Original Article

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Microbiology Section

Microbiological Profile of Osteomyelitis and Antibiotic Resistance Pattern of Bacterial Isolates with Special Reference to MDR Strains at a Tertiary Care Hospital, Kanpur, Uttar Pradesh, India

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ABSTRACT

Introduction: Osteomyelitis is an inflammatory process that affects bone due to the contiguous infection, direct inoculation, or haematogenous spread of microorganisms. It is an infectious disease that is difficult to diagnose and treatment is complex because of its heterogeneity, pathophysiology, clinical presentation and management.

Aim: To determine microbiological profile osteomyelitis and antibiotic resistance pattern of bacterial isolates with special reference to Multidrug Resistance (MDR) strains.

Materials and Methods: A cross-sectional study was conducted in the Department of Microbiology and Department of Orthopaedics, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India. A total of 100 samples from osteomyelitis cases were aerobically cultured and isolates from culture positives were identified by standard procedures. Antimicrobial Susceptibility Testing (AST) was done following Clinical and Laboratory Standards Institute (CLSI) guidelines. Staphylococcal isolates were screened for methicillin resistance and Gram-negative bacilli were screened for MDR production. The data was entered in Microsoft excel.

Results: Out of 100 samples, 76% were culture positive and 24% were culture negative. Males were more affected than females. Staphylococcal spp. (47.4%) was predominant, *E. coli* (14.4%) and *Klebsiell*a spp. (11.8%), *Pseudomonas* spp. (9.2%), *Proteus* spp. (5.2%), Coagulase-Negative Staphylococci (CoNS) (4%). Among the MDR strains, Methicillin-resistant *Staphylococcus aureus* (MRSA) was 44.4%. All the MDR Staphylococcal isolates were 100% sensitive for linezolid. Among the MDR Gram-negative bacilli were Extended Spectrum Beta Lactamases (ESBL) (50%), AmpC (17.6%) and Metallo-beta-lactamases (MBL) (14.7%) and they were 100% sensitive for polymixin B and colistin.

Conclusion: The microbiological profile of osteomyelitis in the present study showed high prevalence of MRSA 44% as the commonest agent, sensitive only to linezolid. *E. coli* ESBL (50%) and MBL 14.7% were sensitive only to colistin and polymixin B, therefore proper infection control practices and antibiotic policy has to be followed to reduce the incidence of MDR strains.

Keywords: Extended spectrum beta lactamase, Metallo-beta-lactamases, Methicillin-resistant *Staphylococcus aureus*, Multidrug resistance

INTRODUCTION

The word “osteomyelitis” is derived from the ancient Greek words *osteo* (meaning bone) and muelinos (meaning marrow) and simply means an infection of medullar portion of the bone [1]. The term osteomyelitis was first used by the French surgeon Edouard Chassaignac in 1852, who defined the disease as an inflammatory process accompanied by bone destruction and is caused by an infecting microorganism [2]. Osteomyelitis is an inflammatory process that affects bone due to the contiguous infection, direct inoculation, or haematogenous spread of microorganisms [3]. Current interest in this condition has increased due to recent changes in the epidemiology, pathogenesis, diagnosis, treatment and prognosis of the disease [4,5].

However, it is not a single entity; this disease is differentiated according to the aetiology, pathogenesis and degree of bone involvement, as well as age and the immune condition of the patient [6]. The reported incidence has increased due to co-morbidities such as diabetes mellitus, peripheral vascular disease, trauma and surgery [7]. After an open fracture, the incidence of osteomyelitis can range from 2-16% depending on the type of injury and the treatment administered [8]. It can involve different structures such as the bone marrow, cortex, periosteum and parts of the surrounding soft tissues, or remain localised [9]. Osteomyelitis mostly affects the growing ends of long bones and it is more common in the lower extremity at metaphysis of femur and proximal end of tibia [10].

