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Bacteriological Profile of Chronic Osteomyelitis with Special Reference to Antibiotic Resistance Mechanisms/Patterns – A Cross-sectional Prospective Study from Tertiary Care Hospital in Central India

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Authors' contributions

This work was carried out in collaboration among all authors. Author AP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PS managed the analyses of the study. Author AJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Chronic osteomyelitis is the infection and inflammation of the bone. Inappropriate use of antibiotics and multidrug resistance has raised the morbidity and mortality rate in chronic osteomyelitis. This study aims to determine the bacterial profile and antimicrobial susceptibility patterns of chronic osteomyelitis with special mention to various resistant mechanisms.
Methods: The study is a prospective design. Hundred (100) clinically diagnosed cases of chronic osteomyelitis of all age group and both sex admitted in a tertiary care hospital at central India, in

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one year were included. Samples like pus, sinus discharge or exudates were collected aseptically and sent for microbiological investigation. Antimicrobial susceptibility of bacterial isolates to the commonly used antibiotics was done by using modified Kirby Bauer disc diffusion method.

Results: The aerobic bacteriological study of chronic osteomyelitis showed *Staphylococcus aureus* is being continued to be major etiological agent followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Gram-positive isolates were sensitive to linezolid, teicoplanin while gram-negative isolates were sensitive to colistin, ciprofloxacin in the majority. The disease occurs mostly due to traumatic injuries commonly affecting the middle age group. In present study prevalence of methicillin-resistant *Staphylococci aureus* and β Lactamase producing (ESBL, Amp-C and MBL) gram-negative bacilli is found to be on the higher side.

Conclusion: It has been the major cause of morbidity for a long time. The emerging multidrug-resistant strain is a major concern for the treatment. Identification of causative isolates and using a judicious selection of antibiotics will help the clinician in starting the empirical treatment accordingly would limit the multidrug resistance strains in the hospital as well as the community.

Keywords: Chronic osteomyelitis; Staphylococcus aureus; antibiotic resistance; methicillin-resistant Staphylococcus aureus.

1. INTRODUCTION

Infection and inflammation of the bone are called osteomyelitis [1]. Although bone cannot be normally colonized by bacteria events such as trauma, ischemia, surgery, the presence of foreign particles, or prostheses placement may disturb the bone integration and eventually leading to the onset of bone infection. A long bone is the most common site for osteomyelitis [2]. Hematogenous spread after bacteremia can also result in osteomyelitis [3]. Osteomyelitis usually starts as an acute infection, but it may eventually turn into a chronic condition [4].

Osteomyelitis is differentiated according to the etiology, pathogenesis, and degree of bone involvement, as well as age and the immune condition of the patient [5]. *S. aureus* is the most common causative microorganism, while other pathogens are less frequently found including *Enterococcus spp., Streptococcus spp., Pseudomonas aeruginosa, Enterobacter spp., Mycobacterium spp.*, anaerobes, and fungi, specifically *Candida* spp. [6].

S. aureus is commonly responsible for both acute and chronic osteomyelitis by forming a biofilm, which rapidly develops antimicrobial resistance and expression of virulence factors, regardless of patients' immune status [7]. In these cases, surgical intervention is necessary to control the infection. Antimicrobial resistance results in a delay in management, increasing the risk of chronicity of the disease and of peri-prosthetic infection [8].

For acute osteomyelitis, the duration of treatment is 4-8 weeks. Osteomyelitis is not common in developed countries, while it still exists in developing countries and the morbidity appears worse in the lower socioeconomic groups [9]. The incidence of chronic osteomyelitis has been lowered due to the rapid diagnosis, newer antibiotics and modern treatment modalities but still, osteomyelitis is a major problem due to the rise of various multi drug-resistant strains and the prevalence of various predisposing conditions such as diabetes mellitus [10].

