International Journal of Research in Medical Sciences
Khatoon R et al. Int J Res Med Sci. 2017 Feb;5(2):482-490
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20170137

Original Research Article

Antibiotic resistance pattern among aerobic bacterial isolates from osteomyelitis cases attending a Tertiary care hospital of North India with special reference to ESBL, AmpC, MBL and MRSA production

Razia Khatoon[1]*, Shameem Ahmad Khan[2], Noor Jahan[3]

[1]Associate Professor, Department of Microbiology, Hind Institute of Medical Sciences, Mau, Ataria, Sitapur, Uttar Pradesh, India
[2]Assistant Professor, Department of Orthopaedics, Hind Institute of Medical Sciences, Safedabad, Barabanki, Uttar Pradesh, India
[3]Associate Professor, Department of Microbiology, Integral Institute of Medical Sciences & Research, Integral University, Lucknow, Uttar Pradesh, India

Received: 10 January 2017
Accepted: 13 January 2017

***Correspondence:**
Dr. Razia Khatoon,
E-mail: drrazia@rediffmail.com

ABSTRACT

Background: Osteomyelitis is a common cause of morbidity in developing countries. Its treatment comprises of surgical debridement of all necrotic bone and soft tissue along with use of appropriate antimicrobial therapy. Treatment is becoming increasingly troublesome due to rise in drug resistant isolates in osteomyelitis cases. The present study was done to determine the antibiotic resistance pattern among aerobic bacterial isolates from osteomyelitis cases.
Methods: 125 samples from osteomyelitis cases were aerobically cultured and isolates from culture positives were identified by standard procedures. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method. Staphylococcal isolates were screened for methicillin resistance and Gram negative bacilli were screened and confirmed for ESBL, AmpC and MBL production.
Results: Out of 125 samples cultured, 20 were culture negative and 105 were culture positive giving rise to 120 isolates (58 Gram positive and 62 Gram negative organisms). The prevalence of methicillin resistant staphylococcal (MRS) isolates, ESBL, AmpC and MBL producers was found to be 43.1%, 51.6%, 24.2% and 14.5% respectively. All the resistant isolates were multidrug resistant, with MRS being 100% sensitive only to vancomycin, linezolid and teicoplanin, ESBL and AmpC producers being 100% sensitive only to imipenem and colistin, and MBL producers being 100% sensitive only to colistin.
Conclusions: Antibiotic therapy on the basis of antibiotic susceptibility pattern helps the clinician to choose appropriate drugs leading to successful treatment and prevention of emergence and dissemination of drug resistant isolates.

Keywords: AmpC, ESBL, Osteomyelitis, MBL, MRSA, Prevalence

INTRODUCTION

Osteomyelitis is a bone infection which occurs due to the extension from an infected joint or by direct invasion as a result of trauma or instrumentation.[1] The two most widely used classification systems for osteomyelitis are by Waldvogel et al. and Cierny et al.[2,3] Under the Waldvogel system, osteomyelitis is first described according to duration, either acute or chronic. Second, the disease is classified according to source of infection, as

hematogenous when it originates from a bacteremia or as contiguous focus when it originates from an infection in a nearby tissue. A final category of the classification is vascular insufficiency. The Cierny-Mader classification is a clinical classification based on anatomic, clinical, and radiologic features.[4]

Acute osteomyelitis is defined as an infection diagnosed within 2 weeks of the onset of symptoms.[5,6]

Chronic osteomyelitis is a relapsing and persistent infection that evolves over months to years and is characterized by low-grade inflammation, presence of dead bone (sequestrum), new bone apposition, and fistulous tracts.[7] Chronic osteomyelitis commonly involves long bones; especially tibia and femur.[8] Unlike the infection in adults, osteomyelitis in children is generally of hematogenous origin and is most often acute. Chronic infections do occur in children, generally as a consequence of failed antimicrobial therapy or the presence of an orthopedic implant.[5,6,9] The most important risk factors of osteomyelitis are trauma (primarily open fractures and severe soft tissues injury), vascular insufficiency, diabetes, elderly, children, obesity, surgical wound infection and haemoglobinopathies such as sickle cell diseases.[8,10] The microorganisms may gain access to the bones during stabilization of fracture or implanting prosthesis. Prosthetic implants create an environment which favors microbial colonization and establishment of infection successfully in the bone.[11] The infective agents adhere to foreign material in the body and secrete glycocalyx (biofilm formation) that inhibits the host defense mechanism and action of antibiotics so that infection can be established which would be difficult to eradicate.[12,13]

A single pathogenic organism is almost always recovered from the bone in hematogenous osteomyelitis, whereas, multiple organisms are usually isolated in contiguous focus osteomyelitis, especially in the diabetic foot.[4,14] The bacteria most commonly isolated from chronic osteomyelitis are *Staphylococcus aureus*, coagulase negative *staphylococci* (especially in implant-associated infections), *Pseudomonas spp., Escherichia coli, Proteus spp., Klebsiella spp., Enterococcus spp., Enterobacter spp.* and anaerobes like *Peptostreptococcus spp., Bacteroides spp., Clostridium spp*. and rarely *Salmonella spp.* (in individuals with sickle cell disease) and *Actinomycetes.*[15]