Various microorganisms can reach to bone through blood and cause inflammation in bone tissue; rarely soft tissue infection may lead to bone damage. Microorganism reach to the metaphysis of bone through blood flow from skin wound, upper respiratory tract infection, periodontitis and any other infectious region. Bone metaphysic is a region full of blood vessels and slow blood stream which can spread the infection. Direct trauma to bone may cause osteomyelitis [11].

The two most widely used classification systems for osteomyelitis are by Waldvogel FA et al., and Cierny G et al., [12,13]. 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 haematogenous 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 [14]. The Cierny-Mader osteomyelitis classification combines both anatomic factors (medullar, superficial, localised, or diffuse osteomyelitis) and physiological classes (healthy host, systemic and/or local compromise, and treatment worse than the disease) [15,16]. This classification applies best to long and large bones and it is not very useful for the digits, small bones, or the skull [17-19].

Diagnosis of this condition mainly depends on strong clinical suspicion in non healing ulcer especially in diabetic patient, radiological findings of translucency of bone with patchy sclerosis and adjacent periosteal bone reaction. Magnetic Resonance Imaging (MRI) and blood culture along with deeper bone biopsy or culture and pus culture are mainstay in management protocol of these patients [20]. The bacteria most commonly causing chronic osteomyelitis are *Staphylococcus aureus,* Coagulase negative *Staphylococcus, Pseudomonas* spp., *E. coli, Proteus* spp., *Klebsiella* spp., *Enterococcus* spp., *Enterobacter* spp. and anaerobes like *Peptostreptococcus* spp., *Bacteroides* spp., *Clostridium* spp. Rarely *Salmonella* spp. and *Actinomycetes* [21], *Staphylococcus aureus* constitutes 50-75% cases of chronic osteomyelitis. In most of the cases infection is monomicrobial, infection with multiple organisms are usually seen in diabetes mellitus patients with ulcer in foot [22].

Osteomyelitis is an ongoing problem due to emergence of Multidrug Resistance (MDR) strains among bacterial pathogens. 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 Intensive Care Unit (ICU) settings [23]. 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 [24]. But extensive and unnecessary use of the carbapenems facilitated the emergence of carbapenem resistant bacteria which produced carbapenem hydrolysing enzyme Metallo Beta Lactamase (MBL), so called because they contain metal ion that works as a co-factor for enzymatic activity [25]. Methicillin-resistant Staphylococcus aureus (MRSA) is prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide [26].

The study was therefore undertaken to determine the microbiological profile of these cases of osteomyelitis and also to ascertain the antibiotic resistance pattern of these isolates and to find out the MDR strains at a tertiary care centre. It will go a long way in helping the clinician in deciding upon the treatment regime for these patients. The data generated by these studies will also help in formulating hospital antibiotic policies.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Department of Microbiology and Department of Orthopaedics, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India, from January to December 2020. Samples from outpatients and inpatients admitted to the orthopaedic ward suspected to have osteomyelitis was collected after obtaining consent from patients. Ethical clearance was taken from the Institutional Ethical Committee (IEC) reference number (MEC/Reg.N./ECR/872/Inst/2016).

Sample size calculation: n=4PQ/L^2 Where, P=Prevalence, Q=100-p, L=Allowable error, If the allowable error is 10% SS (n)=4×57×43/100

Sample size, n=9804/100=98.04

So, in order to cover up the lost to follow-up, drop-out rate and non response rate the sample size taken in present research study was 100 [27].

Inclusion criteria: 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, was included and processed for culture and sensitivity.

Exclusion criteria: Patients with malignant and benign tumours, cysts, non infected, non unions, old trauma and osteomyelitis patients on antibiotic therapy were excluded from the study.

Study Procedure

Sample collection and preliminary identification by biochemical tests: All clinical specimens, sequestrum/excised tissue/pus samples received from orthopaedic outpatient and inpatient department were collected in a sterile container. Then the preliminary identification was done by standard procedures (Gram staining and biochemical tests). The culture isolates were identified by gram stain morphology, colony characters and biochemical reactions [28].

