EVALUATION OF BACTERIOLOGICAL PROFILE OF CHRONIC OSTEOMYELITIS IN A TERTIARY CARE HOSPITAL WITH SPECIAL EMPHASIS ON DOMINANT PATHOGEN STAPHYLOCOCCUS AUREUS

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University

In partial fulfillment of the requirement

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH IV

APRIL 2013

THANJAVUR MEDICAL COLLEGE, THANJAVUR

THE TAMILNADU DR. M. G. R. MEDICALUNIVERSITY

CHENNAI, TAMILNADU

CERTIFICATE

I hereby certify that the Dissertation entitled, **"EVALUATION OF BACTERIOLOGICAL PROFILE OF CHRONIC OSTEOMYELITIS IN A TERTIARY CARE HOSPITAL WITH SPECIAL EMPHASIS ON DOMINANT PATHOGEN STAPHYLOCOCCUS AUREUS"** Submitted to DR. M.G.R MEDICAL University ,in partial fulfillment of regulations required for the award of M.D Degree in microbiology is a record of original research work done **by DR. MOHAMED ALI KMS**, carried out in the Department of microbiology, Thanjavur Medical College , Thanjavur during the period from April 2011 to April 2012 under my guidance and supervision and the conclusions reached in this study are his own.

Dean

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Period of Study : April 2011 to April 2012

College : Thanjavur Medical College

Dissertation Topic : Bacteriological profile of Chronic Osteomyelitis in a tertiary care hospital

The Ethical Committee, Thanjavur Medical College has decided to inform you that your Dissertation is accepted and you are permitted to proceed with the above study.

Place: Thanjavur

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ACKNOWLEDGEMENT

I am immensely grateful to our Honourable Dean, **DR C. GUNASEKARAN MD, DCH , Thanjavur Medical College,** for permitting me to conduct the study.

My sincere thanks to our Professor and Head of Department, **DR. SHANKAR, M.D**, who helped me to choose the topic and for guiding me throughout my work especially in techniques involving MRSA isolation and susceptibility tests. He has always been patient, encouraging and giving valuable suggestions to improve my work.

I am extremely thankful to my professor **DR. LOGESHWARI SELVARAJ , M.D**, Micro for their valuable guidance.

I am extremely grateful to our Associate Professors **DR. S.RADHAKUMARI, M.D, DR. PAVITRA DEVI, M.D**, **DR. LALITHA, M.D**, without whose advice and help I could not have proceeded with my thesis.

I extend my thanks to **DR. AYISHA ,M.D, DR.VASUKI M.D**, our Assistant Professors, whose optimism and encouragement was of immense help in carrying out my dissertation work.

I am thankful to **DR. GHULAM MOHAMED, M.S Ortho, PROFESSOR AND HOD OF ORTHOPAEDIC SURGERY DEPT**, **DR. KUMARAVEL, M.S Ortho, PROFESSOR OF ORTHOPAEDIC SURGERY DEPT**, for his kind support and co-operation.

I am thankful to the Assistant Professor of **ORTHOPAEDICS DR. SIVASENTHL** and **DR. CHINNA DURAI** for their excellent cooperation.

I am thankful to the post graduates of Orthopaedics **DR. MOHAMED FAIZER, D.Ortho (II Yr), DR. RENJIT JOHN MATHEW, M.S Ortho (II Yr), DR. VINODH, M.S Ortho (I Yr), DR. RAEEZ.**

My thanks to **MR. D.SIVA KUMAR, M.Sc, Mrs.S.VASANTHI, M.Sc .** Our non medical demonstrators, who also helped me during my study. My thanks **to Mrs. SARANYA, Mr. GUNASEKAR, Mr. SARAVANAN, Mr BALRAJ,** for helping me whenever I needed to collect medical records of the patients who were followed up.

Last but not the least, I thank my **Colleagues DR. P. SHAMMUGA PRIYA, DR.S. SWARNA, DR.K. FATHIMA, DR.R. SUBBULAKSHMI, DR. K.MALATHI** and my **FAMILY** and **FRIENDS** for their moral support, patient understanding and helping me whenever I needed.