Osteomyelitis is an ongoing problem due to emergence of multi drug resistant strains among bacterial pathogens causing it. Beta lactamases are the most evolving mechanism of antibiotic resistance among the family *Enterobacteriaceae* due to the selective pressure imposed by inappropriate use of third generation cephalosporins, most often encountered in ICU settings.[16] Extended spectrum beta lactamases (ESBL) and AmpC enzymes are the most common known beta lactamases. Carbapenems represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria.[17] But extensive and unnecessary use of the carbapenems facilitated the emergence of carbapenem resistant bacteria which produced carbapenem hydrolyzing enzyme Metallo Beta Lactamase (MBL), so called because they contain metal ion that works as a cofactor for enzymatic activity.[18] *Staphylococcus aureus* infections used to respond to beta lactam and related group of antibiotics but the emergence of Methicillin resistant *Staphylococcus aureus* (MRSA) has posed a serious therapeutic challenge. Proper management of chronic osteomyelitis requires surgical debridement along with accurate microbial isolation and appropriate antibiotic administration. Hence the present study was conducted to determine the bacterial agents causing chronic osteomyelitis and their antibiogram for ensuring proper treatment of the patients.

METHODS

A hospital based cross sectional study was done over a period of 1 year from September 2015 to August 2016, among patients suffering from acute as well as chronic osteomyelitis who attended orthopaedic outpatient department (outpatients) and those admitted in orthopaedic ward (inpatients) of Hind Institute of Medical Sciences, Mau, Ataria, Sitapur, Uttar Pradesh, India to determine the aerobic bacterial profile of the isolates and their antibiotic susceptibility pattern. The study was approved by Institutional Ethical Committee. An informed consent was taken from all the patients included in the study prior to sample collection.

A total of 125 clinically diagnosed cases of osteomyelitis belonging to all age group and both sexes were included in the study whose samples like pus, pus swabs, sequestrum of bone, and synovial fluid, collected under aseptic precautions, were received for culture and sensitivity in clinical bacteriology laboratory of Microbiology department of Hind Institute of Medical Sciences, Mau, Ataria, Sitapur, Uttar Pradesh, India. Patients who were confirmed to be cases of malignant and benign tumors, cysts, non-infected non-unions, old trauma, and bone infarcts were excluded from the study.

All the samples were processed immediately. Direct smear examination was done. The samples were inoculated on Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 24 hours and the growth was identified as per the standard microbiological protocols and procedures.[19]

Antimicrobial susceptibility testing (AST) was done on Mueller Hinton agar (HiMedia Laboratories, India) by Kirby Bauer disk diffusion method using Clinical and Laboratory Standard Institute guidelines (CLSI).[20] Antibiotic disks (HiMedia Laboratories, India) used for testing Gram positive clinical isolates were: penicillin (10 units), gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg), erythromycin (5µg), clindamycin (2µg), co-

trimoxazole (1.25µg /23.75µg), cefoxitin (30µg), linezolid (30µg), vancomycin (30µg) and teicoplanin (30µg). For testing Gram negative isolates antibiotic disks used were: gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg), piperacillin (100µg), piperacillin/ tazobactam (100/10µg), co-trimoxazole (1.25µg /23.75µg), cefoxitin (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefepime (30µg), imipenem (10µg) and colistin (10µg). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as a standard quality control strains.[20] Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes.[21]

Detection of ESBL producers

Isolates of Gram negative bacilli that showed reduced susceptibility to third generation cephalosporins, with zone diameter of ≤22 mm for ceftazidime, ≤25 mm for ceftriaxone and ≤27 mm for cefotaxime were considered as potential ESBL producers, and were subjected to phenotypic confirmatory disk diffusion test recommended by CLSI. A Mueller-Hinton agar (MHA) plate was lawn cultured with the test strain and disks 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 centre to centre apart. The plate was incubated at 37ºC for 18 hours. As shown in Figure 1, an increase of ≥5 mm in zone of inhibition of the combination disks in comparison to the ceftazidime or cefotaxime disk alone was considered to be ESBL producer.[20]

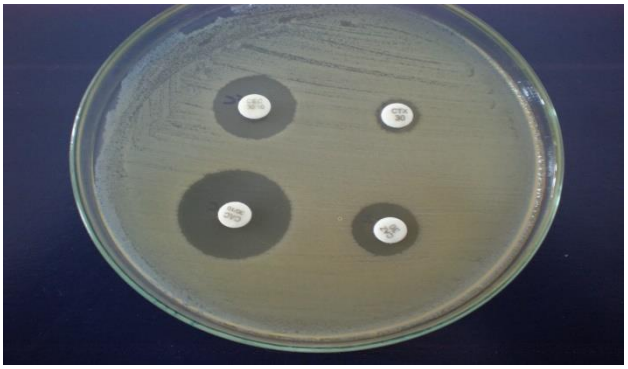

Figure 1: An increase of ≥ 5 mm in zone of inhibition of the combination disks (ceftazidime/clavulanic acid and cefotaxime/clavulanic acid) versus its zone diameter when ceftazidime and cefotaxime are tested alone confirmed an ESBL producing organism.

