ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

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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ABSTRACT

Chronic Osteomyelitis of long bones is often the consequence of an Open, comminuted fracture and inadequately treated infect ion of the fracture site. Rarely it occurs as a complication of acute osteomyelit is now-a-days. The pattern and behaviour of organisms are constantly changing under the pressure of newer antibiotics .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. To study the predisposing factors associated with chronic Osteomyelitis. To study the causative organisms and their antimicrobial susceptibility pattern. To study the resistance pattern in common isolates. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire. Chronic osteomyelitis was conducted to study the Predisposing factors, Etiological agents, Antimicrobial Susceptibility Pattern and Drug resistance pattern among the isolates. Trauma especially road traffic accident with open fracture has been found to be the major predisposing factor. Open fractures leading to Osteomyelitis depends on the type of fracture, the level of contamination, the degree of soft tissue injury and whether local and systemic antimicrobial therapies have been administered. Additional predisposing factors include Post surgical conditions, Diabetesmellitus, Smoking and Alcoholism. The above predisposing factors leads to chronicity of infection, delayed healing and exposure to prolonged antibiotic therapy, resulting in the overgrowth of resistant strains. 56.6% were Aerobic gram positive cocci and 43.3% were Aerobic gram negative bacilli. Among Gram positive cocci, Staphylococcus aureus (36.7%) was the commonest pathogen isolated followed closely by Staphylococcus epidermidis (10.5%).All gram positive cocci except one were sensitive to Vancomycin and Rifampin. Among gram negative bacilli, all were sensitive to Imipenem and 90% to Cefoperazone sulbactum. Pseudomonas had lower sensitivity (76.4%) to Cefoperazone. Multidrug resistance was seen in 40.5% of isolates from chronic Osteomyelitis cases.
Keywords: Antimicrobial susceptibility, osteomyelitis, cross-sectional study, gram-positive cocci.

https://doi.org/10.33887/rjpbcs/2023.14.4.49

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ISSN: 0975-8585

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 [2]. 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) [3]. 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 [4].

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 [5]. 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, and squamous cell carcinoma in scar tissue [6].

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 Department Of Medical Microbiology, Government Cuddalore Medical College, Chidambaram, Cuddalore, Tamil Nadu, India in association with Institute of Orthopaedics, 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 6weeks 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 (Below 12 years)

Collection, transport and processing of samples

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

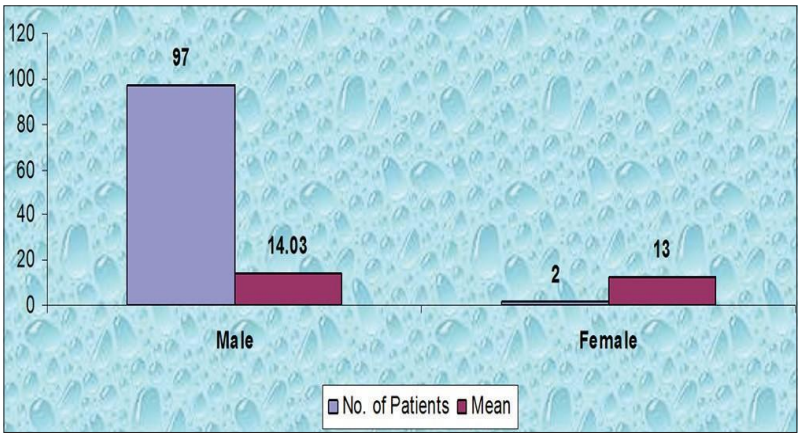
Interpretation of bacterial cultures [5] After 24 h of incubation, identification of bacteria was done by studying the morphology of colony, Gram stain, motility, catalase, and oxidase tests. Single colony was taken and subjected to a battery of tests along with the controls. Test include in bacterial 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

RESULTS

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). In all age groups, males were commonly affected because they were more prone to accidents than females as they do outdoor work, construction work and high altitude work. In this study, 97 were males and 23 were females.

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). 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. 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%). 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). 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 preoperatively followed by sequestrum postoperatively was collected from 7 patients. Among 120 cases studied, culture positivity was seen in 100 patients (83.3%). 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.