Antimicrobial susceptibility test: Antibiotic susceptibility pattern was done on Mueller Hinton Agar by Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines. The plates were then incubated at 37°C for 18-24 hours. The zones of complete growth of inhibition around each of the disc were measured by using a scale. The interpretation of zone size into sensitive, intermediate or resistance was based on the standard zone size interpretant chart as per CLSI guidelines (2020) [29]. The control strains used were *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

STATISTICAL ANALYSIS

The data was entered in Microsoft excel and results were expressed in terms of frequency and percentage.

RESULTS

In the present study, out of 100 samples, there were 76% cases reported for the culture positive and culture negative cases 24%. Tibia was the most common bone involved in osteomyelitis (49%) and commonest predisposing factor was seen in trauma 48 (48%) cases, followed by postoperative infections 20 (20%), orthopaedic implants 18 (18%), implant/diabetes mellitus 8 (8%) and least for trauma/diabetes mellitus 2 (2%) [Table/Fig-1,2]. Out of 100 samples, male were 72% and females were 28%. Staphylococcal spp. (47.4%) was predominant, *E. coli* (14.4%) and *Klebsiell*a spp. (11.8%), *Pseudomonas* spp. (9.2%), *Proteus* spp. (5.2%), CoNS (4%) [Table/Fig-3]. Out of 34 organisms isolated, most effective drug of Gram-negative bacilli was colistin, followed by polymyxin B 100 (%), tigycyclin, meropenem, imipenem, and piperacillin/tazobactum [Table/Fig-4]. Among the MDR Gram-negative bacilli were ESBL (50%), AmpC (17.6) and MBL (14.7%)

Bone involved	N (%)
Tibia	49 (49)
Femur	34 (34)
Fibula	4 (4)
Ulna	3 (3)
Radius	2 (2)
Metacarpal	2 (2)
Metatarsal	2 (2)
Humerus	2 (2)
Calcaneus	2 (2)
Total	100

[Table/Fig-1]: Showing bones involved in osteomyelitis.

Predisposing factor	N (%)
Trauma	48 (48)
Orthopaedic implants	18 (18)
Postoperative infection	20 (20)
Implant/Diabetes mellitus	8 (8)
Postoperative infection/Diabetes mellitus	4 (4)
Trauma/Diabetes mellitus	2 (2)
Total	**100**

[Table/Fig-2]: Showing predisposing factors for osteomyelitis.

and they were 100% sensitive for polymixin B and colistin [Table/Fig-5-7]. Out of 42 organisms isolated, most effective drug of Gram-positive Cocci (GPC) was vancomycin, teicoplanin, followed by gentamicin, amikacin, erythromycin, clindamycin and ciprofloxacin [Table/Fig-8].

Organisms	No. of organisms	Percentage (%)
Staphylococcus aureus	36	47.4
Staphylococcus lugdunensis	3	4.0
CoNS	3	4.0
Escherichia coli	11	14.4
Klebsiella spp.	9	11.8
Pseudomonas spp.	7	9.2
Proteus spp.	4	5.2
Acinetobacter baumanni	3	4.0
Total	76	100

[Table/Fig-3]: Showing various organisms isolated.