The present study was conducted to determine the prevalence of aerobic bacteriological profile of chronic osteomyelitis and various epidemiological/risk factors associated with chronic osteomyelitis. The antibiotic susceptibility pattern of the clinical isolates was done to guide our clinicians to establish empirical treatment guidelines in our hospital.

2. MATERIALS AND METHODS

In this study, a total of 100 clinically diagnosed cases of chronic osteomyelitis of all age group and both sex admitted in orthopaedic ward of Sri Aurobindo Institute of Medical Sciences, a teaching and tertiary care hospital at Indore, Madhya Pradesh, in the duration of one year, January.

2016 to December 2016 were included with the written consent of patients. The inclusion criteria comprised of the aerobic bacteriological profile while anaerobic bacteriological profile, mycobacterium and fungal profile were excluded.

Informed consent from each patient documenting name, age, sex, clinical illness

including chief complains, the previous history of illness, duration of symptoms and predisposing factors were taken.

Samples like pus, sinus discharge or exudates were accepted. Two swabs for each specimen was collected aseptically. After the collection of specimen, they were immediately processed in microbiology laboratory according to the standard operative procedure [11,12].

The first swab was used for direct gram staining and was examined under oil immersion objective for the presence of inflammatory cells and bacteria while second swab was inoculated on blood agar, mac-conkey agar and nutrient agar [13]. All plates were aerobically incubated overnight at 37°C. The isolated organisms were identified according to morphology in gram staining, colony characteristics and biochemical properties [13,12].

Antimicrobial susceptibility of bacterial isolates to the commonly used antibiotics was done by using modified Kirby Bauer disc diffusion method on Mueller Hinton agar equivalent to 0.5 McFarland standard by using following antibiotics & potency [13,12]. The given Table.1 shows total antibiotics and their strength used for various isolates in the present study.

2.1 Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA)

As per CLSI guidelines, for determination of methicillin resistance, cefoxitin (30 µg) disk was used on Mueller-Hinton agar using McFarland 0.5, incubated at 35°C for 18 hours. In case of *Staphylococcus aureus* isolates ≤ 21 mm zone size was considered as methicillin-resistant *Staphylococcus aureus* (MRSA) and for *coagulase-negative staphylococci* (CONS) ≤ 24 mm zone size were considered as methicillin-resistant CONS (MRCONS) [14,15].

2.2 Detection of Extended Spectrum β-Lactamase (ESBL)

2.2.1 Phenotypic confirmatory disk diffusion test

As per CLSI recommendation, Mueller Hinton agar (MHA) was inoculated with test isolates using standard inoculum (0.5 McFarland) incubated at 37ºC for 18 hours. Disc of ceftazidime and cefotaxime (30 µg each) alone and in combination with 10 µg of clavulanic acid were applied on it with individual disks being placed at least 3 cm center to center apart [16]. An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid than ceftazidime or cefotaxime alone was interpreted as ESBL producer [17].

2.3 Detection of Amp-C

2.3.1 Cefoxitin-Cloxacillin Double Disc Synergy test (CC-DDS)

This test was conducted based on the inhibitory effect of cloxacillin on Amp-C production. The isolates were inoculated on Mueller Hinton agar using McFarland 0.5 and incubated at 35°C for 16 to 18 hours. Cefoxitin/cloxacillin disks (30/200 µg) and cefoxitin disk (30 µg) were used in this study. A difference of ≥ 4 mm in the inhibition zones of cefoxitin/cloxacillin and cefoxitin disks was an indication of Amp-C production [18].

2.4 Modified Hodge Test

The antimicrobial susceptibility to carbapenems was done by Kirby Bauer disc diffusion method. according to CLSI recommendations.

GNB isolates, which showed an inhibition zone diameter of ≤ 19 mm to meropenem or imipenem or ertapenem, were tested for carbapenemase production by modified Hodge test. Dilution of the *Escherichia coli* ATCC 25922 in 5 ml of broth or saline was prepared using 0.5 McFarland. Lawn culture on a Mueller Hinton agar plate was done using 1:10 dilution. In the center of the test, 10 µg meropenem susceptibility disk was placed.

Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35 ± 2°C for 16–24 hours in ambient air. As per CLSI guideline, the presence of cloverleaf type of indentation at the intersection of the test organism and ATCC *E. coli* 25922, within the zone of inhibition of meropenem susceptibility disc was interpreted as a positive result for modified Hodge test [19,20].

2.5 Detection of Metallo β -Lactamase (MBL)

Carbapenem resistant clinical isolates were taken as positive for MBL phenotypic screening test. MBL production was detected by the imipenem-EDTA combination disc test. Test organisms were inoculated onto plates of Mueller-Hinton agar using McFarland 0.5.

Table. 1. Antibiotics used for various isolates

Antibiotics disc for gram-positive bacteria	Strength of disc	Antibiotics used for gram negative bacteria	Strength of disc
Penicillin-G	10 unit	Ampicillin	10 µg
Erythromycin	15 µg	Amikacin	30 µg
Clindamycin	2 µg	Gentamicin	10 µg
Ciprofloxacin	5 µg	Piperacillin	100 µg
Gentamicin	10 µg	Piperacillin-Tazobactum	100/10 µg
Rifampicin	5 µg	Cefazolin	30 µg
Tetracycline	30 µg	Cefotaxime	30 µg
Linezolid	30 µg	Cefoxitin	30 µg
Teicoplanin	30 µg	Ceftazidime	30 µg
Daptomycin	0.016-256 mcg/ml	Cefepime	30 µg
Vancomycin	0.016-256 mcg/ml	Cefuroxime	30 µg
Cotrimoxazole	1.25/23.75 µg	Aztreonam	30 µg
--	--	Ciprofloxacin	5 µg
--	--	Imipenem	10 µg
--	--	Cotrimoxazole	1.25/23.75 µg
--	--	Colistin	10 µg

Two 10-µg-imipenem disks were placed on the plate, and 10 µl 0.5 M EDTA solution were added to one of them to obtain the desired concentration and incubated for 16 to 18 hours at 35°C. An increase in zone size ≥ 7 mm around the imipenem –EDTA disc compared to imipenem without EDTA was considered as an MBL producing strain [21,22].

3. RESULTS

During the study period (January 2016 to December 2016), total of 100 samples were received in the microbiology laboratory. Out of 100 cases studied, male predominance (79%) was seen compared to (21%) females. Majority of cases affected were found present in middle 41-60 (48%) years age group. The given Table 2 shows the distribution of patients according to gender in relation to the age groups.

Trauma (47%) was the most common predisposing factor followed by post-operative infection (22%) and implants (21%) cases. Other predisposing factor was diabetes mellitus associated with trauma (4%) cases, post-operative infection (3%) cases and also with cases of orthopedic implants (3%). The Fig. 1 shows the distribution of patients according to predisposing factors.

Femur (38%) was most common bone involved in cases followed by tibia (36%). Other bones involved were fibula (7%), ulna in (5%) cases. Humerus and metatarsal in (4%) cases each, while metacarpal (3%), radius (2%) and calcaneum (1%) were least involved bones in this study. The given Table 3 shows the distribution of patients according to bones involved.

Out of 100 sample were studied, in 85 (85%) the culture was positive, while in 15 (15%) the culture was negative. Gram-positive organism (58.4%) was more common compared to gram-negative organism (41. 6%). Mono-microbial flora (69%) were more common then (16%) poly-microbial flora. 42 (36.2%) *Staphylococcus aureus* was most common isolate, followed by 17(14.7%) of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* each, 15 (12.9%) *Escherichia coli*, 8(6.9%) *Klebsiella pneumoniae* and 2(1.7%) *Proteus mirabilis*. The given Fig. 2 showing the distribution of various isolated organisms.

The commonest organism in polymicrobial flora was *Escherichia coli* + *Staphylococcus aureus* 03(18.75) and *Pseudomonas aeruginosa*+ *Staphylococcus epidermidis* 03(18.75).