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CDC	Centre for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
CNS	Central Nervous System
CONS	Coagulase Negative Staphylococcus aureus
CT	Coagulase Test
CVS	Cardio Vascular System
ESBL	Extended Spectrum Beta Lactamase
ESR	Erythrocyte Sedimentation Rate
ICU	Intensive Care Unit
IMVIC	Indole/Methyl Red /Voges Proskauer /Citrate tests
MHA	Mueller Hinton Agar
MIC	Minimal Inhibitory Concentration
MRI	Magnetic Resonance Imaging
MRSA	Methicillin Resistant Staphylococcus Aureus
MSSA	Methicillin Sensitive Staphylococcus Aureus
NCCLS	National Committee for Clinical Laboratory Standards
PBP	Penicillin Binding Protein
PVL	Panton-Valentine leukocidin
TMCH	Thanjavur Medical College & Hospital
TSI	Triple Sugar Iron Agar
WHO	World Health Organisation

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INTRODUCTION

The first description of chronic osteomyelitis date back to early Sumerian carvings, the fossil was 250 million years old. At that time the mode of treatment was irrigation, immobilisation and bandaging. [1] Traditional treatment included the use of honey, donkey faeces and even wine. In the past three centuries, the treatment involved the use of local ointments.

In 1834, Nelaton coined the term osteomyelitis. [2]

In Greek, ***Osteon*** means bone, ***myelo*** means marrow, ***itis*** means infection.

Osteomyelitis is primarily caused by bacteria . It can also be caused by fungal and even viral infections. Usually occurs in paediatric age group and in immunodeficient individuals.

Haematogenous osteomyelitis is most common in children. Osteomyelitis from adjacent source of infection (diabetic ulcer), post trauma, post operative conditions are common in the elderly age group.

Chronic osteomyelitis is identified radiologically by the presence of dead necrotic bone and new bone formation and surgically by persisting discharging sinus. The fragment of dead bone is called sequestrum.

In Latin, the words sequester means depositary and sequestrate means to give up for safe keeping.

In Latin the word involucrum means “enveloping sheath or envelope”.

In osteomyelitis, there is a process of isolation of infective material and slow resorption of the infective material by the immune system.

Mercer Rang in the book “The story of orthopaedics” appreciated the development of anaesthesia, antisepsis and radiography for the successful development of orthopaedic surgery. [2]

The advent of anaesthetic agent like morphine, heroin increase the number of surgical procedures without antisepsis leading to surgical infections.

In 1848, it was Semmelweiss who demonstrated the use of hand wash in obstetric delivery reducing maternal mortality from 18% to 1%.

It was after the discovery of use of disinfectant for surgical hand wash by Joseph lister, the father of antiseptic surgery post operative infection

decreased dramatically. Use of carbolic acid has reduced mortality from 43% to 15% in amputation patients.

Only in the 19th century, Osteomyelitis was understood as bone marrow infection. With the use of antibiotics the incidence of Osteomyelitis has decreased significantly.

The commonest causative organism of Chronic Osteomyelitis is

- Staphylococcus aureus [32]
- Coagulase negative staphylococci
- Pseudomonas
- Proteus
- Escherichia coli and
- Enterococci

Staphylococcus aureus constitutes 50% – 75% cases of Chronic Osteomyelitis [10]

There is emergence of Gram negative organisms as predominant pathogens in Chronic Osteomyelitis following

- Injury
- Adjacent septic focus and
- Prolonged hospital stay of the patient

The incidence of anaerobic Osteomyelitis is on the rise because of low oxygen tension at the infection site due to the presence of devitalized tissue.

In diabetic patient, Osteomyelitis secondary to foot ulcer is a common occurrence.

