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Etiological profile of Osteomyelitis at a Tertiary care hospital in Hyderabad

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ABSTRACT:

Introduction: Osteomyelitis is defined as an inflammation of the bone caused by an infecting organism.The most common etiologic agents causing osteomyelitis are: aerobic bacteria like Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella, Staphylococcus epidermidis, etc. and anaerobic bacteria like Bacteroides species, Peptostreptococcus, Bifidobacterium, etc. Some osteomyelitis infections could be polymicrobial and most of these bacteria have become multi drug resistant pathogens like MRSA, ESBL & MBL producers. Hence, early detection of such drug resistant isolates helps in early institution of appropriate antimicrobial therapy and thus prevents the development and dissemination of these multidrug resistant strains in the hospital as well as in the community.

Materials and Methods: A total of 100 samples (pus, wound swabs, sequestrum, aspirated fluid, blood) were collected from clinically diagnosed cases of Osteomyelitis (between age groups 16 and 65) attending Orhtopaedic OPD and from those admitted in the wards. 3 specimens were collected from each patient and processed as follows: the first specimen was used for Gram staining and microscopic examination. The second one for isolation of aerobic bacteria and the third specimen was inoculated in pre-reduced RCM broth, for the isolation of anaerobic bacteria. All the specimens were processed according to standard microbiological procedures and AST done by modified Kirby Bauer Disk Diffusion method. For all Staphylococcus aureus isolates, MRSA detection was carried out and for all the Gram negative isolates, ESBL & MBL detection was done.

Results: Among the 100 samples processed, 81 were culture positive, out of which, 71 showed monomicrobial growth of either aerobes or anaerobes and 10 samples showed polymicrobial growth of both aerobe and anaerobic isolates. S.aureus was the predominant isolate(53.52%) followed by Pseudomonas and Klebsiella. Out of the 38 S.aureus isolates, 20(52.63%) were MRSA.Out of the 24 GNBs isolated, [Pseudomonas(13), Klebsiella(08) and E.coli(03)], 09(37.5%) were ESBL and MBL producers. Among the 20 anaerobes isolated, Bacteroides species (08/40%) were predominant.

Conclusion: The present study highlights the importance of microbiological identification of all the organisms causing osteomyelitis and detection of resistant strains among the isolates inorder to initiate appropriate antimicrobial therapy and thereby curb multidrug resistance.

KEYWORDS: Osteomyelitis, S.aueus, MRSA, ESBL, MBL, multidrug resistance.

I. INTRODUCTION:

Osteomyelitis is defined as an inflammation of the bone caused by an infectingorganism, which might reach the bone through blood orevents such as trauma,surgery, or presence of foreign bodies, that disrupt bony integrity and predispose to the onset of infection. [1] The infection may be limited to a single portion of thebone or may involvenumerous regions, such as the marrow, cortex, periosteum, and the surroundingsoft tissue. The infection generally is due to a single organism, but polymicrobialinfections can occur, especially in the diabetic foot. [1,2]

Osteomyelitis can be acute or chronic.Chronic osteomyelitis is acatastrophicsequel of delay in diagnosis and management of the acute stage of thedisease. It presents complex therapeutic challenges and imposes a high cost onhealth care provision. In developing countries, inadequate health care settingfrequentlycombined with patient malnutrition makes chronic osteomyelitis longlasting and causing more severe disability, if not managed adequately and may result in amputation, sepsis or death. [3]

The mostcommonbacteria causing Osteomyelitis are Staphylococcusaureus, Coagulase negative Staphylococcus, Pseudomonas species., E. coli,Proteus species., Klebsiella species., Enterococcus species., and Enterobacter species.Fungal agents are rarely encountered (< 5 % of cases) in causing Osteomyelitis,which include Candida spp., Aspergillus spp., and Dimorphic fungi. [4]Anaerobic bacteria are also emerging as an important cause ofOsteomyelitis. The most frequently isolated anaerobic bacteria arePeptostreptococcus species., Bacteroides species., Propionibacterium species., Bifidobacterium species., Veillonella species, Fusobacterium species, and Porphyromonas species. [5]

Osteomyelitis is an infectious disease that is difficult to diagnose, because of its heterogeneity, pathophysiology, clinical presentation and management. Progressive destruction of the bone and formation of sequestra are characteristics of this disease. [6]Though advanced treatment options are available,Osteomyelitis still continues to be a majorcause of morbidity due to the difficulty in establishing the correct etiologic agent, and treatment failures due to multidrug resistance.[1,7, 8]

Therefore, early investigations to determine the right etiological agent and their susceptibility to antimicrobials plays a crucial role in proper management of the disease, preventing chronicity, reducing hospital stay and complications like amputation. [9,10]

II. MATERIALS AND METHODS:

A cross-sectional study was conducted in the department of Microbiology, Osmania General Hospital, Hyderabad, from August 2019 to October 2020. Institutional Ethics Committee approval was obtained prior to the study. A total of 100 samples were collected from patients aged 16 to 65years, of both sexes, attending Orthopaedic OPD and those admitted in Othopaedic wards, with complaints and clinical signs of Osteomyelitis. The samples included syringe aspirated pus, synovial fluid aspirate, sequestrum, unhealthy granulation tissue and blood. Patients <16yrs of age and >65yrs of age, and those who were not diagnosed nor clinically suspected as osteomyelitis, were excluded from the study.