Antibiotic susceptibility pattern of most of the gram positive isolates was sensitive to linezolid (100%), teicoplanin (100%), vancomycin (100%) followed by daptomycin (88.13%). The given Table 4 is showing various antibiotic susceptibility pattern of gram positive organisms.

Antibiotic susceptibility pattern of most of the gram-negative isolates was sensitive to colistin (100%), ciprofloxacin (71.42%) followed by imipenem (66.66%). The given Table 5 is

showing the antibiotic susceptibility pattern of gram-negative organism.

Methicillin resistant *Staphylococcus aureus* among isolates were 83% out of 42 *Staphylococcus aureus*. Distribution of Extended Spectrum β lactamase (ESBL) producer was 54.76%. Distribution of Amp-C producer was 35.71%. 21.42% were Carbapenemase producer found in total 42 gram negative isolates and out of those, 11.90% were Metallo Beta lactamase producers. Given Table 6 is showing distribution of various resistance mechanisms in isolated organisms.

4. DISCUSSION

Osteomyelitis is characterized by infection of bone caused by bacteria. Chronic osteomyelitis is one of the most persistent diseases among most of the developing countries like India. In the absence of early diagnosis and increase in antibiotic resistance strains causing failure in antibiotic therapy, chronic osteomyelitis has become prominent cause of morbidity. Proper management of chronic osteomyelitis requires careful isolation of microorganisms and regular monitoring of susceptibility pattern of antimicrobial agents.

Hence this present study was done to know the aerobic bacteriological profile of chronic osteomyelitis and antibiotic susceptibility pattern of various isolates along with study of common predisposing factors.

In present study, chronic osteomyelitis was commonly seen in 41-60 (48%) year's age group followed by 21-40 (37%) year's age group which correlates with study conducted by Mita et al. [23] and also with study of Suguneswari et al. [3] conducted in patients with chronic osteomyelitis. There were more number of males in each age group in comparison to the females, showing a male preponderance. In present study out of 100 cases studied 79(79%) were males and 21(21%) were females which were in correlation with Peng et al. [24] and also with study conducted by Mita et al. [23] and Suguneswari et al. [3].

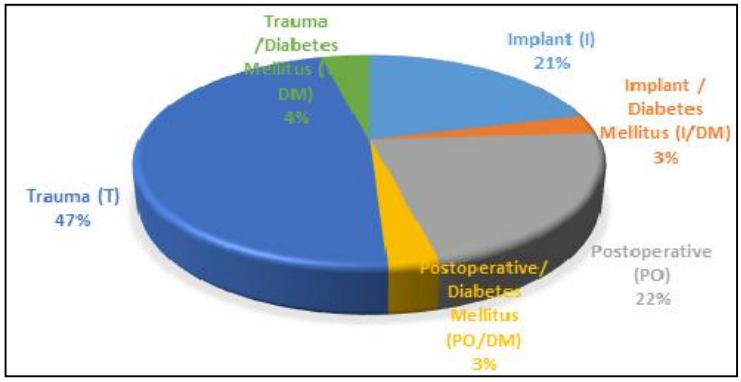

Fig. 1. Distribution of predisposing factors

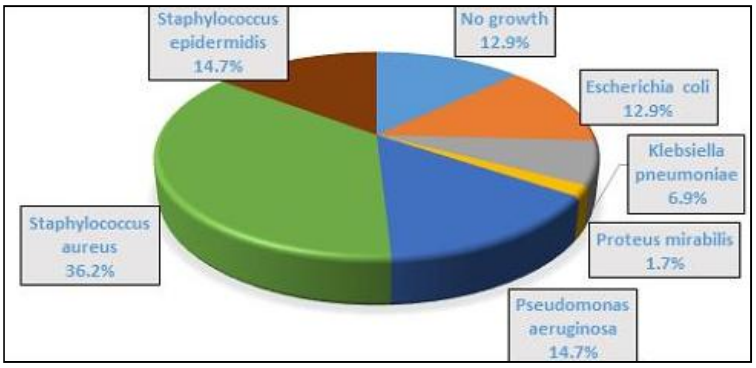

Fig. 2. Distribution of various isolated organisms

Table 2. Distribution of patients according to age and gender (N= 100)