Diabetic patients have 15% lifetime risk to develop pedal ulcer. [46-48] In diabetic foot ulcer, the underlying bone gets infected in 66% of the patients. [49]

Apart from having severe infections, prevalence of osteomyelitis in diabetic foot ulcers is about 10% to 20%. [50, 51]

The mortality, morbidity as a result of Osteomyelitis in diabetic patient is very high. Chronic Osteomyelitis is difficult to treat and the occurrence of relapse is very high even after successful treatment.

Removal of dead bone is the gold standard of treatment. [25]

The relapse of Osteomyelitis even after 80 years has been documented.

Most common causes of treatment failure is due to

- Inadequate bone debridement
- Presence of prosthetic materials

- Bacteria hiding in the host endothelial cells and
- Existing as dormant form in the biofilm

Due to this Orthopaedic surgeon treat the patient with high dose parenteral antibiotic therapy it takes 3 to 4 weeks for the infected bone to revascularise.

The antimicrobial agent of choice depends on

- the type of organism isolated,
- their anti microbial susceptibility,
- pharmaco kinetic factors like bone penetration
- vascularity of the affected area ,
- presence of any prosthetic material and
- the patient tolerance to the first line antibiotic.

The age of the patient and the presence of any vascular insufficiency affect the management and prognosis of the patient.

The use of oral antibiotics in paediatric Osteomyelitis is successful.

In adults, the duration of treatment is greater than six weeks parenteral for the drug to attain adequate concentration in the bone due to vascular insufficiency.

The literatures at present are inadequate to guide us about the oral or parenteral antibiotic therapy and duration of treatment.

Epidemiology of Osteomyelitis

In UK incidence of acute haematogenous Osteomyelitis is about 10-100 / 100,000 of population per year.

In United States Osteomyelitis incidence is below 2% per annum (Paluska).

It is observed that under the age of 1, the Incidence is more.

In children the existence is 1 out 5,000 (King).

In subjects treated for acute osteomyelitis, the occurrence of chronic osteomyelitis is in the range 5% to 25% in US (Khan).

Occurrence can go as high as 30% to 40% in people with diabetes and 16% after foot puncture (King).

According to Gustilo [11], the occurrence of infection in open fractures is 2% to 50%.

Gustilo and Anderson [12] have classified open fractures depending on the extent of soft tissue injury as type 1, 2, 3, 4.

The infection rate in type 1 and 2 open fracture is 2%.

Type 3 and 4, the infection rate is 10 to 50%. [11, 12]

increased incidence of infection in Type 3 and Type 4 open fracture is due to gross contamination of wound, loss of overlying skin coverage and bone fracture.

Tibia is the most common site of open fracture and infection.

In a retrospective cohort study, open tibial fracture reported 56% of infection rate. [13]

The incidence of post operative infection in simple spinal surgeries is 1% to 2%. In spinal fusion surgery the infection rate is high 3 to 6% [32, 33] due to blood loss, soft tissue injury, and increased duration of surgery.

Spinal implants act as source of infection and the infection rate is 6 to 8%. [34, 35] The overall prevalence of Osteomyelitis is 5 to 6%.

In a cohort study involved 8905 patients; the incidence of diabetic foot ulcer is 5.8% of which 15% developed Osteomyelitis.

AIM OF STUDY

- To determine the bacteriological profile of Chronic Osteomyelitis.

- To determine the antimicrobial susceptibility of the bacterial isolates of Chronic Osteomyelitis.

- To find out the prevalence of MRSA in Chronic Osteomyelitis patients.

- To provide guidelines for empirical antibiotic treatment.

