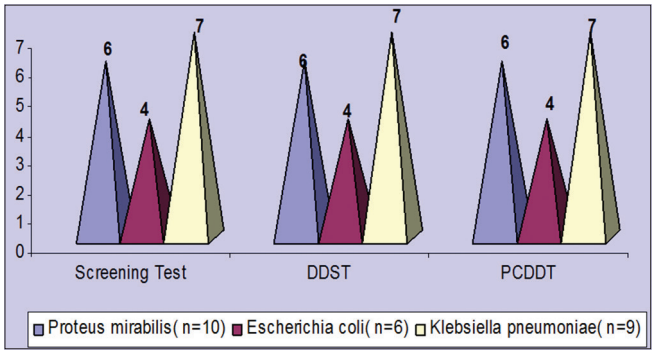

Figure 6: Detection of extended-spectrum beta-lactamases producers among the Gram-negative bacilli

Pathogens isolated were monomicrobial in 92 cases, polymicrobial in 8 cases. Of the 63 sequestrum/intraoperative collection of pus and fluids, 59 were monomicrobial, and only one was polymicrobial. *M. tuberculosis* and *Candida tropicalis* were isolated only from sequestrum samples. Of the 57 swabs, 33 were monomicrobial and 7 were polymicrobial.

Table 11 shows S. aureus and P. aeruginosa were the most common isolated in mixed infections in 37.5% followed by S. schleiferi and E. fecalis (12.5%).

All Gram positive organisms showed 100% sensitivity to rifampin. All Gram positive organisms except one strain of S. aureus were sensitive to vancomycin. S. schleiferi and E. fecalis were totally resistant to fluoroquinolones [Table 12].

Most of the gram negative bacilli showed high level resistance to third generation cephalosporins. All Gram negative organisms showed 100% sensitivity to imipenem. Except Pseudomonas aeruginosa and Escherichia coli all Gram negative organisms showed good sensitivity to cefoperazone sulbactum [Figure 5 and Table 13]. Figure 6 shows detection of extended-spectrum beta-lactamases producers among the Gram-negative bacilli.

Among the 106 aerobic bacteria isolated, 43 (40.5%) were multidrug resistant.

Table 1: Age and sex distribution

Age (years)	Number of patients		Total *n*=120 (%)
	Male *n*=97 (%)	Female *n*=23 (%)	
<20	18 (18.5)	5 (21.7)	23 (19.1)
21–30	21 (21.6)	7 (30.4)	28 (23.3)
31–40	25 (25.7)	5 (21.7)	30 (25)
41–50	18 (18.5)	2 (8.6)	20 (16.6)
51–60	9 (9.2)	4 (17.3)	13 (10.8)
61–70	6 (6.1)	0	6 (5)

Chi-square: 4.471, *P*=0.484 (not significant). The mean age of male is 35.94. The mean age of female is 31.78. Out of 120 patients, 30 patients belonged to age group 31–40 years (25% of total cases), 28 patients belonged to the age group 21–30 years (23% of total cases)

Table 2: Duration of illness

Duration in months	Number of patients *n*=120 (%)
2–6	19 (15.8)
7–12	48 (40)
13–24	43 (35.8)
25–36	10 (8.3)

Most patients (40%) were commonly affected between 7 and 12 months after injury. The patients followed this 13–24 months (35.8%) who developed infection between 13 and 24 months after injury

Table 3: Correlation of sex and duration of illness

Duration actual months	Sex	*n*	Mean	SD	SEM	Significance
	Male	97	14.03	7.555	0.767	*P*=0.574
	Female	2	13.00	9.200	1.918	

The mean duration of illness in males is 14.03. *P*=0.574 [not significant]. The mean duration of illness in females is 13.00. SD: Standard deviation, SEM: Standard error mean

Table 4: Correlation of age and duration of illness

Groups	*n*	Mean±SD
1	19	29.47±16.473
2	48	30.33±10.041
3	43	39.02±15.056
4	10	52.30±12.962
Total	120	35.14±14.772

Groups: 1-duration of illness 2–6 months, 2-duration of illness 7–12 months, 3-duration of illness 13–24 months, 4-duration of illness 25–36 months. SD: Standard deviation

Coagulase negative staphylococci (54.5%) exhibit more resistance to methicillin than S. aureus (48.7%). Among Enterobacteriaceae, Klebsiella showed higher level of ESBL production (77.7%) [Table 14]. Table 15 shows detection of extended-spectrum beta-lactamases producers among the Gram-positive bacilli.