Figure 1: Correlation of sex and duration of illness



ISSN: 0975-8585

Figure 2: Correlation of age and duration of illness

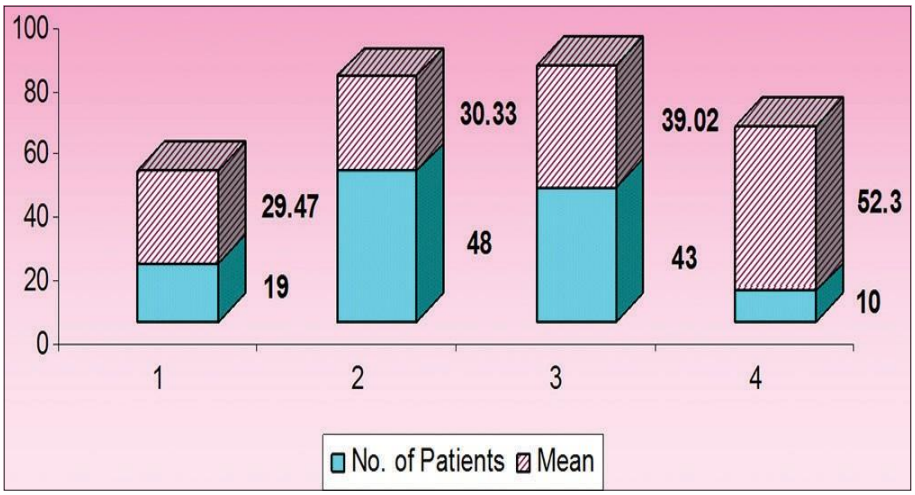


Figure 3: Site of bone*Age group cross tabulation. *P* = 0.140. There is no statistical significance exists among different age groups with respect to thebone site

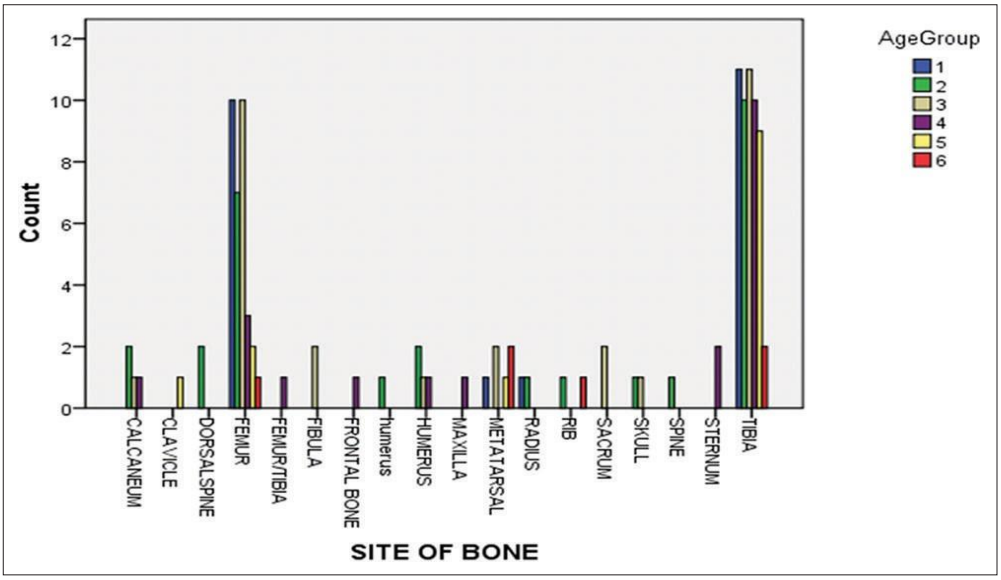


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Figure 4: Organisms isolated in chronic osteomyelitis