Age group	Female (%)(N=21)	Male (%)(N=79)	Total (%)
<=20 years	01 (4.8%)	02 (2.5%)	03 (3%)
21-40 years	09 (42.9%)	28 (35.4%)	37 (37%)
41-60 years	09 (42.9%)	39 (49.4%)	48 (48%)
61-80 years	02 (9.50%)	10 (12.7%)	12 (12%)
Total	21 (100%)	79 (100.0%)	100 (100%)

Table 3. Distribution according to bones involved (N=100)

Bones involved	No.	Percentage
Calcaneum	1	1
Femur	38	38
Fibula	7	7
Humerus	4	4
Metatarsal	4	4
Metacarpal	3	3
Radius	2	2
Tibia	36	36
Ulna	5	5
Total	100	100

Table 4. Showing antibiotic susceptibility pattern of gram positive organism

Antibiotic drugs	*S. aureus* (N=42) (%)	*S. epidermidis* (N=17) (%)
Penicillin-G	0(0)	(0)
Erythromycin	24(56.10)	11(64.71)
Clindamycin	23(53.66)	8(47.06)
Ciprofloxacin	14(34.15)	11(64.71)
Gentamycin	24(56.10)	15(88.24)
Rifampicin	36(87.80)	14(82.35)
Tetracycline	37(90.24)	14(82.35)
Linezolid	42(100)	17(100)
Teicoplanin	42(100)	17(100)
Daptomycin	37(90.24)	15(88.24)
Vancomycin	42(100)	17(100)
Cotrimoxazole	7(17.07)	5(29.41)

Table 5. Showing antibiotic susceptibility pattern of gram negative organisms

Antibiotics drugs	*E. coli* N=15 (%)	*K. pneumoniae* N=08 (%)	*P. mirabilis* N=02(%)	*P. aeruginosa* N=17 (%)
Ampicillin	3(20)	2(25)	1(50)	1 (5.88)
Amikacin	8(53.33)	5(62.50)	1(50)	11(64.71)
Gentamicin	11(73.33)	3(37.5)	1(50)	9(52.94)
Pipperacillin-Tazobactum	3(20)	0(0)	1(50)	1(5.88)
Ceftazidime	4 (26.37)	0(0)	1(50)	3 (17.65)
Cefepime	5 (33.33)	0(0)	1(50)	3 (17.65)
Aztreonam	6 (6.67)	0(0)	1(50)	1 (5.88)
Imipenem	10(66.67)	7 (82.5)	0(0)	11 (64.71)
Ciprofloxacin	10(66.67)	6 (75)	1(50)	13 (76.47)
Cotrimoxazole	10(66.67)	5 (62.50)	1(50)	--
Colistin	15(100)	08 (100)	02(100)	17 (100)

Table 6. Distribution of various resistance mechanisms in isolated organism

Distribution of methicillin resistant *Staphylococcus aureus* (N=42)			
Organism	**Total isolate no.**	**MRSA Isolate**	
S. aureus	42	35 (83%)	
Distribution of Extended Spectrum Beta Lactamase (ESBL) Producer (N=42)			
Organisms	**Total isolate no.**	**ESBL Isolate**	
Escherichia coli	15	9 (21.42%)	
Klebsiella pneumoniae	8	5 (11.90%)	
Proteus mirabilis	2	0 (0%)	
Pseudomonas aeruginosa	17	9 (21.42%)	
Total (%)	42	23 (54.76%)	
Distribution of Amp-C producer (N=42)			
Organisms	**Total isolate no.**	**Amp-C isolate**	
Escherichia coli	15	6 (40%)	
Klebsiella pneumoniae	8	4 (26.67%)	
Proteus mirabilis	2	1 (6.67%)	
Pseudomonas aeruginosa	17	4 (26.67%)	
Total (%)	42	15 (35.71%)	
Distribution of carbapenamase producer (N=42)			
Organisms	**Total isolate no.**	**Total carbapenamase producer (%)**	**Total metallo beta lactamase producer (MβL) (%)**
Escherichia coli	15	2 (4.76%)	0 (0%)
Klebsiella pneumoniae	8	1 (2.38%)	1 (2.38%)
Proteus mirabilis	2	0 (0%)	0 (0%)
Pseudomonas aeruginosa	17	6 (14.28%)	4 (9.52%)
Total (%)	42	9 (21.42%)	5 (11.90%)