Table 16 shows the drug susceptibility pattern of different antibiotics against M. tuberculosis.

Among 39 *S. aureus*, 38 strains showed sensitivity while one strain showed intermediate sensitivity against vancomycin [Table 17].

Table 18 shows sensitivity of different antifungal agents (fluconazole, amphotericin B, itraconazole, and voriconazole) against C. tropicalis. C. tropicalis was sensitive to all the agents.

Discussion

This study was conducted in the Institute of Microbiology in association with Institute of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai - 600 003. 120 patients with chronic osteomyelitis were included in the study. In this study, 97 (80.83%) were males and 23 (19%) were females. Rao *et al.*[10] also showed nearer percentage of males (77%) in their study. In this study, male to female ratio was (4.2:1) [Table 1]. Turek[11] also showed similar sex ratio distribution of male to female (4:1).

40% of the patients had a duration of the illness from 7 to 12 months. 35.8% of patients had illness ranged from 13 to 24 months. About 55.8% of patients presented within 1 year [Table 2]. This was similar to the study conducted by Srivastava *et al.*, Varanasi, which showed 55%.

In this study, about 50.8% of patients had a compound fracture due to trauma being the most common predisposing factor [Table 5]. This was slightly higher than the study conducted by Dartnell *et al.*[12] The next most common predisposing factor was post-surgical (20%) followed by diabetes mellitus with vascular insufficiency (14.1%), followed by smoking/alcohol (10.8%) as the most common predisposing factor. Gustilo,[13] showed infection rate in the setting of open fracture was 50% which was similar to this study. Suedkamp *et al.*[14] in his retrospective study showed 56% of cases to be post-traumatic, which was slightly higher than this study. Thomas *et al.*[15] stated that prevalence of osteomyelitis in diabetic foot ranges from 10% to 20%. This study showed 14% prevalence.

Tibia (45%) was the most common bone involved, followed by femur (29.1%) [Table 6]. Dartnell *et al.*,[12] Muggeridge *et al.*[16] from Australia, Jayasimha *et al.*[17] from Belgaum also showed a similar pattern of involvement.

Of the samples collected, 63 (52.5%) were sequestrum/per-operative collections of pus and tissue fluids and 57% (47.5%) were swabs [Table 7]. Of the 63 per-operative samples/sequestrum, 59 (93.6%) was monomicrobial, and 1 was polymicrobial. In contrast, an increased number of polymicrobial (12.2%) infections were noted in swabs, though the monomicrobial infection was the most common type even in swabs (57.8%) [Table 9]. Waldvogel *et al.* (1970), Bhattacharya and Gupta,[18] and Arora and Tyagi[19] showed culture positivity of 95.3%, 95.2%, and 95%, respectively. In this study, culture positivity

Table 5: Organisms isolated in chronic osteomyelitis

Organisms	*n* (%)
Aerobic bacterial isolates *n*=106	
GPC	
S. aureus	39 (36.7)
S. epidermidis	11 (10.3)
S. schleiferi	3 (2.8)
S. saprophyticus	2 (1.8)
S. lugdunensis	2 (1.8)
S. warneri	2 (1.8)
E. fecalis	1 (1)
GNB	
P. aeruginosa	17 (16)
P. mirabilis	10 (9.4)
K. pneumoniae	9 (8.4)
E. coli	6 (5.6)
A. baumannii	4 (3.7)
Acid-fast bacilli *n*=1	
M. tuberculosis	1 (1.08)
Fungal *n*=1	
Candida tropicalis	1 (1.08)