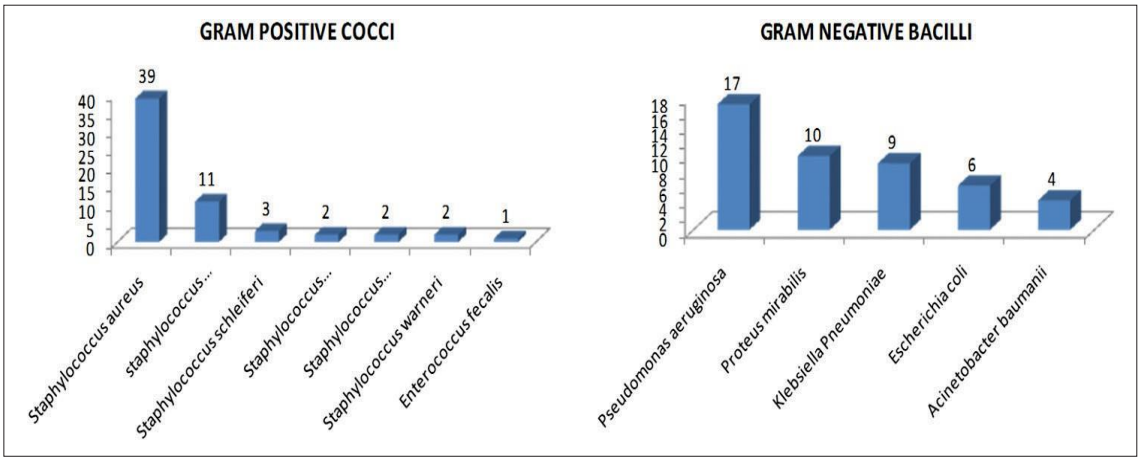


Figure 5: Antimicrobial sensitivity patterns of Gram-negative bacilli

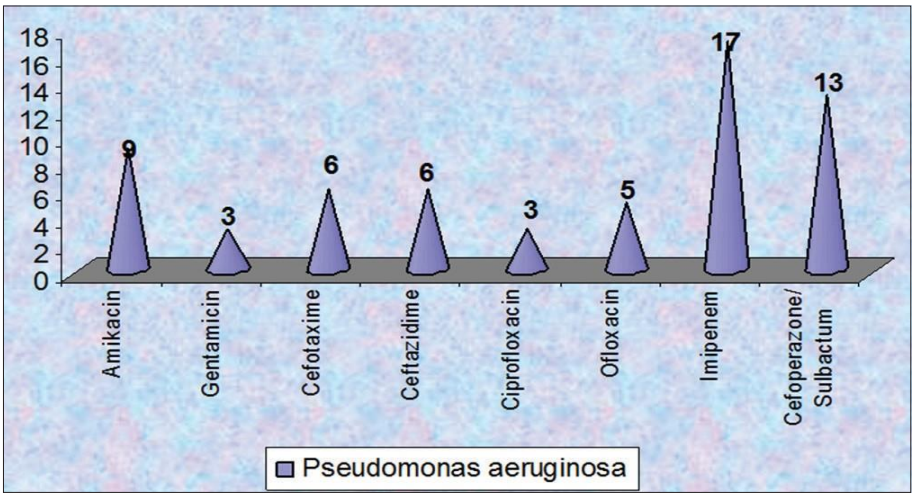
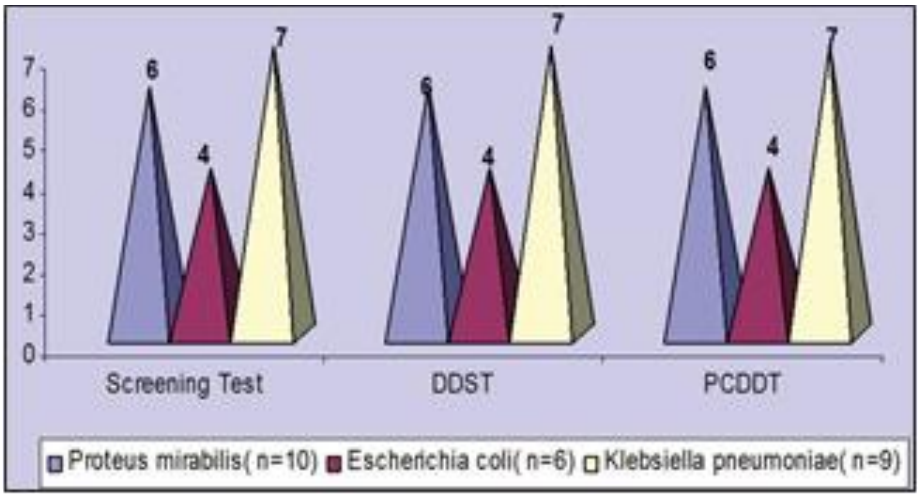