After taking informed consent, 3 swabs were collected from each patient, of which the 1st one was used for Gram stain and direct microscopy, 2nd swab for for isolation of aerobic bacteria and 3rd one was inoculated onto SDA for isolation of fungi. Syringe aspirated specimens were inoculated in RCM broth and overlaid with liquid paraffin for isolation of anaerobic bacteria. All the specimens were further processed according to standard microbiological procedures and and AST done by Modified Kirby Bauer Disk Diffusion method.

Staphylococcus aureus isolates which were resistant to Cefoxitin with a zone diameter of <21mm were further subjected tophenotypic detection of MRSA and VRSA.Gram negative isolates like Pseudomonas, E.coli and Klebsiella which showed resistance to Cefotaxime with a zone diameter of <22mm, were further subjected to ESBL detection and Pseudomonas isolates which showed resistance to Imipenem with a zone diameter of <15mm were further processed for MBL detection.

Phenotypic Detection of MRSAand VRSA by Cefoxitin Vancomycin E-strip test: [11,12]

Test organisms were lawn cultured on MHA plates containing 2% NaCl and EZY CX-VA E-STRIPS (HIMEDIA) containing gradient concentrations of Cefoxitin on one half and Vancomycin on the other half of the strip, were placed carefully under aseptic conditions. Plates were incubated at 37°C for 16-18 hours and MIC values for each drug noted. All the isolates showing an MIC of <6µg/ml for Cefoxitin and <4µg/ml for Vancomycin were considered as Cefoxitin and Vancomycin resistant respectively.

Phenotypic detection of ESBL by Combined Disk Test: [13]

Test organisms matching 0.5 McFarland turbidity were lawn cultured on MHA plates.Cefotaxime 30µg and Cefotaxime + Clavulanate 30/10µgdisks were placed 15mm apart and plates incubated at 37°C for 16-18 hours. An isolate which showed an increase of >5mm zone diameter around the combined disk alone was considered an ESBL producer.

Phenotypic detection of MBL by Combined Disk Test: [14,15]

Test organisms matching 0.5 McFarland turbidity were lawn cultured on MHA plates. 10µg Imipenem disk and 750µg of Imipenem + EDTA Disk were placed 15mm apart and plates incubated at 37°C for 16-18 hours. An isolate which showed an increase of >7mm zone diameter around the combined disk alone was considered an MBL producer.

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Presumptive identification of Anaerobic isolates:[16]

Direct smear was prepared from the syringe aspirated specimens and gram stained. Inoculated RCM broth tubes which showed abundant gas, turbidity and foul smell after 2 to 7 days of incubation, were presumed to have grown anaerobes. A drop of such inoculum was subcultured onto anaerobic BA plates (HiMedia) supplemented with 5% sheep blood,vitamin K (1mg/ml) and hemin (5mcg/ml). Metronidazole(5µg) disk was placed in the well and Kanamycin (1000µg), Colistin (10µg) andVancomycin (5µg) disks were placed on the streak lines with the help of sterile forceps. For achieving anaerobiasis, the plates were incubated at 37°C in McIntosh Fildes Jar and a sachet of Anaero gas pak(HiMedia) was placed inside the jar. The jar was then tightly sealed with the help ofsterile grease and metallic clips were applied to the rim tomaintain anaerobiasis.

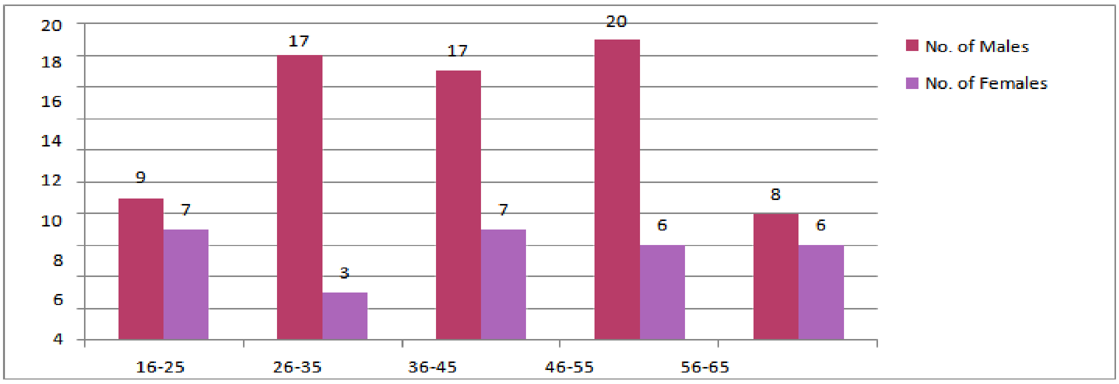
After 48 hours of incubation, the plates were examined for bacterial growth and further identification of the organisms was carried out based on biochemical reactions and AST.