The above table shows, 56.6% of isolation of GPC, 43.3% of isolation of GNB, one acid-fast bacillus mycobacterium tuberculosis and one yeast *C. tropicalis* was also isolated in this study. *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, S. schleiferi: Staphylococcus schleiferi, S. saprophyticus: Staphylococcus saprophyticus, S. lugdunensis: Staphylococcus lugdunensis, S. warneri: Staphylococcus warneri, E. fecalis: Enterococcus fecalis, P. aeruginosa: Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis, K. Pneumoniae: Klebsiella Pneumoniae, E. coli: Escherichia coli, A. baumanii: Acinetobacter baumannii, M. tuberculosis: Mycobacterium tuberculosis, C. tropicalis: Candida tropicalis,* GPC: Gram-positive cocci GNB: Gram-negative bacilli

Table 6: Samples collected from the study group

Samples	*n*=120 (%)	LCL in percentage	UCL in percentage
Discharge from sinus	57 (47.5)	38.31	56.82
Sequestrum	41 (34)	25.76	43.38
Intraoperative collection of pus/tissue fluids	22 (18.3)	11.86	26.43

LCL: Lower confidence limit, UCL: Upper confidence limit

Table 7: Culture positivity

Culture	*n*=120 (%)	Lower confidence limit in percentage	Upper confidence limit in percentage
Positive	100 (83.3)	75.44	89.51
No growth	20 (16.6)	10.49	24.56

Table 8: Correlation between type of specimen collected and type of pathogens isolated

Type of pathogen	Swab *n*=57 (%)	Sequestrum/intraoperative collection of pus and tissue fluids *n*=63 (%)	Total *n*=120
Monomicrobial	33 (57.8)	59 (93.6)	92
Polymicrobial	7 (12.2)	1 (1.5)	8
No growth	17 (29.8)	3 (4.7)	20

Chi-square: 21.4, $P=0.0000253<0.0001$ significant

Table 9: Combination of bacterial isolates in mixed infections

Organisms	No of patients *n*=8 (%)
S. aureus and *P. aeruginosa*	3 (37.5)
S. schleiferi and *E. fecalis*	1 (12.5)
S. epidermidis and *K. pneumoniae*	1 (12.5)
P. mirabilis and *E. coli*	3 (37.5)

S. aureus, P. aeruginosa, P. mirabilis, E. coli were commonly isolated in mixed infections. *S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, S. schleiferi: Staphylococcus schleiferi, E. faecalis: Enterococcus faecalis, S. epidermidis: Staphylococcus epidermidis, K. pneumoniae: Klebsiella pneumoniae, P. mirabilis: Proteus mirabilis, E. coli: Escherichia coli*

Table 10: Antimicrobial sensitivity patterns of GPC

Antibiotics	Isolate (%)						
	S. aureus n=39	*S. epidermidis* n=11	*S. schleiferi* n=3	*S. lugdunensis* n=2	*S. saprophyticus* n=2	*S. warneri* n=2	*E. faecalis* n=1
Amikacin	25 (64)	8 (72.7)	3 (100)	1 (50)	1 (50)	1 (50)	1 (100)
Ciprofloxacin	10 (25.6)	3 (27.2)	0 (0)	1 (50)	1 (50)	1 (50)	0 (0)
Chloramphenicol	25 (64.1)	6 (54.5)	1 (33.3)	2 (100)	1 (50)	1 (50)	1 (100)
Cotrimoxazole	23 (58.9)	3 (27.27)	1 (33.3)	1 (50)	0 (0)	1 (50)	0 (0)
Cephalexin	21 (53.8)	3 (27.27)	1 (33.3)	1 (50)	0 (0)	0 (0)	0 (0)
Erythromycin	25 (64.1)	2 (18.18)	1 (33.3)	1 (50)	0 (0)	0 (0)	1 (100)
Penicillin	20 (51.2)	5 (45.4)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
Rifampin	39 (100)	11 (100)	3 (100)	2 (100)	2 (100)	2 (100)	1 (100)
Vancomycin	38 (97.4)	11 (100)	3 (100)	2 (100)	2 (100)	2 (100)	1 (100)