Detection of AmpC β-lactamase producers

The isolates of Gram negative bacilli which showed reduced susceptibility to cefoxitin (zone diameter <18 mm) and resistance to 3rd generation cephalosporins were considered as screen positive, and were confirmed by putting AmpC disk test. Lawn culture of *Escherichia coli* ATCC 25922 was done on MHA. Cefoxitin (30µg) disc was placed on it. Sterile disk (6mm) was moistened with sterile saline and inoculated with several colonies of the test organism. The inoculated disk was then placed beside cefoxitin disk almost touching it. The plate was incubated at 35ºC for 16 to 18 hours. As shown in Figure 2, flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test organism disk was considered as positive for AmpC β-lactamase production, whereas, negative test had an undistorted zone.[22]

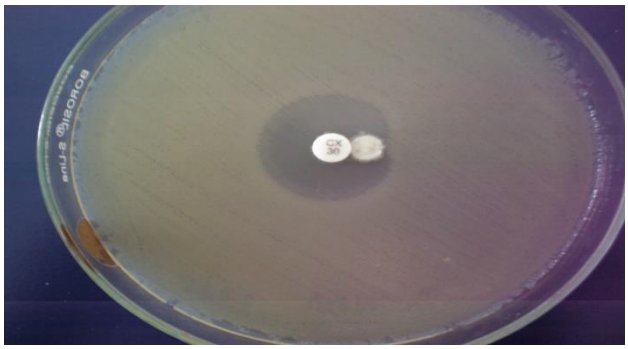

Figure 2: AmpC disk test shows flattening of the cefoxitin (CX) inhibition zone in the vicinity of the test organism disk confirming AmpC β-lactamase producing organism.

Detection of MBL producers

Isolates of Gram negative bacilli which were found to be resistant to imipenem were considered to be screening positive and were confirmed by putting imipenem - EDTA combined disk test. A MHA plate was lawn cultured with test organism and two (10 µg) imipenem disks were placed at a distance of 20 mm from center to center on it, and 10 µl of 0.5 M EDTA solution was added to one disk. The plate was incubated at 35°C for 16-18 hours. As shown in Figure 3, a zone diameter difference between the imipenem and imipenem + EDTA of ≥ 7 mm was interpreted as a positive result for MBL production.[23]

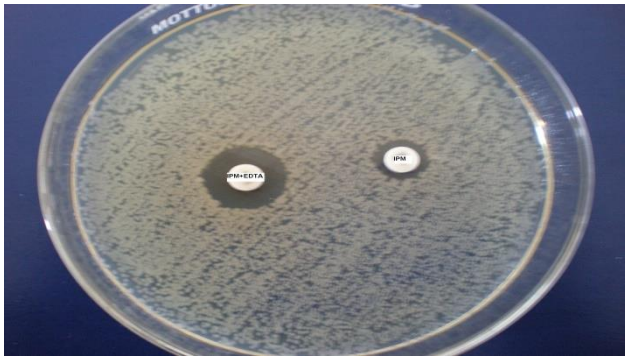

Figure 3: A ≥7 mm difference in diameter of zone of inhibition between the imipenem (IPM) disk and imipenem (IPM) + EDTA shows MBL producing organism.

Detection of MRSA and MRCoNS

Methicillin resistance was determined using cefoxitin (30µg) disk on Mueller-Hinton agar as per CLSI guidelines, and results were read after 18 hours of incubation at 35°C. The *Staphylococcus aureus* isolates which showed zone size ≤21mm were considered as methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (CoNS) which showed zone size ≤24mm were considered as methicillin-resistant CoNS (MRCoNS).[20]

Statistical analysis

Data were collected and transferred to computer and statistically analyzed using SPSS Data Editor Software, Chicago, version 20. Frequency and percentage were used for the categorical and ordinal variables. Mean, range (minimum and maximum values) and standard deviation (SD) were used for the continuous variables. Chi-square test was performed and p value ≤0.05 were considered statistically significant.

RESULTS

A total of 125 clinically diagnosed cases of osteomyelitis were included in the study, of which 110 (88.0%) were males and 15 (12.0%) were females. The mean age of the patients was 28.5 (±12.7) years which ranged from 5-65 years. Table 1 shows that maximum osteomyelitis cases belonged to age group 31-40 years (32.8%), followed by 21-30 years (25.6%) and 11-20 years (18.4%).

Table 1: Distribution of osteomyelitis cases according to their age groups.

Age groups	Number of osteomyelitis cases N = 125 (%)
1-10 years	12 (9.6%)
11-20 years	23 (18.4%)
21-30 years	32 (25.6%)
31-40 years	41 (32.8%)
41-50 years	11 (8.8%)
51-60 years	04 (3.2%)
61-70 years	02 (1.6%)

N = Number of osteomyelitis cases included in the study.

Table 2 depicts that amongst the various predisposing factors for osteomyelitis; accidents leading to open fracture accounted the maximum cases (45.6%), followed by post-operative infections (27.2%), orthopaedic implants (24.0%) and diabetes mellitus (3.2%). It was found that tibia was the commonest bone affected by osteomyelitis (44.0%), followed by femur (40.8%), metatarsals (4.0%), humerus and calcaneum (2.4% each), as shown in Table 3. Out of 125 non-duplicate samples aerobically cultured in the microbiology laboratory, 105 (84%) were culture positive and 20 (16%) were culture negative. Monomicrobial flora was seen in 90 culture positives, whereas, 15 culture positives yielded polymicrobial flora, giving rise to a total of 120 isolates.