III. RESULTS:

Out of the 100 patient specimens processed, 81 were culture positives while 19 showed no bacterial growth. Among the 81 culture positives, 71 (87.6%) specimens showed monomicrobial growth of predominantly aerobes and 10 (12.3%) specimens showed polymicrobial growth, predominantly, anaerobes.

Age and Sex wise distribution of cases:

Agegroup(Years)	**No. of Males**	**No. of Females**	**Total**
16 – 25	9	7	16
26 – 35	17	3	20
36 – 45	17	7	24
46 – 55	20	6	26
56 – 65	8	6	14
Total	71	29	100



Out of 100 cases studied,71(71%) were males and29(29%) were females, indicating male predominance and the most common age group affected was 46 – 56 years.

Distribution of Acute and Chronic cases:

Age group (years)	**Acutecases <6weeks Duration**	**Chronic cases >6 weeks Duration**	**Total**
16-25	5	11	16
26-35	6	14	20
36-45	4	20	24
46-55	3	23	26
56-65	0	14	14
Total	**18 (18%)**	**82 (82%)**	**100**

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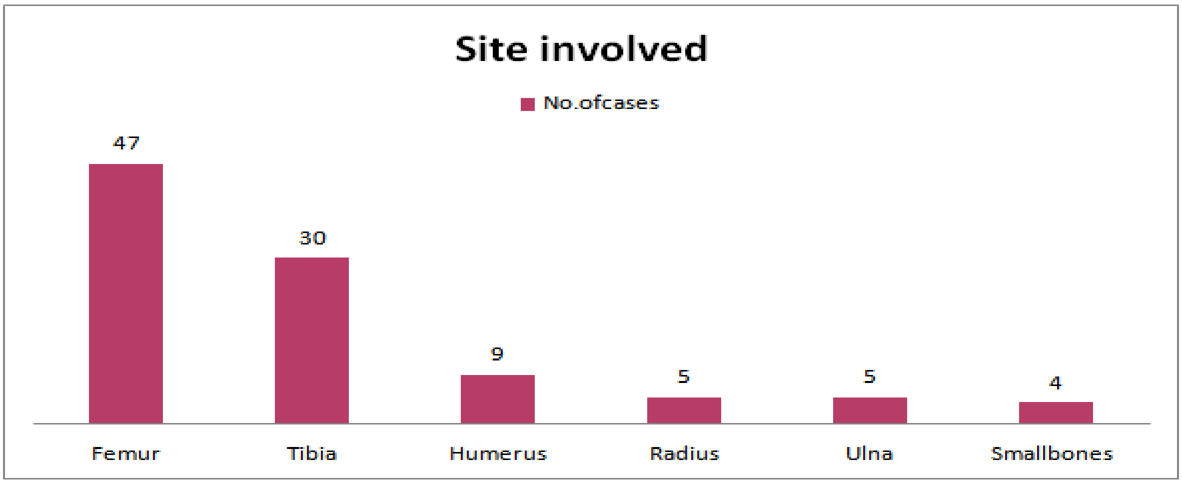
In the present study, 82% of the cases were chronic, indicating that osteomyelitis is more of a chronic than acute disease, leading to prolonged hospital stay, high treatment cost, complications and morbidity.

Various samples studied:

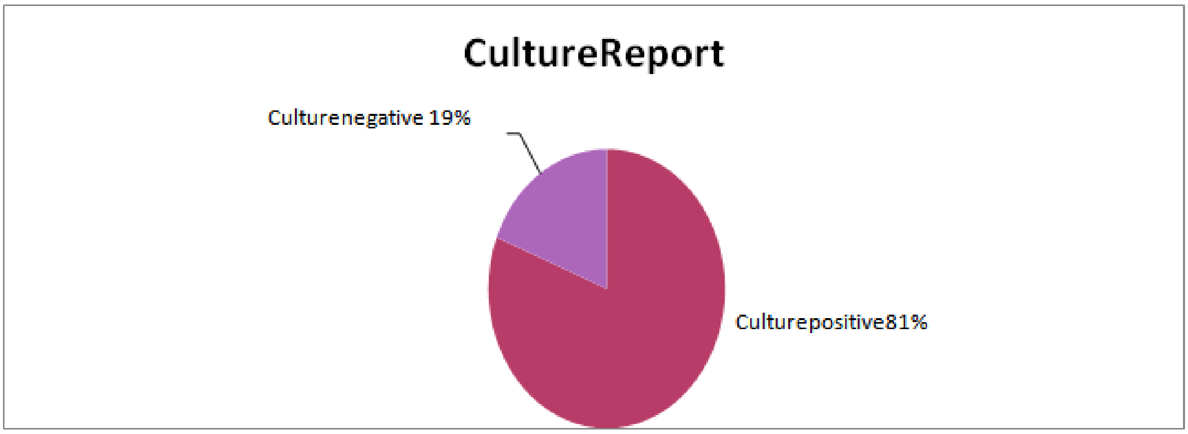
TypeofSamples	**No.ofsamples**
Woundswab	37 %
Syringe Aspirate	29%
Blood	16%
KneeAspirate	11%
Unhealthygranulationtissue	4%
Sequestrum	3%
Total	100%

Site involved:



In the present study, the most common site involved was lower end of femur (47% of cases) followed by upper end of tibia (30%) indicating knee joint is more commonly affected leading to chronic disability. And the most common predisposing factor for osteomyelitis was 'trauma' which was ignored as trivial.

Distribution of Culture positive & Culture Negative cases:



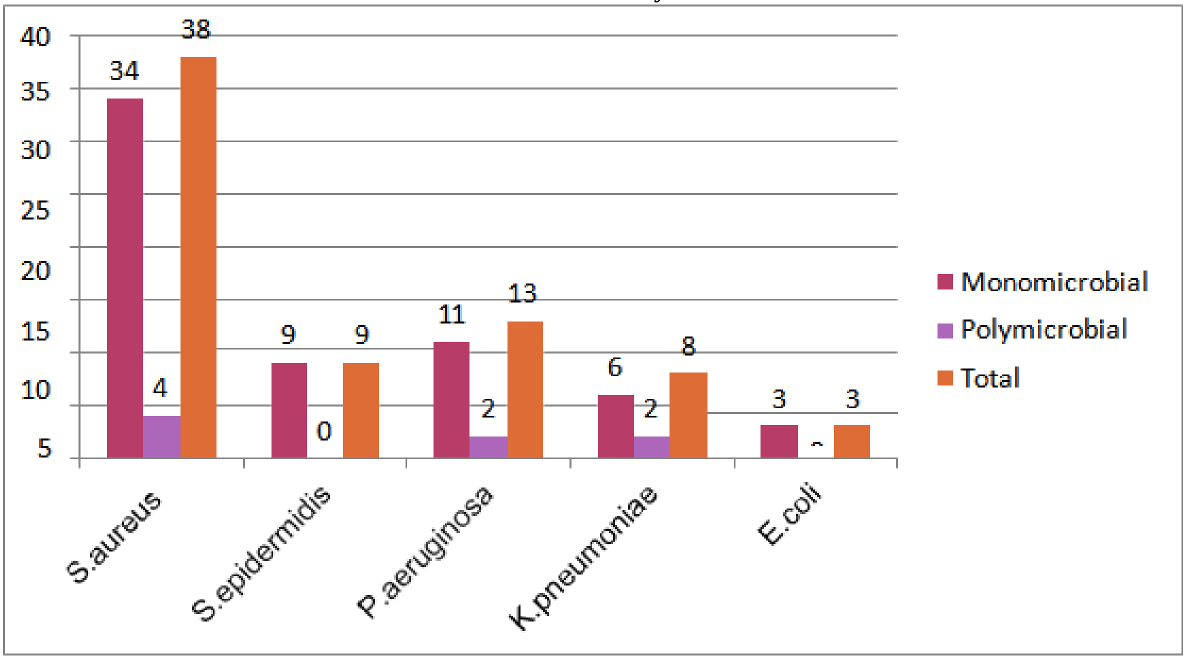
In the present study, out of 100 patient samples processed, 81% of the specimens showed culture positivity whereas 19% of the samples did not show any bacterial growth. This could be attributed to empirical treatment given before sending for culture/sensitivity.

Distribution of micro bialflora among culture positive cases:

Microbialgrowth	**Aerobic Bacteria (71cases)**	**Anaerobes (10cases)**	**Totalno. of organisms**
Monomicrobialgrowth	63	8	71
Polymicrobialgrowth	8	12	20
Totalorganisms	**71**	**20**	**91**

Out of the 81 were culture positivesand91 microorganisms, Monomicrobial growth was seen in 71 cases (out of which 63 were aerobes and 8 anaerobes) and Polymicrobial growth was seen in 10 cases, indicating that monomicrobial growth of aerobes is predominant among osteomyelitis cases. In the 10 specimens which showed polymicrobial growth, anaerobes (12) were predominant than aerobes (8). No fungi were isolated in the present study.