Antibiotics	*E. coli* (11)	*Klebsiella* spp. (9)	*Pseudomonas* spp. (7)	*Proteus* spp. (4)	*Acinetobacter* spp. (3)
Amoxyclav	1 (9%)	0	0	2 (50%)	0
Gentamicin	7 (63.6%)	5 (55.5%)	4 (57.2%)	3 (75%)	1 (33.3%)
Amikacin	8 (72.7%)	5 (55.5%)	4 (57.1%)	3 (75%)	1 (33.3%)
Ciprofloxacin	2 (18.2%)	0	1 (14.2%)	1 (25%)	0
Cotrimoxazole	2 (18.2%)	0	0	3 (75%)	0
Cefoxitin	3 (27.3%)	0	0	3 (75%)	0
Piperacillin	1 (9%)	0	4 (57.2%)	4 (100%)	0
Piperacillin/ Tazobactum	4 (36.4%)	0	7 (100%)	4 (100%)	0
Ceftazidime	5 (45.4%)	0	4 (57.2%)	2 (50%)	0
Aztreonem	4 (36.4%)	2 (22.2%)	5 (71.4%)	2 (50%)	0
Ceftriaxone	1 (9%)	0	3 (42.8%)	2 (50%)	0
Cefotaxime	1 (9%)	0	1 (14.2%)	2 (50%)	0
Cefepime	2 (18.25)	0	0	2 (50%)	0
Meropenem	11 (100%)	9 (100%)	4 (57.2%)	3 (75%)	3 (100%)
Imipenem	11 (100%)	9 (100%)	4 (57.2%)	3 (75%)	3 (100%)
Colistin	11 (100%)	9 (100%)	6 (85.7%)	4 (100%)	3 (100%)
Polymyxin B	11 (100%)	9 (100%)	6 (85.7%)	4 (100%)	3 (100%)
Tigecycline	11 (100%)	8 (88.8%)	0	2 (50%)	3 (100%)

[Table/Fig-4]: Antibiotic sensitivity pattern of Gram-negative bacilli.

Organisms	No. of isolates	ESBL producers no. (%)
E. coli	11	5 (45.4)
Klebsiella spp.	9	7 (77.7)
Acinatobacter spp.	3	3 (100)
Pseudomonas spp.	7	1 (14.2)
Proteus spp.	4	1 (25)
Total	34	17 (50)

[Table/Fig-5]: Showing Extended Spectrum β Lactamases (ESBL) producers.

Organism	No. of isolates	MBL producers no. (%)
E. coli	11	0
Klebsiella spp.	9	0
Acinatobacter spp.	3	0
Pseudomonas spp.	7	4 (57.1%)
Proteus spp.	4	1 (25%)
Total	34	5 (14.7%)

[Table/Fig-6]: Showing Metallo β Lactamases (MBL producers).

The MRSA was found to be 44.4%. All the MDR Staphylococcal isolates were 100% sensitive for linezolid [Table/Fig-9].

Oragnism	No. of isolates	AmpC producers no. (%)
E.coli	11	0
Klebsiella spp.	9	0
Acinetobacter spp.	3	1 (33.3)
Pseudomonas spp.	7	4 (57.1)
Proteus spp.	4	1 (25)
Total	34	6 (17.6)

[Table/Fig-7]: Showing AmpC producers.

Antibiotics	*S. aureus* (36)	*S. lugdunensis* (3)	CONS (3)
Penicillin	0	0	0
Ampicillin	1 (2.7%)	0	0
Gentamicin	34 (94.4%)	3 (100%)	3 (100%)
Amikacin	31 (86.1%)	0	3 (100%)
Ciprofloxacin	6 (16.6%)	0	0
Erythromycin	20 (55.5%)	0	1 (33.3%)
Clindamycin	20 (55.5%)	0	1 (33.3%)
Cotrimoxazole	14 (38.8%)	1 (33.3%)	0
Oxacillin	16 (44.4%)	0	1 (33.3%)
Cefoxitin	16 (44.4%)	0	1 (33.3%)
Linezolid	35 (97.2%)	3 (100%)	3 (100%)
Vancomycin	36 (100%)	3 (100%)	3 (100%)
Teicoplanin	36 (100%)	3 (100%)	3 (100%)

[Table/Fig-8]: Antibiotic sensitivity pattern of Gram-positive isolates.