Figure 6: Detection of extended-spectrum beta-actamases producers amongthe Gram-negative bacilli



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DISCUSSION

COSM was found more in 21-30 and 31-40 years of age group. In the present study, prevalence of OSM was found to be more in males (68.70%) as compared to females (31.30%) with Male to Female ratio was 2.2:1. Similar result was observed by Carvalho VC et al [11] affecting 63.4% males and 36.6% females with OSM. Similar result was obtained by Waldvogel et al (1970)9 with Male to Female ratio was 2:1, Hassani U et al [12] observed male predominance with Male to Female ratio was 1.95:1, and Wadekar MD et al [13] observed Male to Female ratio was 2.7:1. While Izadi et al [14] observed, prevalence of COSM was (81.7%) in males and (18.3%) in females, Ali M et al [15] noted incidence of OSM in males (84%) and in females (16%). In the present study, bones involved in AOSM were femur (70.59%), tibia (11.76%), humerus (11.76%). whereas in COSM were femur (59.18%), tibia (30.61%). Similarly, Ali M et al [15] observed bones involved in COSM were femur (46%), tibia (30%), humerus (4%). Wadekar MD et al [13] observed femur (48%), tibia (23%), humerus (9%) and ulna (4%). Whereas, Izadi et al [14] found COSM mostly affected tibia (33%), femur. Out of the 16 culture positive samples from AOSM, 20 organisms were isolated. S. aureus leads followed by K. pneumoniae and A. baumannii. Carvalho VC et al [11] found A. baumannii (21.4%), P. aeruginosa (19.8%), K. pneumoniae (8.2%) and E.coli (4.9%) in AOSM. Mirnejad R et al [16] found S. aureus (55.9%), Klebsiella spp. (14.8%), Coagulase negative Staphylococcus (7.4%) Acinetobacter spp. (3.7%) as cause of AOSM. Craigen MAC et al [17] observed S. aureus (88.2%), S. pyogenes (3.8%) and E.coli (0.6%) isolates in AOSM. In the present study, out of the 85 culture positive samples from COSM, 96 organisms were isolated. Staphylococcus aureus 40 (41.67%) was the most common isolated followed by K. pneumoniae and A. baumannii. Among the fungal agents, Candida albicans was isolated. In this study, S. aureus was the most predominant isolate as also seen in other studies such as Rahbar M et al (2010)18 as (26.3%), Wadekar MD et al [19] as (32.9%), Izadi M et al [14] as 48.9%, Wirbel R et al [20] as (74%). Ali M et al [15] observed S. aureus (58%), Coagulase negative Staphylococcus (14%). Thus, Staphylococcus aureus has been found to be the major etiological agent in our study, which is similar to other studies. Three cases in the present study were of sickle cell disease presenting with chronic osteomyelitis. Of which two cases yielded Salmonella Typhi from the pus aspirate and in one case Staphylococcus aureus was isolated. Thanni LOA et al isolated 304 cases of COSM with sickle cell disease, out of which 129 were S. Typhi and 82 were S. aureus. All the isolates of S. aureus showed 100% sensitivity to Vancomycin, Amikacin, Netilmicin, Chloramphenicol and resistant to Penicillin G, Rifampicin. Ali M et al [15] found S. aureus was 100% sensitive to Vancomycin, highly resistant to Cephalosporins, least sensitive to Ciprofloxacin, Amikacin and Gentamicin. Izadi et al (2012)14 found S. aureus was most sensitive to Vancomycin (97.7%) and least sensitive to Penicillin (7%). Wadekar MD et al [13] observed that in COSM S. aureus was (100%) sensitive to Vancomycin, (97.1%) to Linezolid and resistant to Ciprofloxacin, Erythromycin, Gentamicin, Clindamycin and Amikacin. Increasing resistance to Penicillin has been observed over the years. The studies in literature clearly show this resistance pattern. In the study done by Izadi et al [14] 93% of the S. aureus were found resistant to penicillin. In the present study, out of 48 isolates of S. aureus, 37.50% were MRSA, 6.25% were ICR, 14.58% were MRSA + ICR found. By E-strip method, 95.83% of the S. aureus isolates were sensitive and 4.17% were intermediate sensitive (IS) to Vancomycin. Ali M et al [15] observed 42% of MRSA strains in COSM. Wadekar MD et al [19] found 40% MRSA in COSM. Izadi M et al [14] found 75% of MRSA in COSM. Whereas, Wirbel R et al [20] observed 10% of MRSA in COSM. The probable reason for this can be, the difference in location of samples and consequently difference in strains.14 K. pneumoniae showed 100% resistant to Ampicillin, followed by Amoxyclav, Cefuroxime, Cefoxitin and Ceftazidime. Similarly, Ali M et al [15] observed K. pneumoniae was 100% resistant to Ampicillin, followed by Gentamicin and Cefuroxime. 100% sensitive to Imipenem followed by Amikacin, Ciprofloxacin, Cefotaxime and Ceftazidime. Whereas, Wadekar MD et al [13] found K. pneumoniae was 100% resistant to Ampicillin, Gentamicin, followed by Cefotaxime, Amikacin and 78.5% sensitive to Imipenem. A. baumannii were 100% sensitive to Polymyxin B (300), followed by Imipenem, Aztreonam and Amikacin. A. lwoffii was sensitive to Gentamicin, Amikacin, Tobramycin, Ciprofloxacin and Polymyxin B (300). Carvalho VC et al [11] observed that A. baumannii was sensitive to Imipenem (62%), Gentamicin (54%) and Amikacin (27%). P. aeruginosa isolates were 90% sensitive to Imipenem, 60% to Ciprofloxacin, Piperacillin, Aztreonam and 40% sensitive to Piperacillin-Tazobactam, Amikacin, Gentamicin. Ali M et al [15] reported that Pseudomonas aeruginosa, was 100% sensitive to Imipenem, 60% to Ceftazidime, 40% to Amikacin and Ciprofloxacin. Wadekar MD et al [13] observed that Pseudomonas aeruginosa, was 76.40% sensitive to Imipenem, 58.80% to Amikacin. In the present study, ESBL rates for K. pneumoniae isolates is 20 %, whereas Wadekar MD et al [19] observed that ESBL rates were 85.7% for K. pneumoniae isolates and 75% for