Table 2: Distribution of osteomyelitis cases according to its various predisposing factors.

Predisposing factors	Number of osteomyelitis cases N = 125 (%)
Accidents leading to open fractures	57 (45.6%)
Post-operative infections	34 (27.2%)
Orthopaedic implants	30 (24.0%)
Diabetes mellitus	04 (3.2%)

N = Number of osteomyelitis cases included in the study.

Table 3: Distribution of osteomyelitis cases on the basis of the affected bone.

Bone involved	Number of osteomyelitis cases N = 125 (%)
Tibia	55 (44.0%)
Femur	51 (40.8%)
Fibula	01 (0.8%)
Humerus	03 (2.4%)
Radius	02 (1.6%)
Ulna	02 (1.6%)
Calcaneum	03 (2.4%)
Metatarsals	05 (4.0%)
Metacarpals	03 (2.4%)

N = Number of osteomyelitis cases included in the study.

Of these 120 isolates, 95 were recovered from samples received from outpatients and 25 isolates were recovered from samples received from inpatients. These isolates were identified by standard microbiological procedures. The distribution of the clinical isolates is shown in Table 4.

Table 4: Distribution of pathogenic organisms isolated from osteomyelitis cases.

Isolates	Number of Organisms (%)
Staphylococcus aureus	41 (34.2%)
Coagulase negative staphylococci	17 (14.2%)
Escherichia coli	19 (15.8%)
Klebsiella spp.	15 (12.5%)
Pseudomonas spp.	22 (18.3%)
Proteus spp.	06 (5.0%)
Total	120 (100%)

Of these 120 isolates, 58 (48.3%) were Gram positive cocci and 62 (51.7%) were Gram negative bacilli. Maximum numbers of isolates recovered from osteomyelitis cases were *Staphylococcus aureus* isolates (34.2%, 41/120). Amongst the Gram negative isolates, maximum were *Pseudomonas spp*. (35.4%, 22/62),

followed by *Escherichia coli* (30.6%, 19/62), *Klebsiella spp.* (24.2%, 15/62) and *Proteus spp.* (9.7%, 6/62). All the isolates were tested for antibiotic susceptibility for commonly used antibiotics by using Kirby-Bauer disk diffusion method. The Gram negative isolates were screened and confirmed for ESBL, AmpC, MBL production and the Gram positive isolates were screened for MRSA production.

Out of 58 Gram positive isolates tested, 25 (43.1%) were methicillin resistant *staphylococci* (MRS) and 33 (56.9%) were methicillin sensitive *staphylococci* (MSS). Out of 25 MRS isolates, 18 (72.0%) were methicillin resistant *Staphylococcus aureus* (MRSA) and 07 (28.0%) were methicillin resistant coagulase negative *staphylococci* (MRCoNS). Thus, the overall prevalence of MRS was 43.1%, with higher prevalence of resistance among *Staphylococcus aureus* isolates (43.9%, 18/41) as compared to MRCoNS (41.2%, 07/17). Table 5 shows the comparative evaluation of antibiotic resistance pattern among MRS and MSS.

It was found that both MRS and MSS were highly resistant to penicillin (100% and 93.9% respectively). The resistance pattern of MRS was found to be significantly different statistically from that of MSS for antibiotics amikacin (p = 0.028), gentamicin (p = 0.001), ciprofloxacin (p = 0.006), co-trimoxazole (p = 0.003) and erythromycin (p = 0.004). All the MRS isolates were 100% sensitive to vancomycin, linezolid and teicoplanin, followed by sensitivity of 68% to amikacin and 64% to clindamycin.

Table 5: Comparison of antibiotic resistance pattern among methicillin resistant *staphylococci* (MRS) and methicillin sensitive *staphylococci* (MSS) isolated from osteomyelitis cases.

Antibiotics tested		Methicillin Resistant *Staphylococci* (MRS), N = 25 (%)	Methicillin Sensitive *Staphylococci* (MSS), N = 33 (%)	Chi-Square ($\chi 2$) & *p value
Penicillin	Resistant	25 (100%)	31 (93.9%)	$\chi 2 = 1.569$; df = 1; p = 0.210
	Sensitive	0 (0)%	2 (6.1%)	
Amikacin	Resistant	8 (32.0%)	3 (9.1%)	$\chi 2 = 4.857$; df = 1; p = 0.028
	Sensitive	17 (68.0%)	30 (90.9%)	
Gentamicin	Resistant	13 (52.0%)	4 (12.1%)	$\chi 2 = 10.918$; df = 1; p = 0.001
	Sensitive	12 (48.0%)	29 (87.9%)	
Ciprofloxacin	Resistant	15 (60.0%)	8 (24.2%)	$\chi 2 = 7.600$; df = 1; p = 0.006
	Sensitive	10 (40.0%)	25 (75.8%)	
Cefoxitin	Resistant	25 (100%)	0 (0%)	$\chi 2 = 58.000$; df = 1; p <0.001
	Sensitive	0 (0%)	33 (100%)	
Co-trimoxazole	Resistant	19 (76.0%)	12 (36.4%)	$\chi 2 = 8.981$; df = 1; p = 0.003
	Sensitive	6 (24.0%)	21 (63.6%)	
Erythromycin	Resistant	18 (72.0%)	11 (33.3%)	$\chi 2 = 8.507$; df = 1; p = 0.004
	Sensitive	7 (28.0%)	22 (66.7%)	
Clindamycin	Resistant	9 (36.0%)	5 (15.2%)	$\chi 2 = 3.376$; df = 1; p = 0.066
	Sensitive	16 (64.0%)	28 (84.8%)	
Vancomycin	Resistant	0 (0%)	0 (0%)	NA
	Sensitive	25 (100%)	33 (100%)	
Linezolid	Resistant	0 (0.0%)	0 (0.0%)	NA
	Sensitive	25 (100%)	33 (100%)	
Teicoplanin	Resistant	0 (0.0%)	0 (0.0%)	NA
	Sensitive	25 (100%)	33 (100%)	