All GPC showed 100% sensitivity to rifampin. All GPC except one strain of *S. aureus* were sensitive to vancomycin. (97.4%) *S. schleiferi* and *E. fecalis* were totally resistant to fluoroquinolones. GPC: Gram-positive cocci, *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, S. schleiferi: Staphylococcus schleiferi, S. lugdunensis: Staphylococcus lugdunensis, S. saprophyticus: Staphylococcus saprophyticus, S. warneri: Staphylococcus warneri, E. fecalis: Enterococcus fecalis*

Table 11: Antimicrobial sensitivity patterns of GNB

Antibiotics	Isolate (%)				
	P. aeruginosa n=17	*K. pneumoniae* n=9	*P. mirabilis* n=10	*E. coli* n=6	*A. baumannii* n=4
Amikacin	9 (52.9)	8 (88.8)	6 (60)	4 (66.6)	2 (50)
Gentamicin	3 (17.6)	5 (55.5)	2 (20)	1 (16.6)	2 (50)
Cefotaxime	6 (35.2)	2 (22.2)	4 (40)	2 (33.3)	1 (25)
Ceftazidime	6 (35.2)	2 (22.2)	4 (40)	2 (33.3)	1 (25)
Ciprofloxacin	3 (17.6)	1 (11.1)	1 (10)	1 (16.6)	1 (25)
Ofloxacin	5 (29.4)	5 (55.5)	2 (20)	1 (16.6)	4 (100)
Imipenem	17 (100)	9 (100)	10 (100)	6 (100)	4 (100)
Cefoperazone/sulbactum	13 (76.4)	8 (88.8)	9 (90)	4 (66.6)	4 (100)

Most of the GNB showed high-level resistance to third generation cephalosporins. All GNB showed 100% sensitivity to imipenem. Except for pseudomonas aeruginosa and Escherichia coli, all GNB showed good sensitivity to cefoperazone sulbactam. GNB: Gram-negative bacilli, *P. aeruginosa: Pseudomonas aeruginosa, K. pneumoniae: Klebsiella pneumoniae, P. mirabilis: Proteus mirabilis, E. coli: Escherichia coli, A. baumannii: Acinetobacter baumannii*

Table 12: Drug resistance mechanism among the pathogens isolated from chronic osteomyelitis

Total pathogens (n=106)	Number of multidrug resistant isolate n=43 (%)	LCL	UCL
S. aureus (n=39)			
MRSA	19 (48.7)	32.42	54.21
VISA	1 (2.5)		
S. epidermidis (n=11)	6 (54.5)		
MRSA		23.38	83.25
P. mirabilis (n=10)	6 (60)		
ESBL	4 (66.6)	26.24	87.8
E. coli (n=6)			
ESBL	4	12.78	66.36
K. neumoniae (n=9)			
ESBL	7 (77.7)	43.79	96.09

Among the 106 aerobic bacteria isolated, 43 (40.5%) were multidrug-resistant. Coagulase-negative staphylococci (54.5%) exhibit more resistance to methicillin than *S. aureus* (48.7%). Among *Enterobacteriaceae, Klebsiella* showed higher level of ESBL production (77.7%). LCL: Lower confidence limit, UCL: Upper confidence limit, *S. aureus: Staphylococcus aureus,* ESBL: Extended-spectrum β lactamases, *S. epidermidis: Staphylococcus epidermidis, E. coli: Escherichia coli,* VISA: Vancomycin-intermediate *Staphylococcus aureus,* MRSA: Methicillin-resistant *Staphylococcus aureus, P. mirabilis: Proteus mirabilis, K. neumoniae: Klebsiellap neumoniae*