Antibiotics	MRSA (20)	MSSA (16)
Penicillin	0	0
Ampicillin	0	1 (6.2%)
Gentamicin	19 (95%)	15 (93.7%)
Amikacin	16 (80%)	15 (93.7%)
Ciprofloxacin	4 (20%)	2 (12.5%)
Erythromycin	11 (55%)	9 (56.2%)
Clindamycin	11 (55%)	9 (56.2%)
Cotrimoxazole	8 (50%)	6 (37.5%)
Oxacillin	0	16 (100%)
Cefoxitin	0	16 (100%)
Linezolid	19 (95%)	16 (100%)
Vancomycin	20 (100%)	16 (100%)
Teicoplanin	20 (100%)	16 (100%)

[Table/Fig-9]: Antibiotic sensitivity pattern of MRSA, MSSA.

DISCUSSION

Osteomyelitis is an inflammatory process that affects the bone due to the contiguous infection, direct inoculation, or haematogenous spread of microorganisms [1]. It is an infectious disease that is difficult to diagnose, and treatment is complex because of its heterogeneity, pathophysiology, clinical presentation and management.

In the present study, an attempt was made to know the microbiological profile of osteomyelitis and their antibiotic sensitivity pattern. The results for culture positive was observed to be 76% and 24% were culture negative. This study was parallel to the study performed by the other authors where the culture positive results was found to be 86% and 89%, whereas culture negative was observed to be 14% and 11%, respectively [30,31]. There was the another study performed by Shah RV and Sanghavi RV, and Khatoon R et al., results of their study were also in correlation to the present study where the culture positive reported was 64% and 84% and the culture negative observed was 36% and 16% [32,33]. In the study

by Padmini B and Deepa S, reported the rate of culture positive to be 87% and the culture negative was observed to be 13% [34]. Several predisposing factors associated with osteomyelitis in the present study is comparable with the studies done by various studies [Table/Fig-10] [30-33,35].

Study series	Publication year	Trauma (%)	Orthopaedic implants (%)	Postoperative infections (%)	Diabetes (%)
Suguneswari G et al., [35]	2013	53.0	4	26	17
Wadekar MD et al., [30]	2014	44	21	23	12
Singh A et al., [31]	2016	48	28	21	-
Shah RV and Sanghavi RV [32]	2017	76	21	40	13
Khatoon R et al., [33]	2017	57	30	34	04
Present study	2023	48	18	20	14

[Table/Fig-10]: Comparison of predisposing factors [30-33,35].

In the present study, the commonest bone affected in osteomyelitis was Tibia, followed by femur, which was in accordance with the studies done by other workers [Table/Fig-11] [30,33,35].

Bone involved	Suguneswari G et al., [35] (2013)	Wadekar MD et al., [30] (2014)	Khatoon R et al., [33] (2017)	Present study (2023)
Tibia	58	23	55	**49**
Femur	31	48	51	**34**
Fibula	-	1	01	**4**
Ulna	2	4	02	**3**
Radius	1	3	02	**2**
Metacarpal	2	4	03	**2**
Metatarsal	1	3	05	**2**
Humerus	3	9	03	**2**
Calcaneus	2	-	03	**2**
Malleolli	-	3	-	-
Patella	-	2	-	-

[Table/Fig-11]: Comparison of different bones affected in osteomyelitis with other workers studies [30,33,34].

In the present study, total of 76 organisms were isolated. The predominant organisms isolated were *S. aureus* followed by *E. coli*, which was in accordance with other studies [Table/Fig-12] [30-33,35].

Antibiotic sensitivity was carried out for 100 isolates by Kirby-Bauer disc diffusion method. Of 42 Gram-positive isolates, were 100% sensitive to vancomycin to linezolid and teicoplanin. Among 34 Gram-negative isolates were 100% sensitive to meropenem, imipenem and polymixin B and colistin. Similar sensitivity was reported by Khatoon R et al., [33]. AST pattern of GPC and Gram-negative bacilli (GNB) of present study and other studies is shown in [Table/Fig-13] [30,32,33].