N = Number of isolates. NA = Not Applicable. *p value < 0.05 was considered as statistically significant.

Out of 62 Gram negative isolates included in the study only 6 isolates were found to be uniformly sensitive to all the routine antimicrobials tested. Table 6 shows the distribution of Gram negative bacilli according to their resistance pattern. The prevalence of ESBL, AmpC β-lactamase and MBL producers in present study was 51.6%, 24.2% and 14.5% respectively. Maximum ESBL production was seen in *Klebsiella spp.* (66.7%), followed by *Escherichia coli* (57.9%) and *Pseudomonas spp.* (45.5%). Maximum AmpC β-lactamase production was found in Proteus spp. (33.3%) followed by *Klebsiella spp.* (26.7%) and *Escherichia coli* (26.3%).

Maximum MBL production was seen in *Pseudomonas spp.* (31.8%), followed by Proteus spp. (16.7%) and *Klebsiella spp.* (6.7%). All ESBL, AmpC and MBL

producers were found to be multidrug resistant. Table 7 shows that ESBL producers are 100% sensitive to imipenem, colistin and cefoxitin followed by sensitivity to amikacin (84.4%) and piperacillin/ tazobactam (75.0%).

Table 6: Distribution of Gram negative organisms isolated from osteomyelitis cases on the basis of their resistance pattern.

Organisms	Sensitive isolates N (%)	ESBL producers N (%)	AmpC producers N (%)	MBL producers N (%)
Escherichia coli (N = 19)	3 (15.8%)	11 (57.9%)	5 (26.3%)	0 (0%)
Klebsiella spp. (N = 15)	0 (0%)	10 (66.7%)	4 (26.7%)	1 (6.7%)
Pseudomonas spp. (N = 22)	1 (4.5%)	10 (45.5%)	4 (18.2%)	7 (31.8%)
Proteus spp. (N = 06)	2 (33.3%)	1 (16.7%)	2 (33.3%)	1 (16.7%)
Total (N = 62)	6 (9.7%)	32 (51.6%)	15 (24.2%)	9 (14.5%)

N = Number of isolates.

Table 7: Antibiotic sensitivity pattern of ESBL producers isolated from osteomyelitis cases.

Antibiotic tested	ESBL producers N = 32 (%)	
	Sensitive N (%)	Resistant N (%)
Amikacin	27 (84.4%)	5 (15.6%)
Gentamicin	18 (56.2%)	14 (43.8%)
Piperacillin	7 (21.9%)	25 (78.1%)
Piperacillin/ Tazobactam	24 (75.0%)	8 (25.0%)
Cefoxitin	32 (100%)	0 (0%)
Cefotaxime	2 (6.2%)	30 (93.8%)
Ceftazidime	1 (3.1%)	31 (96.9%)
Ceftriaxone	1 (3.1%)	31 (96.9%)
Cefepime	9 (28.1%)	23 (71.9%)
Ciprofloxacin	6 (18.7%)	26 (81.3%)
Co-trimoxazole	8 (25.0%)	24 (75.0%)
Imipenem	32 (100%)	0 (0%)
Colistin	32 (100%)	0 (0%)

N = Number of isolates

Table 8 depicts that AmpC producers are 100% sensitive to imipenem and colistin. As shown in Table 9, MBL producers were found to be 100% resistant to imipenem and 100% sensitive to colistin. It was found in our study that the organisms isolated from samples received from hospitalized patients (inpatients) were more resistant than those recovered from samples of outpatients (Table 10).This difference was found to be statistically significant (p = 0.002). Out of 120 isolates tested, majority (81 isolates) were resistant to the routine antimicrobials tested. Out of 39 sensitive isolates, majorities (33 isolates) were Gram positive organisms and only 6 isolates were Gram negative organisms. All the 39 sensitive isolates were derived from outpatients, whereas, all the isolates derived from inpatients were found to be resistant to the antimicrobials tested. Maximum resistance was in the form of ESBL production (26.7%), followed by methicillin resistance among staphylococci (20.8%).

Table 8: Antibiotic sensitivity pattern of AmpC β-lactamase producers isolated from osteomyelitis cases.