REVIEW OF LITERATURE

In 2008, Alok.C.Agrawal et al, In India found, Staphylococcus aureus-21, Streptococcus -7, Klebsiella-9, Proteus-7, E.coli-38 and Pseudomonas-29 out of 111 cases of chronic Osteomyelitis. [29]

In 2011, Vladimir Cordeiro et al, In chronic Osteomyelitis patients observed Enterobacter-24.7%, Acinetobacter baumannii-21.4%, Pseudomonas aeruginosa-19.8%, Klebsiella pneumonia-8.2%, Serratia marcescens-6.6%, Proteus mirabilis-5.7%, Escherichia coli-4.9%, Providencia stuarti -2.4%, Morganella morganii-2.4%, Stenotrophomonas maltophilia-1.6%, Lecleria adecarboxylata-0.8%, Pantoea agglomerans-0.8%. They observed below in their study of 121 cases. [28]

In 2010, Dr.Mita D. Wadekar et al, observed Staphylococcus aureus - 43%, Pseudomonas aeruginosa- 10%, Proteus species - 6%, E.coli - 5%, Klebsiella species - 5%, Staphylococcus epidermidis - 4%, Enterobacter species - 3%, Streptococcus pyogenes - 2% and Enterococcus species - 2%. [56]

In 2008, Kaur J et. al, observed Staphylococcus aureus - 43%, Proteus species - 6%, Escherichia coli - 5%, Enterobacter species -3%, Klebsiella species - 5%. Beta-lactamase resistance in 81.4% strains, Methicillin resistance 27.9% in chronic Osteomyelitis patients. [27]

In 2001, Haider abdul lateef mousa et. al, observed Staphylococcus - 45.2% in haematogenous osteomyelitis , Pseudomonas aeruginosa-25% in postoperativeosteomyelitis, Anaerobes-26%, Proteus-12.9%. [53]

In 2008, Ethan Rubinstein et. al, found that hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) accounts for 20%–40% of MRSA patients. All isolates are resistant to erythromycin and β-lactams. They also found, Out of 396 patients, 203 received aztreonam and linezolid, and 193 received vancomycin and aztreonam. MRSA pneumonia was diagnosed in 32 patients. Success rate was 66% for patients on linezolid treatment and 68% for patients on vancomycin. [26]

Iran, during a 15-month period (January 2004-March 2005), Oxacillin resistance was present in 99 of 277 cases (36%) of Staphylococcus aureus isolates from blood and wounds. All MRSA isolates were susceptible to teicoplanin,vancomycin, , tigecycline and linezolid. Marked resistance of MRSA to beta-lactam drugs and high resistance (> 95%) to tetracycline, azithromycin, kanamycin, erythromycin, gentamicin, and ciprofloxacin is observed. [14]

335 cases out of 358 (93.57%) is Vancomycin sensitive. Sixteen cases showed vancomycin intermediate resistance. Vancomycin resistance was found in remaining seven isolates. All isolated VRSA were resistant to

rifampicin, ceftazidime. 86% were tetracycline susceptible and 71.4% were susceptible to chloramphenicol and clarithromycin. [15]

In eastern Uttar Pradesh India, the prevalence of methicillin resistant Staphylococcus aureus (MRSA) is studied, 301 out of 549 specimens of Staphylococcus aureus (54.85%) were found to be methicillin resistant. MRSA resistance to penicillin, cotrimoxazole, ciprofloxacin, gentamicin, erythromycin, tetracycline is >80% , 60.5% to amikacin and 47.5% to netilmicin. No vancomycin resistance was appreciated. 32.0% of MRSA strains were multi-drug resistant. [17]

In a study conducted South Africa in 2006 for the prevalence of MRSA in the KwaZulu-Natal (KZN) province, 26.9% were MRSA and all strains were susceptible to teicoplanin, vancomycin. [18]

In the study conducted during Nov-1998 to Feb-2000, out of 91 strains of Staphylococcus aureus, 52 isolates MSSA and 39 MRSA. All MSSA were susceptible to vancomycin, gentamicin, teicoplanin, ciprofloxacin, rifampicin, linezolid and quinupristin-dalfopristin. 90% were erythromycin susceptible. All the MRSA were susceptible to vancomycin, teicoplanin, rifampicin and linezolid, 92% to gentamicin. None of MRSA was erythromycin susceptible or ciprofloxacin susceptible. [19]