In the present study, it was observed that the rate of MRSA was found to be (44.4%), ESBL (50%), AmpC (17.6%) and MBL (14.5%). This study was in support with the study performed by Khatoon R et al., where the rate of MRSA was (43.1%), ESBL (51.6%) and AmpC (24.2%) and MBL(14.5%) [33]. In the current study, MRSA isolated was observed to be 16 (44.4%) which was in accordance with the study by Khatoon R et al., [33]. There were another study also performed by the other author where the rate of MRSA isolated was observed to be 52% and the study by Padmini B and Deepa S, also supported present study where the rate of MRSA was observed to be 66% [31,34]. There was a study by Suguneswari G et al., which was in contrast with the current study where the MRSA isolates was observed to be 23% [35].

Clinical symptoms of osteomyelitis can be non specific and difficult to recognise. Signs and symptoms change depending on the category of infection, organism and anatomical location of the disease. From the present study, it was quite clear that drug resistance bacteria along with MRSA strains are becoming alarming because of their increased resistance towards antibiotics-like amikacin, netilmycin, and to a lesser extent to vancomycin and linezolid that leaves the clinicians with less choice to use the appropriate drug for treatment of chronic osteomyelitis. It is high time to emphasise on surveillance to monitor change in aetiology and to follow one health policy to impede the menace created by MDR bacteria.

Study series	Publication year	*S. aureus* (%)	*S. lugdunensis* (%)	*S. epidermidis* (%)	CoNS (%)	*E. coli* (%)	*Klebsiella* spp. (%)	*Acinetobacter* spp (%)	*P. mirabilis* (%)	*P. aeruginosa* (%)
Suguneswari G et al., [35]	2013	53.8	-	13.9	-	-	5.82	6.97	9.30	10.6
Wadekar MD et al., [30]	2104	33	-	-	13.0	12	14	-	3	17
Singh A et al., [31]	2016	53	-	-	5.0	7	9	-	2	2
Shah RV and Sanghavi RV [32]	2017	60.6	-	-	-	4.04	13.1	6.06	-	13.1
Khatoon R et al., [33]	2017	34.2	-	-	14.2	19	12.5	-	5.0	18.3
Present study	**2023**	**47.4**	**4**	**-**	**4**	**14.4**	**11.8**	**4**	**5.2**	**9.2**

[Table/Fig-12]: Comparison of organisms isolated by various workers studies [30-33,35].

Publication year	Study	Antibiotic sensitivity GPC isolates	Antibiotic sensitivity GNB isolates	Place
2014	Wadekar MD et al., [30]	Amikacin, linezolid, vacomycin	Amikacin, imipenem	Mysore Medical College, Karnataka, India
2017	Shah RV and Sanghavi RV [32]	Linezolid, vancomycin	Piperacillin/tazobactum and ceftazidime	Shri MP SHAH G Medical College, Gujarat, India
2017	Khatoon R et al., [33]	Vancomycin, linezolid, teicoplanin	Imipenem, colistin	Integral University Lucknow, UP, India
2023	Present study	Vancomycin, linezolid, teicoplanin	Meropenem, imipenem colistin, polymysin B	RMCH&RC Kanpur, UP, India

[Table/Fig-13]: Comparision of antibiotic susceptibility pattern of (GPC, GNB) with other workers [30,32,33].

Limitation(s)

The drawback of the present research study was the small sample size. More insights about the microbiological profile of osteomyelitis and its antibiotic resistance pattern would have been generated by a large sample size. Also, the present work was self-supported so there was a lack of financial help because of which the gene responsible for MDR could not be targeted.

CONCLUSION(S)

Isolation of causative organism and performance of antibiotic sensitivity studies are critical in the selection of antimicrobial agents. Therefore, antibiotic therapy should be guided carefully by culture and sensitivity is an effective treatment modality. This will prevent development of drug resistance and indiscriminate use of antibiotics.

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