Antibiotic tested	AmpC β-lactamase producers N = 15 (%)	
	Sensitive N (%)	Resistant N (%)
Amikacin	5 (33.3%)	10 (66.7%)
Gentamicin	3 (20.0%)	12 (80.0%)
Piperacillin	1 (6.7%)	14 (93.3%)
Piperacillin/ Tazobactam	4 (26.7%)	11 (73.3%)
Cefoxitin	0 (0%)	15 (100%)
Cefotaxime	1 (6.7%)	14 (93.3%)
Ceftazidime	1 (6.7%)	14 (93.3%)
Ceftriaxone	0 (0%)	15 (100%)
Cefepime	2 (13.3%)	13 (86.7%)
Ciprofloxacin	2 (13.3%)	13 (86.7%)
Co-trimoxazole	4 (26.7%)	11 (73.3%)
Imipenem	15 (100%)	0 (0%)
Colistin	15 (100%)	0 (0%)

N = Number of isolates.

DISCUSSION

Osteomyelitis is one of the most inconvenient diseases among most of the developing countries like India. An increase in the emergence of drug resistant strains makes treatment even more complicated.[1] Chronic osteomyelitis is notoriously resistant to treatment and requires aggressive surgical debridement in addition to antibiotic therapy.[24] The advent of prosthetic joints has added new dimensions to the challenges of septic arthritis and osteomyelitis, as these are prone to become infected by a wide range of organisms.[25] Chronic osteomyelitis may require antimicrobial therapy for months to years, sometimes with antibiotics that are invaluable for the hospital environment, such as glycopeptides and carbapenems. This situation makes the accurate identification of the pathogen an absolute cornerstone of

antimicrobial therapy.[26] Widespread use of antibiotics has altered etiological pattern of infections and their antibiotic susceptibility. Hence continuous monitoring of susceptibility pattern needs to be carried out in individual setting so as to detect the true burden of antibiotic resistance among organisms and prevent their further emergence by judicious use of drugs.[25]

Table 9: Antibiotic sensitivity pattern of MBL producers isolated from osteomyelitis cases.

Antibiotic tested	MBL producers N = 9 (%)	
	Sensitive N (%)	Resistant N (%)
Amikacin	3 (33.3%)	6 (66.7%)
Gentamicin	1 (11.1%)	8 (88.9%)
Piperacillin	0 (0%)	9 (100%)
Piperacillin/ Tazobactam	0 (0%)	9 (100%)
Cefoxitin	0 (0%)	9 (100%)
Cefotaxime	0 (0%)	9 (100%)
Ceftazidime	0 (0%)	9 (100%)
Ceftriaxone	0 (0%)	9 (100%)
Cefepime	0 (0%)	9 (100%)
Ciprofloxacin	2 (22.2%)	7 (77.8%)
Co-trimoxazole	2 (22.2%)	7 (77.8%)
Imipenem	0 (0%)	9 (100%)
Colistin	9 (100%)	0 (0%)

N = Number of isolates

In present study, maximum osteomyelitis cases belonged to younger age groups of 31-40 years (32.8%) followed by 21-30 years (25.6%), with accidents resulting in open fractures as the major predisposing factor of oseteomyelitis (45.6%), followed by post-operative infections (27.2%) and orthopaedic implants (24.0%). Both the above findings are supported by another study which also reported higher cases of osteomyelitis among younger age groups with 29% cases among 30-40 years followed by 23% among 20-30 years, with accidents as its commonest predisposing factor (53%), followed by post-surgical wounds (26%) and prosthesis and other causes (20%).[1] Other previous done studies also reported maximum cases of osteomyelitis belonging to younger age groups of 21-40 years (39.6%) followed by 41-60 years (28.7%) and trauma as the commonest cause of osteomyelitis (44.0%).[15,27]

The present study found that tibia was the commonest bone affected by osteomyelitis (44.0%), followed by femur (40.8%), metatarsals (4.0%) and fibula being the least affected (0.8%). This finding was supported by a study which showed that tibia was most commonly affected (58%) followed by femur (31%).[1] Another study also reported highest incidence of osteomyelitis affecting leg (30.7%) followed by thigh (27.7%).[27]

However, in contrast to present finding another study reported highest incidence of osteomyelitis in femur (48%), followed by tibia (23%) and humerus (9%).[15] The present study yielded 105 (84%) culture positives with monomicrobial flora in 90 (85.7%) culture positives, and polymicrobial flora in 15 (14.3%) culture positives giving rise to a total of 120 isolates. Another study yielded 87% culture positives with monomicrobial flora in 67% and polymicrobial flora in 20% culture positives.[15]

Table 10: Distribution of pathogenic isolates derived from inpatient and outpatient osteomyelitis cases on the basis of their antibiotic susceptibility pattern.