In the study of anti-biotic sensitivity of Staphylococcus aureus Malaysian hospitals, Resistance to penicillin was 94.1%, methicillin 39.7%, ciprofloxacin 29.2%, clindamycin 2.1%, erythromycin 45.9%, gentamicin 40.5%, tetracycline 47.2%, co-trimoxazole 38.5%. All isolates are vancomycin sensitive. Erythromycin, gentamicin, tetracycline and ciprofloxacin are least susceptible to MRSA. [20]

Pathogenesis of chronic Osteomyelitis

Normally adults are resistant to bone infection. Yet there are chances for infections that occurs as follows

- Size of inoculum large, greater than 10^5 organisms per gram of tissue. [7]
- Presence of devitalised bone and soft tissue. [8,9]
- Presence of foreign body.
- The microorganisms which reach the bone or the adjacent muscle via blood are from adjacent source of infection or open wound contamination.

Foreign elements like metal ware and bone cement provides surface for bacterial colonisation. According to Elek and Conan [23], the presence of foreign elements greatly reduces the amount of innoculum required to initiate an infection.

Haematogenous spread is an important mode of spread of staphylococcus aureus infection. With the help of receptors bacteria attaches host proteins.

A biofilm is an collection of microorganisms embedded in glycocalyx. [23] Planktonic Bacterias colonising the biofilm is responsible for more than 65%of bacterial infections .The slime layer containing glycocalyx help the bacteria to evade the host immune system like complement and phagocytosis and impart resistance to conventional anti bacterial therapy. The organisms attached to the dead bone forms bio films. [24]

Pathogenesis of Diabetic Osteomyelitis

Peripheral neuropathy is the predominant factor for development of diabetic osteomyelitis. [22]

Bacteriology of Chronic Osteomyelitis

According to Gustilo and Anderson cultures from open fractures give positive results in 70% of cases [36, 37].

Staphylococcus aureus is the predominant organism in children contributing more than 90%.

In adults, staphylococcus aureus contribute 50% to 75% cases of Chronic osteomyelitis. [21]

Coagulase negative staphylococcus (staphylococcus epidermidis) and gram negative bacilli contribute to 1/3 of infection. [43]

Commonest organisms:

Gram positive:

1. Staphylococcus aureus
2. Staphylococcus epidermidis

Gram negative:

1. Escherichia coli
2. Pseudomonas aeruginosa

Occasionally encountered organisms:

Gram positive:

1. Streptococcus viridans
2. Enteroccocus faecalis
3. Diphtheroids

Gram negative:

1. Enterobacter cloacae
2. Klebsiella pneumonia

3. Acinetobacter baumanii
4. Serratia marcescens

Anaerobic organisms:

1. Propionibacterium acnes
2. Peptococcus species
3. Peptostreptococcus species
4. Bacteroides fragilis
5. Clostridium difficile

Staphylococcus aeurus:

Staphylococcus aeurus is a normal commensal organism of anterior nares.

The staphylococcus are gram positive cocci arranged in clusters and they are non sporing, non motile, catalase positive organisms.

Staphylococcus contains bound coagulase which binds to fibrinogen in the plasma and cause aggregation of staphylococcus.

In tube coagulase test, free coagulase causes the plasma to clot. Hence staphylococcus is also called coagulase positive staphylococcus. Staphylococcus aureus causes pyogenic infections. [6]

Morphology and Culture Characteristics: [5]

- Staphylococcus aureus is 1 micro meter in size.
- On nutrient agar after 18-24 hrs incubation at 37 deg C forms colonies of 1 to 3 mm in diameter, smooth glistening densely opaque colonies.
- In blood agar, the colonies are surrounded by a zone of haemolysis.
- It produces cream to gold colour pigmentation on nutrient agar.
- In mannitol salt agar it forms yellow colonies 1 mm in diameter surrounded by yellow medium due to acid formation.

Bio Chemical Reactions:

- Tube Coagulase test positive
- Bound Coagulase test positive.
- Voges-Proskauer test positive
- Lactose fermentation variable

Staphylococcus epidermidis:

Staphylococcus epidermidis is the normal commensal of the skin and mucous membrane occasionally involved in infective endocardits.