In our study the most common predisposing factor of chronic osteomyelitis was trauma (47%) followed by postoperative infection (22%) which was correlated with study of Mita et al. [23], Khatoon et al. [25], Suguneswari et al. [3] and also study conducted by Peng et al. [24]

The most common bone involved in chronic osteomyelitis is femur (38%) followed by tibia (36%) which was similar to Mita et al. [23] In study of Suguneswari et al. [3] most common bone involved was tibia with (58%) followed by femur (31%) which was similar to study conducted by Gopi et al. [26] and Khatoon et al. [25]. but differ from our present study in case of predominance of femur along with tibia. While other studies like Peng et al. [24] showed predominance of tibia followed by fibula.

Out of 100 swab sample, 85 (85%) the culture was positive, while in 15 (15%) the culture was negative. Positive culture was more common in chronic osteomyelitis in comparison with negative culture in this study which was correlating with most of the other studies like Mita et al. [23] Gopi et al. [26] Khatoon et al. [25] and Peng et al. [24] incidence of positive culture depends on Collection of specimen before the administration of antibiotics, use of proper transport medium and other related factors.

In present study, the common cause of chronic osteomyelitis is gram positive organism (58.4%) compared to gram negative organism (41.6%). This was similar to Peng et al. [24] Suguneswari et al. [3] and also with Khatoon et al [25]. But not with Mita et al. [23] where gram positive organism was (45.8%) less comparatively to gram negative organism (54.2%).

In our study, there were (69%) cases showing monomicrobial flora, while 16 (16%) cases were showing polymicrobial flora which is correlating with most of studies like Peng et al. [24], Mita et al. [22], Khatoon et al. [25] and also with study conducted by Gopi et al. [26] in this study *Escherichia coli* was most common polymicrobial strain where as in study conducted by Gopi et al. [26] *Staphylococcus aureus* was one of the most common organism found in polymicrobial flora. Most of osteomyelitis in diabetic foot infections and ischemic ulcers is polymicrobial and includes mixtures of aerobic and anaerobic organisms [23].

Staphylococcus aureus (36.2) was most common gram positive organism found in present study along with *Pseudomonas aeruginosa* (14.7) was most common gram negative organism. This study was similar to Suguneswari et al. [3] Khatoon et al. [25] and also correlating with Mita et al. [23] while in other studies like Peng et al. [24] *Escherichia coli* (15.38%) was most common gram negative organism and also in Gopi et al. [26] *Klebsiella penumonie (*11%) was most common gram negative organism. *Staphylococcus aureus* was predominantly most common gram positive organism in all studies mentioned above. Causative agent is often associated with mode of infection and age of the patient [26].

Most of the gram positive isolates were sensitive to linezolid (100%), teicoplanin (100%), vancomycin (100%) followed by daptomycin (88.13%) and tetracycline (86.44%) in this study. daptomycin and teicoplanin were the newer drugs added in CLSI guidelines for treatment of gram positive isolates [27]. This study was correlating with Khatoon et al. [25] Mita et al. [23], Peng et al. [24] and also with study conducted by Suguneswari et al. [3].