Antibiotic susceptibility pattern	Patient status			Chi-Square ($\chi 2$) & *p value
	Outpatient N = 95 (%)	Inpatient N = 25 (%)	Total N = 120 (%)	
ESBL producers (N = 32)	22 (23.2%)	10 (40.0%)	32 (26.7%)	$\chi 2 = 16.499$
AmpC producers (N = 15)	9 (9.5)%	6 (24.0%)	15 (12.5%)	df = 4
MBL producers (N = 9)	7 (7.4%)	2 (8.0%)	9 (7.5%)	p = 0.002
MRS (N = 25)	18 (18.9%)	7 (28.0%)	25 (20.8%)	
Sensitive organisms (N = 39)#	39 (41.1%)	0 (0%)	39 (32.5%)	

N = Number of isolates. MRS = Methicillin resistant *staphylococci.* # means 39 sensitive organisms comprised of 33 Gram positive isolates and 6 Gram negative isolates. *p value < 0.05 was considered as statistically significant.

Even though the gram negative organisms are increasing rapidly since longer time, still *staphylococcus* remained the most common isolate of osteomyelitis with methicillin resistant strains aggravating the disease further. Present study reported that *Staphylococcus aureus* was the commonest isolate from osteomyelitis cases (34.2%), followed by *Pseudomonas spp.* (18.3%), *Escherichia coli* (15.8%) and coagulase negative *staphylococci* (14.2%). This finding is in concordance with another study which also showed highest incidence of *Staphylococcus aureus* (32.9%) among osteomyelitis cases followed by *Pseudomonas aeruginosa* (15.8%), *Klebsiella pneumoniae* and coagulase negative *staphylococci* (13.0% each).[15] Various previously done studies also reported staphylococcus as the major isolate from osteomyelitis cases.[1,28,29]

Present study reported the prevalence of methicillin resistant *staphylococci* (MRS) to be 43.1%, with 43.9% among *Staphylococcus aureus* isolates and 41.2% among coagulase negative *staphylococci*. All the MRS isolates were 100% sensitive to vancomycin, linezolid and teicoplanin, followed by sensitivity to amikacin (68%) and clindamycin (64%). This is similar to a previous done study which reported prevalence of MRSA to be 40%, with 100% sensitivity of MRSA isolates to vancomycin and linezolid, followed by sensitivity to amikacin (78.5%) and co-trimoxazole (50%).[30] Other studies also reported 100% sensitivity of MRSA isolates to vancomycin and 91.66% sensitivity to levofloxacin.[1,25]

Although Gram-negative bacilli (GNB) represent a minor portion of all the pathological agents isolated in osteomyelitis cases, they are of major clinical importance due to the peculiarities of their antimicrobials susceptibility pattern.[27] In present study amongst the Gram negative organisms isolated (62 isolates) from osteomyelitis cases, maximum were *Pseudomonas spp.* (35.5%), followed by *Escherichia coli* (30.6%), *Klebsiella spp.* (24.2%) and *Proteus spp.* (9.7%), with the prevalence of ESBL, AmpC and MBL production of 51.6%, 24.2% and 14.5% respectively.

All the resistant isolates were uniformly resistant to 3rd generation cephalosporins (cefotaxime, ceftazidime and ceftriaxone) with 100% sensitivity to imipenem and colistin, followed by sensitivity to amikacin (84.4%) and piperacillin/ tazobactam (75%) among ESBL producers, 100% sensitivity to imipenem and colistin among AmpC producers and 100% sensitivity to colistin among MBL producers.

This is in accordance with another study which also showed that most of the Gram negative bacilli belonging to *Enterobacteriaceae* and non-fermenters showed resistance against 3rd generation cephalosporins. Among the *Enterobacteriaceae*, imipenem was more sensitive whereas among non – fermenters aztreonam and levofloxacin were the most active drugs.[1] In another study, the prevalence of ESBL and MBL among 58 Gram negative isolates was 68.9% and 18.9% respectively. Most of the ESBL producers were sensitive to imipenem (82.5%), amikacin (52.5%) and ciprofloxacin (45.0%) and MBL producers to amikacin (45.4%).[30]