Table 13: Detection of ESBL producers among the GNB

Pathogens	Number of positive isolates					
	Screening test		DDST		PCDDT	
P. mirabilis (n=10)	6	60	6	60	6	60
E. coli (n=6)	4	66.6	4	66.6	4	66.6
K. pneumoniae (n=9)	7	77.7	7	77.7	7	77.7

PCDDT: Phenotypic confirmatory disk diffusion test, DDST: Double disk diffusion synergy test. 77.7% of *Klebsiella*, 66.6% of *E. coli,* 60% of *P. mirabilis*, were ESBL producers. ESBL: Extended-spectrum beta-lactamases, GNB: Gram-negative bacilli, DDST: Double disk diffusion synergy test, PCDDT: Phenotypic confirmatory disk diffusion test, *P. mirabilis: Proteus mirabilis, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae*

was 83.3% [Table 8]. This is in close correlation with Dich *et al.*[20] (1975) and Kaur *et al.*[21] who showed 85% and 80%, respectively. The common organism isolated was *S. aureus* (36.7%) [Table 10]. Zulauaga *et al.* 42%(2002), Arora and Tyagi 42%, Henry *et al.* 42.2% (1990), and Kaur *et al.*[21] 43% (2008) also had a lower incidence of *S. aureus* isolation. The isolation rate of *Klebsiella* (8.4%) was slightly higher than Henry *et al.* who showed 6.9% of isolation. Kaur *et al.* and Ako-Nai *et al.* showed 5% and 5.1% of *E. coli* in their studies. Out of 92 pure cultures yielded, one showed the growth of acid-fast bacilli *M. tuberculosis* (0.8%) [Table 10]. Rieder *et al.*[22] have stated that only 1–2% of all tuberculosis cases affect the bone which coincided

Table 14: Drug susceptibility pattern of *M. tuberculosis*

Drugs	Results
Streptomycin	S
Isoniazid	S
Rifampicin	S
Ethambutol	S
Kanamycin	S
Ethionamide	S
Ofloxacin	S

M. tuberculosis: Mycobacterium tuberculosis, S: Sensitive

Table 15: Interpretation of MIC of vancomycin for *S. aureus*

S. aureus n=39	MIC value	Interpretation
38	≤2 µg/l	Sensitive
1	8 µg/l	Intermediate

One strain of *S. aureus* showed intermediate sensitivity to vancomycin. MIC: Minimum inhibitory concentration, *S. aureus: Staphylococcus aureus, S. aureus: Staphylococcus aureus*

Table 16: MIC values of antifungal agents for *C. tropicalis* (n=1)

Drug	MIC value (µg/ml)	Interpretation
Fluconazole	2	S
Amphotericin B	0.25	S
Itraconazole	0.25	S
Voriconazole	0.0625	S

S: Sensitive, MIC: Minimum inhibitory concentration, *C. tropicalis: Candida tropicalis*

with this study. In mixed infections, *S. aureus and P. aeruginosa* were commonly isolated in combinations. *E. coli and Proteus mirabilis* were also isolated in equal proportions [Table 11].

S. aureus which was the most common bacteria isolated in this study showed 100% sensitivity to Rifampin, 97.4% sensitivity to vancomycin, 64% sensitivity to amikacin, chloramphenicol and erythromycin, 51.2% to penicillin, and 51.2% were sensitive to cefoxitin [Table 12]. Raviprakash *et al.*[23] showed 53.3% of MSSA which was slightly higher than the present study (51.2%). Mujumder *et al.* showed 47.10% of MSSA in his study.