Most of the gram negative isolates were sensitive to colistin (100%), ciprofloxacin (71.42%) followed by imipenem (66.66%) and amikacin (59.52%) in this study. This study was similar to Khatoon et al. [25]. While in Mita et al. [23] gram negative organisms were sensitive to imipenem (81%), amikacin (56.8%) followed by ciprofloxacin (39.6%). Piperacillin/ tazobactum was most sensitive followed by levofloxacin, amikacin and aztreonam in study conducted by Suguneswari et al. [3] which was almost similar to Peng et al. [24]

In this study, out of 42 isolated *Staphylococcus aureus*, 35(83) were methicillin resistant *Staphylococcus aureus* which was correlating with Khatoon et al. [25] (72%) and lower incidence was found in studies by Mita et al. [23] (40%), Peng et al. [24] (43.59) and Gopi et al. [26] (5%). methicillin sensitive *Staphylococcus* was 17% in present study which was correlating with Khatoon et al. [25] (28%) while in other studies MSSA had higher incidence 76.08%, 69.64% in Suguneswari et al. [3] and Peng et al. [24] respectively.

Out of 42 gram negative isolates, (54.76) were ESBL producers in present study which was correlating with Khatoon et al. [25] (51.6%). Incidence of ESBL producer was higher in study conducted by Mita et al. [23] (70.6%). In our study, out of 15 *Escherichia coli* and 17 *Pseudomonas aeruginosa*, 9(21.42) were ESBL producer each where as in Khatoon et al [25] *Klebsiella Pneumoniae* followed by *Escherichia coli* were the highest ESBL producer.

In this study Out of 42 gram negative isolates, (35.71) were Amp-C producer. Similar incidence was not found in my knowledge but lower Incidence was found in study of Khatoon et al [25] (24.2%). In present study *Escherichia coli* (40) and *Pseudomonas aeruginosa* (26.67) were highest Amp-C producer while in study done by Khatoon et al. [25], *Proteus* spp. followed by *Klebsiella penumoniae* were highest Amp- C Producer.

In present study, 9 (21.42) were Carbapenemase producer found in total 42 gram negative isolates and out of those, 05(11.90) were Metallo Beta lactamase producers which was correlating with study done by Khatoon et al [25] while higher incidence was found in study conducted by Mita et al. [23]. *Pseudomonas aeruginosa* (9.52) followed by *Klebsiella pneumonia* (2.38) were highest MBL producer in present study similar to study of Khatoon et al [25] where *Pseudomonas aeruginosa* was highest MBL producer.

5. CONCLUSION

Chronic osteomyelitis is continuous to be a therapeutic challenge. It has been the major cause of morbidity since long time. Emerging multidrug resistant strain is major concern for the treatment. The high recurrence rate of chronic osteomyelitis due to post traumatic and post – surgical has leads to development of few new treatment options such as auto vaccination, hyperbaric oxygenation of the affected limb, local antibiotics perfusion system, intra –arterial antibiotics therapy and implantation of aminoglycosides impregnated beads [28,29,30] though diagnosis of the route cause remains the best modality for the early start of treatment.

The aerobic bacteriological study of chronic osteomyelitis showed *Staphylococcus aureus* is being continued to be major etiological agent followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. In present study prevalence of methicillin resistant *Staphylococci* and β Lactamase producing (ESBL, Amp-C and MBL) gram negative bacilli is found to be on higher side among the organisms isolated from chronic osteomyelitis patients. The small size

was first and most common limitation. Our studies were single center based cross sectional study.

Moreover, genotypic identification and tests, specific molecular diagnosis like PCR was not done due to limitations of resources. Identification of causative isolates and using judicious selection of antibiotic by using appropriate antibiotic sensitivity profile would limit the emerging drug resistance strains and will help the clinician in treatment accordingly and thus avoid the development and dissemination of these multidrug resistance strains in the hospital as well as community.

CONSENT

Informed written consent from each patient documenting name, age, sex, clinical illness including chief complains, previous history of illness, duration of symptoms and predisposing factors were taken.

ETHICAL APPROVAL

The study was approved by institutional ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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