CONCLUSION

Osteomyelitis has been the major cause of morbidity since long. Emerging multidrug resistant strains is of major concern as they pose challenge in the treatment of osteomyelitis. In present study the prevalence of β-lactamase producing (ESBL, AmpC and MBL) Gram negative bacilli and methicillin resistant *staphylococci* is found to be quite high among the organisms isolated from osteomyelitis patients. The early detection of such drug resistant isolates may help in appropriate antimicrobial therapy since beginning and thus avoid the development and dissemination of these multidrug resistance strains in the hospital as well as in the community. Present study highlights the importance of culture-directed antibiotic therapy and thus helps the clinician in choosing appropriate antibiotics which not only contribute to better treatment but there judicious use will also help in preventing emergence of resistance to the drug which are still sensitive.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Suguneswari G, Singh AH, Basu R. Bacteriological profile of osteomyelitis in a tertiary care hospital at Visakhapatnam, Andhra Pradesh. Int J Cur Res Rev. 2013;5:52-8.
2. Waldvogel FA, Medoff G, Swartz MN. Osteomyelitis - a review of clinical features, therapeutic considerations and unusual aspects. 3: osteomyelitis associated with vascular insufficiency. N Engl J Med. 1970;282:316-22.
3. Cierny G III, Mader JT, Penninck JJ. A clinical staging system for adult osteomyelitis. Clin Orthop Relat Res. 2003;414:7-24.
4. Calhoun JH, Manring MM, Shirtliff M. Osteomyelitis of the Long Bones. Semin Plast Surg. 2009;23:59-72.
5. Lew DP, Waldvogel FA. Osteomyelitis. Lancet. 2004;364:369-79.
6. Krogstad P. Osteomyelitis. In: Feigin RD, Cherry JD, Demmler-Harrison GD, Kaplan SL, editors. Textbook of Pediatric Infectious Diseases. 6th Edition. PA, USA: Saunders Elsevier. 2009;725-42.
7. Zuluaga AF, Galvis W, Saldarriaga JG, Agudelo M, Salazar BE, Vesga O. Etiologic diagnosis of chronic osteomyelitis a prospective study. Arch Intern Med. 2006;166:95-100.
8. Abid AS, Ehan AH, Yonis AR. Epidemiological and bacteriological study of chronic osteomyelitis. Tikrit Med J. 2008;14:59-62.
9. Gutierrez K. Bone and joint infections in children. Pediatr Clin N Am. 2005;52:779-94.
10. Ako-Nai AK, Ikem IC, Aziba A, Ajayi AA, Onipede OA. Bacteriological examination of chronic osteomyelitis cases in ILE-IFE, Southwestern Nigeria. Af J Clinical & Exp Microbiology. 2003;4:41-51.
11. Carek PJ, Dickson LM, Sack JL. Diagnosis and management of osteomyelitis. Am Fam Physicians. 2001;63:2413-21.
12. Gilbert P, McBain AJ. Biocides usage in the domestic setting and concern about antibacterial and antibiotic resistance. J Infect. 2001;43:85-91.
13. Gilbert P, McBain AJ. Biofilms: their impact upon health and their recalcitrance towards biocides. Am J Infect Cont. 2001;29:252-5.

14. Canale ST, James HB. Campbell's Operative Orthopaedics, 11th ed. St Louis Missouri: Mosby. 2008;695-709.
15. Wadekar MD, Anuradha K, Venkatesha D. Chronic osteomyelitis: aetiology and antibiotic susceptibility pattern. Int J Recent Trends Sci Tech. 2014;9:337-40.
16. Rudresh SM, Nagarathnamma T. Extended spectrum β-lactamase producing Enterobacteriaceae & antibiotic coresistance. Indian J Med Res. 2011;133:116-8.
17. Hodiwala A, Dhoke R, Urhekar AD. Incidence of metallo-betalactamase producing Pseudomonas, Acinetobacter & Enterobacterial isolates in hospitalised patients. Int J Pharmacy Biol Sci. 2013;3:79- 83.
18. Chakraborty D, Basu S, Das S. A study on infections caused by metallo beta lactamase producing Gram negative bacteria in intensive care unit patients. Am J Infect Dis. 2010;6:34-9.
19. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Mackie & McCartney Practical Medical Microbiology. 14th edition. Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Edinburgh: Churchill Livingstone. 2006:131-49.
20. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement. CLSI document M100–S25. CLSI, Wayne, Pennsylvania, USA, 2015.
21. Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D. ICMR-ESBL study group. Phenotypic and molecular characterization of AmpC β-lactamases among Escherichia coli, Klebsiella spp. & Enterobacter spp. from five Indian Medical Centers. Indian J Med Res. 2012;135:359-64.
22. Doddaiah V, Anjaneya D. Prevalence of ESBL, AmpC and carbapenemase among gram negative bacilli isolated from clinical specimens. Am J Life Sci. 2014;2:76-81.
23. Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int J Curr Res Aca Rev. 2013;1:89-95.
24. Mackowiak PA, Jones SR, Smith JW. Diagnostic value of sinus-tract cultures in chronic osteomyelitis. JAMA. 1978;239:2772-5.
25. Kaur J, Gulati VL, Aggarwal A, Gupta V. Bacteriological profile of osteomyelitis with special reference to Staphylococcus aureus. Indian Journal for the Practising Doctor. 2008;4:6.
26. Zuluaga AF, Galvis W, Jaimes F, Vesga O. Lack of microbiological concordance between bone and non-bone specimens in chronic osteomyelitis: an observational study. BMC Infect Dis. 2002;2:1-7.
27. de Carvalho VC, de Oliveira PRD, Dal-Paz K, de Paula AP, Felix CDS, Lima LLM. Gram-negative osteomyelitis: clinical and microbiological profile. Braz J Infect Dis. 2012;16:63-7.
28. Rao PS, Beena VK, Rao PS, Shivnanda PG. Bacteriological study of bone and joint infections with special reference to anaerobes. Indian J Orthopaedics. 1997;31:171-4.
29. Along TO, Ogunlade SO, Fashina AN. Microbial isolates in chronic osteomyelitis- A guide to management from department of surgery, college of medicine, Nigeria ; African J Med Sciences. 2002;31:167-9.
30. Wadekar MD, Naganath M, Venkatesha D. Detection of ESBL, MBL and MRSA among isolates of chronic osteomyelitis and their antibiogram. Int J Curr Microbiol App Sci. 2015;4:289-95.

Cite this article as: Khatoon R, Khan SA, Jahan N. Antibiotic resistance pattern among aerobic bacterial isolates from osteomyelitis cases attending a Tertiary care hospital of North India with special reference to ESBL, AmpC, MBL and MRSA production. Int J Res Med Sci 2017;5:482-90.