Distribution of Aerobes isolated in Monomicrobial and Polymicrobial cultures:



Out of the 71 cases which showed which showed aerobic growth, Staphylococcus aureus was predominant (53.5%) among the monomicrobial and polymicrobial cultures, followed by Pseudomonas aeruginosa (18.3%), and Klebsiella (11.2%).

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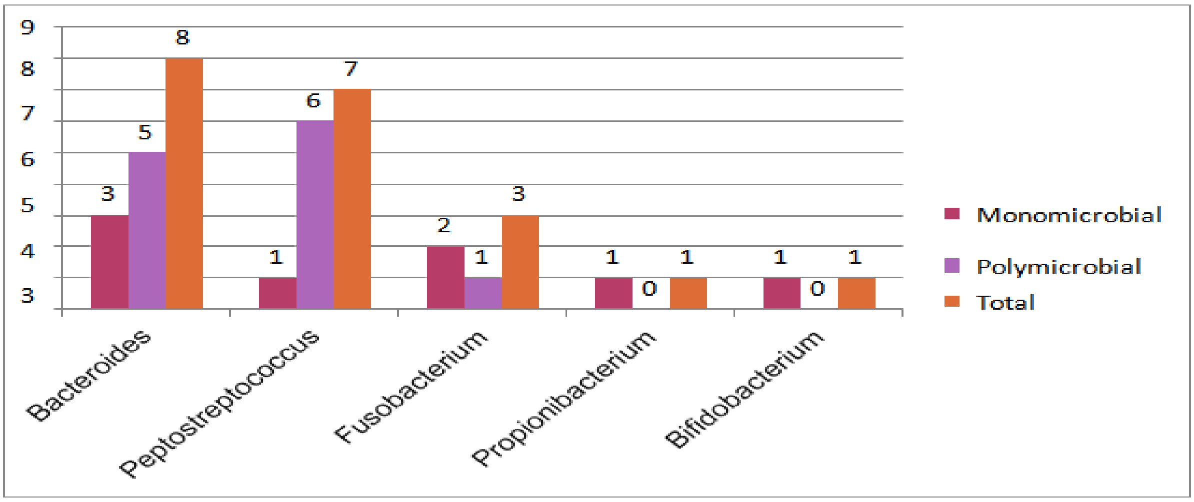
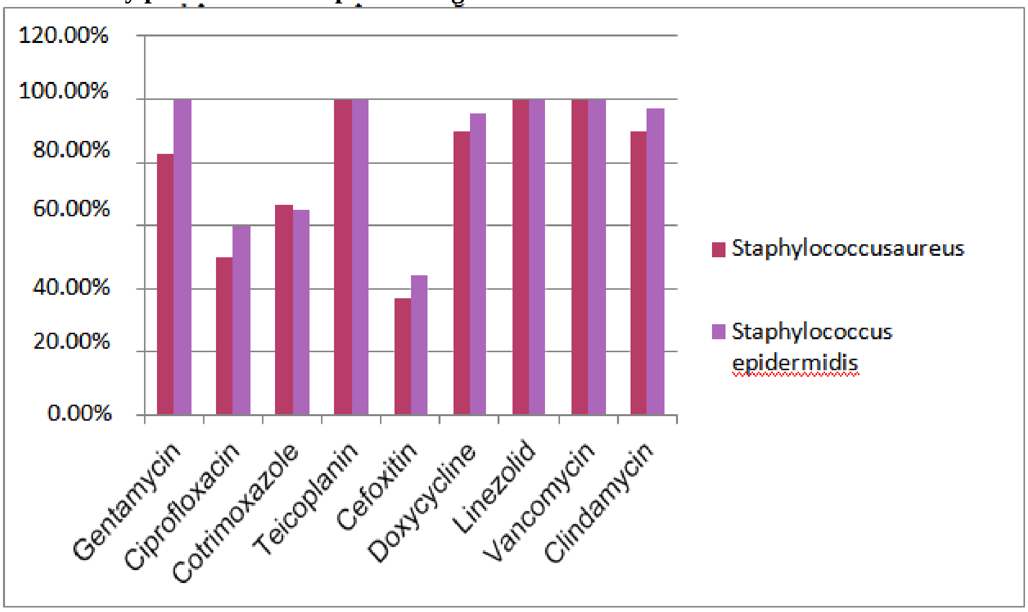
Distribution of Anaerobes isolated in cultures:



Organisms isolated in Polymicrobial growth:

Organisms (Aerobic + Anaerobic)	No. of cases
Staphylococcusaureus+Bacteroidesspecies	3
Staphylococcusaureus+Fusobacteriumspecies	1
Pseudomonasaeruginosa+Peptostreptococcus	2
Klebsiellaspecies+Peptostreptococcispecies	2
Bacteroidesspecies+Peptostreptococcispecies	2
Total No. of Cases	**10**
TotalNo. of Organisms	**20**

Antibiotic sensitivity pattern of Gram positive organisms isolated:



Among the 47 Gram positive isolates, 38 were Staphylococcus aureus and 9 were Staphylococcus epidermidis, all of which were sensitive to Linezolid, Teicoplanin and Vancomycin. Out of the 38 S.aureus isolates, 24 showed Cefoxitin resistance and 5 out of 9 isolates of S.epidermidis showed Cefoxitin resistance. Therefore a total of 29 (24 + 5) Staphylococcal isolates were further subjected to MRSA detection by E-strip test.

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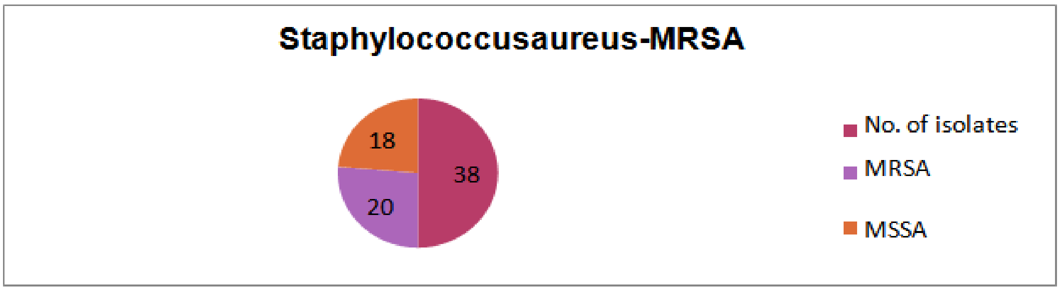
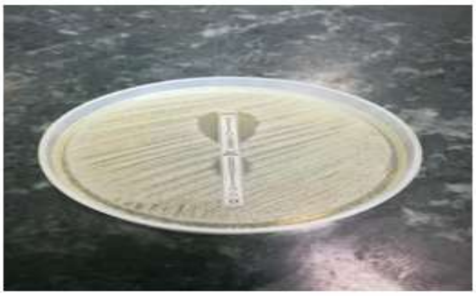
Grampositiveisolates	Cefoxitin resistance by Disk Diffusion Method	Cefoxitin resistance by E-Strip Method
Staphylococcusaureus(38)	24(63.15%)	20 (52.63%)
Staphylococcus epidermidis(9)	5(55.55%)	4 (44.44%)

No. of MRSA detected among S.aureus isolates:



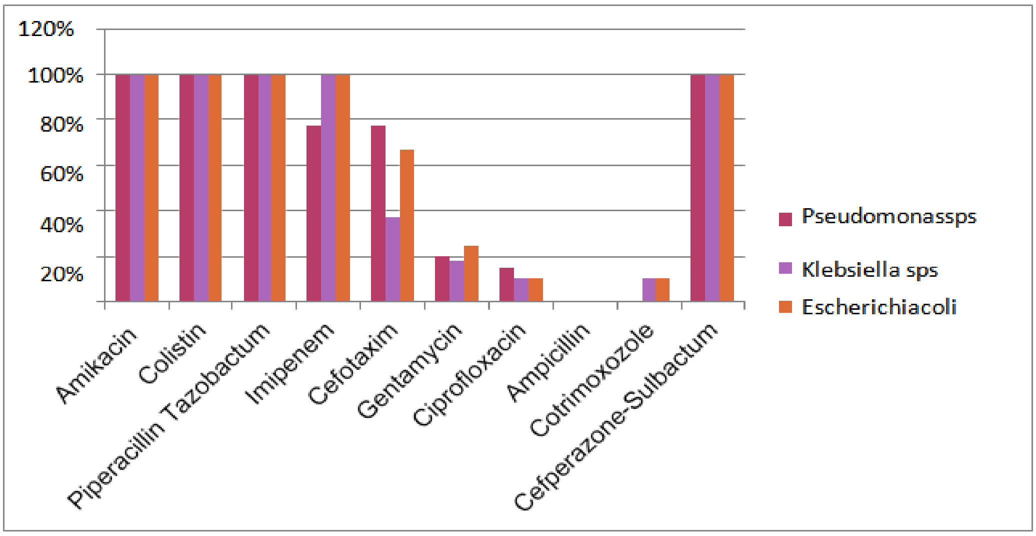
20 out of 38 S.aureus isolates were found to be MRSA whereas none of the Gram positive isolates showed Vancomycin resistance by E-strip Test.