Enterobacter isolates in their study. In this study we observed, 4 (6.57%) AmpC producers, while AmpC production reported by Rawat et al (2013)22 was 20.8% and 36.6% by Haider et al [23] All these were Inducible AmpC producers. However, Haider et al have found only 21.7% Inducible AmpC producers and 78.3% non-inducible AmpC producers, which is an alarmingly high percentage of derepressed mutants. Co-expression of different beta lactamase enzymes were found in the present study. There were three strains showing ESBL and AmpC co-production (4.92%). Rawat et al have found that ESBL and AmpC were co-produced by 25% isolates in their study. Out of 13 Acinetobacter spp. isolated, ESBL was found in 1 (7.69%). Goel V et al (2013)24 found that 17.9% of A. baumannii to be ESBL producers. In our study we observed that 23.08% A. baumannii and 10% P. aeruginosa were MBL producers, whereas Goel V et al found that 48.72% A. baumannii and 53.85% P. aeruginosa were plasmid mediated MBL enzyme producing strains detected by Imipenem-EDTA disk method. OSM resulting from fungi is uncommon.25 In the present study, 2 (1.72%) isolates of Candida albicans were isolated from cases of OSM.

CONCLUSION

Osteomyelitis is found to be highest in third decade, with the males being predominantly affected. Acute osteomyelitis is predominantly seen in children, whereas chronic osteomyelitis in adults. Even though Staphylococcus aureus has always remained the most common etiological agent of osteomyelitis, increasing infections due to Gram negative bacilli and even poly-microbial infections are gaining importance. MRSA infection is known to increase postoperative complications. Introduction of MBL or carbapenemase production in Gram negative bacilli is a matter of great concern. Timely knowledge of aetiology and antimicrobial resistance pattern of osteomyelitis isolates can help in rational use of antibiotics and control of drug resistance.

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