48.7% of *S. aureus* and 54.5% of *Staphylococcus epidermidis* were found to be methicillin resistant. Raviprakash *et al.* showed 46.67% of methicillin-resistant *S. aureus*. Among coagulase-negative staphylococci, *Staphylococcus schleiferi* showed 100% resistance to ciprofloxacin and penicillin. Similarly, *Staphylococcus saprophyticus and Staphylococcus warneri* were also totally resistant to cephalexin, erythromycin, and penicillin. Other than *S. epidermidis*, other species of coagulase-negative staphylococci showed multidrug resistance pattern. However, all of them showed 100% sensitivity to vancomycin. Sudharani *et al.* (2004) from Tirupathi also showed 100% sensitivity of CONS to vancomycin.

Vancomycin sensitivity was detected by macrobroth dilution method. 38 out of 39 showed MIC within sensitive range (<2µg/ml). One isolate showed MIC range of 8 µg/ml and it was identified as vancomycin intermediate *S.aureus* (VISA).

Table 17: Predisposing factors

Duration in months	Number of patients n=120 (%)	Lower confidence limits in percentage	Upper confidence limits in percentage
Compound fracture due to road side accidents	61 (50.8)	41.92	59.71
Post-surgical	24 (20.1)	13.25	28.28
Diabetes mellitus with vascular insufficiency	17 (14.1)	8.474	21.71
Smoking/alcoholism	13 (10.8)	5.896	17.81
Hematogenous	5 (4.1)		

Table 18: Site of infection

Bone	No. of Patients n=120	Male n=97	Female n=23	Percentage
Tibia	53	43	10	44.2
Femur	33	24	9	27.5
Metatarsal	6	6	0	5
Humerus	5	5	0	4.1
Calcaneum	4	4	0	3.3
Dorsal Spine	3	2	1	2.5
Frontal Bone	3	1	2	2.5
Fibula	2	2	0	1.6
Radius	2	2	0	1.6
Rib	2	2	0	1.6
Sacrum	2	2	0	1.6
Sternum	2	2	0	1.6
Maxilla	1	1	0	0.8
Clavicle	1	1	1	0.8
Femur/Tibia	1	1	0	0.8
Total	120	97	23	100

Among Gram-negative isolates, *P. aeruginosa* was the most common isolate which showed 100% sensitivity to imipenem, 76.4% to cefoperazone sulbactam, 52.9% to amikacin, and 35.2% to cefotaxime and ceftazidime [Table 13]. Kaur *et al.*[21] from Amritsar, showed 89.5% sensitivity to cefoperazone sulbactam and amikacin which were higher than this study. *P. mirabilis* showed 100% sensitivity to imipenem, 90% to cefoperazone/sulbactam, 60% to amikacin, and 30% to cefotaxime and ceftazidime. Kaur *et al.* from Amritsar, showed 89.5% sensitivity to cefoperazone sulbactam and 60.3% to amikacin which were closely similar to the present study. *Klebsiella pneumoniae* showed 100% sensitivity to imipenem, 90% to cefoperazone/sulbactam and amikacin [Table 13]. *Klebsiella, Proteus,* and *E. coli* were screened for ESBL production and confirmed by PCDDT and DDST. The acid-fast bacilli *M. tuberculosis* was sensitive to isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, ethionamide, and ofloxacin. MIC of Fluconazole, Amphotericin B, Itraconazole, Voriconazole for *C. tropicalis* was determined by microbroth dilution method. MIC of four drugs was within their sensitivity ranges for the isolate.

Conclusion

56.6% were aerobic Gram-positive cocci (GPC) and 43.3% were aerobic GNB. Among GPC, *S. aureus* (36.7%) was the most common pathogen isolated followed closely by *S. epidermidis* (10.5%). All GPC except one were sensitive to vancomycin and rifampin. Among GNB, all were sensitive to imipenem and 90% to cefoperazone sulbactam. Pseudomonas had lower sensitivity (76.4%) to cefoperazone. Various factors in open fracture leading to chronic osteomyelitis each patient has to be routinely monitored after trauma and treatment for developing osteomyelitis. Treatment given in the early stage will prevent dreadful complications and sequelae.

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