EZYCX-VAE-STRIP(HiMedia)



Cefoxitin resistance in S.aureus with MIC 8µg/ml

Antibiotic sensitivity pattern of Gramnegative bacteria:



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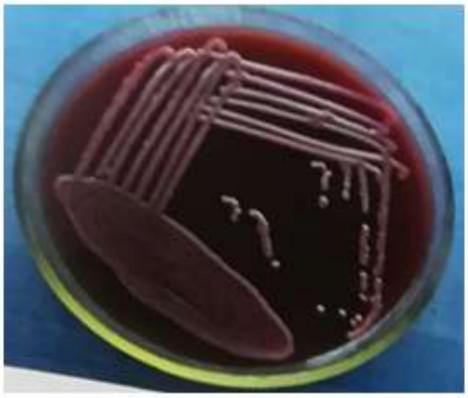
Out of the 24 GNBs isolated, 13 were Pseudomonas, 8 Klebsiella and 3 E.coli, all of which were sensitive to Cefoperazone-Sulbactam, PTZ, Colistin and Amikacin. Out of the 24 GNBs, 9 isolates (3 Pseudomonas + 5 Klebsiella + 1 E.coli) which showed Cefotaxime resistance were further processed for ESBL detection by Combined Disk test (CDT). All 9 isolates were found to be ESBL producers by CDT.

Among 13 Pseudomonas isolates, 3 showed Imipenem resistance too whereas all of the E.coli & Klebsiella isolates were sensitive to Imipenem. The 3 Imipenem resistant isolates of Pseudomonas were subjected to MBL detection by CDT and all 3 were found to be MBL producers.

Pseudomonas – MBL producer



Klebsiella colonies on Blood Agar

AntibioticSensitivityPatternofAnaerobicOrganisms:

Organism	**Metronidazole(5µg)**	**Vancomycin(5µg)**	**Kanamycin(1000µg)**	**Colistin (10µg)**
Bacteroidessps	S	R	R	R
Peptostreptococcussps	S	S	S	R
Fusobacteriumsps	S	R	S	S
Propionibacteriumsps	S	R	S	S
Bifidobacteriumsps	S	S	S	S

All anaerobes were 100% sensitive to Metronidazole.

Anaerobic Blood Agar plate

Anaero Gas Pack for Anaerobiasis

IV. DISCUSSION:

Osteomyelitis is a chronicdisease, difficultto treat or eradicate completely. An increase in the emergence of drug resistant strains makes treatment even more complicated and therefore osteomyelitis still continues to be a amajor problemdue to treatment failures and multidrug resistance.The present study was undertaken toknowthe etiology ofOsteomyelitisand their antibiotic sensitivity pattern.

In the present study, the most common age group affected was between 46 – 55 yr olds with male a predominance, which correlated with the study done by SamuelBA et al **[17]**andAnupama Singh et al **[18]**.The most common predisposing factorfor osteomyelitis in the present study was found to be ‘trauma’, which correlated well with the studies done by Anupama Singh et al **[18]**andHumaira Bashir et al **[19]**. In the present study, monomicrobial growth was predominant than polymicrobial growth and the most common organism isolated was Staphylococcus aureus, which correlated well with the studies done by Aparna Pandey et al [20]. Among the anaerobes isolated, Bacteroides and Peptostreptococcus were predominant and none of the isolates showed resistance to metronidazole.

The present study also focused on detecting MRSA by E-Strip method and found that all the MRSA isolates were sensitive to Vancomycin, indicating that early detection of such isolates can be effectively treated. Among the gram negative isolates, ESBL and MBL detection also was carried out to necessitate appropriate antibiotic therapy.

V. CONCLUSION:

To conclude, the present study highlights the importance of accurate identification and isolation of the etiological agents of osteomyelitis because the prevalence of polymicrobial etiology and missing the identification of anaerobes may delay the diagnosis and lead to unnecessary usage of antibiotics. The study also emphasizes the need to detect drug resistant strains like MRSA, ESBLs and MBLs and thus prevents the development anddissemination of these multidrug resistant strains in the hospital as well as in thecommunity.

REFERENCES:

[1]. Ruchi V. Shah. “Bacteriological Profile in Chronic Osteomyelitis PatientsandTheirAntibiogramin A Tertiary Care Hospital, Jamnagar, Gujarat,India.”IOSRJournalofDentalandMedical Sciences(IOSR- JDMS) , vol. 16, no. 10, 2017, pp. 47–50

[2]. Naseel SalimandJ.Lancy.2017. Bacteriological profile of post traumatic osteomyelitis in a Tertairy care centre. Int.J.Curr.Microbiol.App.Sci. 6(1): 367-372. doi: http://dx.doi.org/10.20546/ijcmas.2017.601.044

[3]. Kuzma Jerzy1,* and Hombhanje Francis2 Chronic Osteomyelitis BacterialFlora,AntibioticSensitivityandTreatmentChallenges,The Open Orthopaedics Journal, 2018, 12, 153-163http://dx.doi.org/10.2174/1874325001812010153

[4]. Canale ST, JamesHB. Campbell’sOperativeOrthopaedics, 11thed. St Louis Missouri: Mosby 2008; 1: 695-709.

[5]. Haitham M Al-Habib*, Mahmood A Aljumaily** *Department of Microbiology,**DepartmentofSurgery,CollegeofMedicine,Universityof Mosul. Bacterial etiology of chronic osteomyelitis involving anaerobes (Ann. Coll. Med. Mosul 2010; 36 (1 & 2): 106-113)

[6]. ShanimolePuthenpurayilEbrahimkutty,KL SaradaDevi,KArun,Ramani BaiJosephTheodore.Prognosisandoutcome ofexogenousbacterial osteomyelitis: A prospective cohort study . **DOI:** 10.4103/0972- 1282.184758

[7]. RezaM,ShahabF,JalalK,FarhadJ,MehdiK, NematollahJ,FarshidA. Epidemicassessment of bacterial agentsinosteomyelitis and their antibiotic resistance patterndetermination.Journal ofBiologicalSciences2008;8(2): 478-481

[8]. ZuluagaAF,GalvisW,VesgaOetal.Etiologic diagnosisofchronic osteomyelitis. Arch Intern Med 2006; 166: 95-100.

[9]. A.K. Ako-Nai, I.C.Ikem, A. Aziba et al. Bacteriological examination of chronicosteomyelitiscasesinILE-IFE,SouthwesternNigeria.African Journal of Clinical and Experimental Microbiology 2003; 4(2): 41-51

[10]. Prieto-PérezL,Pérez-TanoiraR,Petkova-SaizE,etal. Osteomyelitis: a descriptive study. Clin OrthopSurg. 2014;6(1):20-5. Nelson CL, Duthie RB, Bentley U. Mercer’s Orthopaedic surgery, 9thed.London: Arnold 2003; 561-77.

[11]. Kale SS et al.The study of reduced susceptibility of methicillin resistant

Staphylococcus aureus to various antibiotics with special reference to glycopeptides in a tertiary care hospital in central IndiaInt J Community Med Public Health. 2019 Apr;6(4):1426-1433.

[12]. Moses, Kuruvilla and Thomas, A prospective study to evaluate methods of MRSA detection in patients with soft tissue and bone infection in a tertiary care centre, Indian Journal of Microbiology Research 2020;7(2):154–160.

[13]. Tantry et al. Detection of Extended-Spectrum ß-lactamases Production by Escherichia coli: A Phenotypic Comparative Study, J Pure Appl Microbiol, **12**(**4**), 2245-2252 Dec. 2018,http://dx.doi.org/10.22207/JPAM.12.4.64.

[14]. Timothy R Walsh,Mark A Toleman,Laurentpoirel and Patrice Nordmann. Metallo-β- lactamases:the quiet before the storm?Clin.Microbiol.Rev.Apr 2005;18(2):306-325.

[15]. Gales, A. C., L. C. Menezes, S. Silbert, and H. S. Sader. 2003. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant Pseudomonas aeruginosa producing SPM metallo--lactamase. J. Antimicrob. Chemother. 52:699– 702.

[16]. Padmaja Ananth Shenoy et al., Anaerobic Bacteria in Clinical Specimens, Journal of Clinical and Diagnostic Research. 2017 Jul, Vol-11(7): DC44-DC48.

[17]. Samuel BA, Raphael OA. Chronic osteomyelitis in Ilorin, Nigeria. South African Journal of Surgery 2008; 46(4): 116-118.

[18]. Singh A, Biswas PP, Sen A. Bacteriological profile of osteomyelitis cases with special reference to antibiotic susceptibility pattern of isolates in a tertiary care hospital of eastern India. J Evolution Med Dent Sci. 2016;5(53):3496- 3501, DOI: 10.14260/jemds/2016/80.

[19]. Humaira Bashir1 and Asifa Nazir1*Clinico-microbilogical Profile of Chronic Osteomyelitis in a Tertiary Care Hospital of North India, Department of Microbiology, Government Medical College and Associated Hospitals, Srinagar, Jammu and Kashmir, India. Microbiology Research Journal International 30(2): 11-18, 2020; Article no.MRJI.55027 ISSN: 2456-7043 (Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140.

[20]. Aparna Pandey1*, Prachi Shaw1 and Aamir Johar,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,Journal of Advances in Medicine and Medical Research 32(3): 43-52, 2020; Article no.JAMMR.54452 ISSN:2456-8899 (Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965) DOI:10.9734/JAMMR/2020/v32i330381.