Coagulase negative Staphylococcus is the commonest organism involved in the prosthetic joint infection followed by Staphylococcus

aureus. Staphylococcus epidermidis together with gram negative baclli constitute one-third of cases of chronic osteomyelitis . [43]

Staphylococcus epidermidis secretes biofilm made of glycocalayx which enhances the adherence of bacteria to any necrotic bone or any bone implants and bone cement. [41,42,38,39,40]

Pseudomonas aeruginosa:

Pseudomonas aeruginosa is widely prevalent in nature. Exotoxin A kills host cells by disrupting protein synthesis.

Osteomyelitis caused by Pseudomonas aeruginosa is more common in Intravenous drug addicts.

The carrier rate of Pseudomonas aeruginosa in human faeces is less than 10%. Prolong hospital stay increases the carrier rate to 30% after 3 weeks.

Morphology and Culture Characteristics: [5]

- Pseudomonas aeruginosa is a gram negative bacilli, catalase positive, oxidase positive, motile organism.
- It produces pigments like pyocyanin, pyorubrin and pyomelanin.

- On nutrient agar after 24 hrs incubation at 37 deg C produces large low convex colonies oval with the long axis in the line of innoculum.
- In MacConKey agar, it produces colourless colonies.
- It gives characteristic grape like smell due to amino aceto phenone.
- Presence of blue, green pigmented colonies confirms the presence of Pseudomonas aeruginosa.

Bio Chemical Reactions:

- Indole Test negative
- Methyl Red Test negative
- Voges-Proskauer Test negative
- In triple sugar iron agar test, sugars are not fermented and H_2S not produced.

Escherichia coli:

Escherichia coli is a member of entero bacteriaceae.

Morphology and Culture Characteristics:

- 2-4 micrometer x 0.6 micro meter in size, gram negative bacilli.

- On nutrient agar ,after 18-24 hrs incubation at 37 deg C forms colonies of 2 to 3 mm in diameter, smooth low convex colonies.
- In MacConKey agar, it produces lactose fermenting pink colour colonies.
- In blood agar ,the colonies are surrounded by a zone of haemolysis.

Bio Chemical Reactions:

- Indole positive
- MR positive
- Voges-Proskauer test negative
- Citrate test negative,
- Urease test negative
- In triple sugar iron agar test, acid slant by acid butt no H_2S production

Klebsiella pneumoniae:

Klebsiella pneumoniae is a member of enterobacteriaceae family.

It is a gram negative bacilli, capsulated organism, non motile, oxidase negative, catalase positive organism.

Morphology and Culture Characteristics:

- On MacConKey agar, after 18-24 hrs incubation at 37 deg C, it forms lactose fermenting colonies of 2 to 3 mm in diameter, smooth convex colonies.
- In blood agar, large greyish white mucoid colonies are produced.

Bio Chemical Reactions:

- Indole negative
- MR negative
- Voges-Proskauer test positive
- Citrate test positive
- Urease test positive
- In triple sugar iron agar test, acid slant by acid butt and abundant gas production.

Proteus:

Proteus is gram negative bacilli, motile, non lactose fermenting, catalase positive organism.

Proteus mirabilis is the most common species of Proteus.

Morphology and Culture Characteristics:

On MacConKey agar after 18-24 hrs incubation at 37 deg C, it forms non lactose fermenting colonies.

In young cultures, the bacteria are filamentous reaching upto 80 micro meter in length.

In blood agar plate, swarming of proteus is seen.

Bio Chemical Reactions:

- Indole variable
- MR positive
- Voges-Proskauer test negative
- Citrate test variable
- Urease test positive

In triple sugar iron agar test, alkaline slant by acid butt with abundant H_2S production.

MATERIALS AND METHODS

APPROVAL FROM THE ETHICAL COMMITTEE

The study was approved by the ethical committee of Thanjavur Medical College. The informed consent was obtained from all patient who are participating in this study.

STUDY PERIOD

April 2011 to April 2012

PLACE OF STUDY

The study was carried out in the department of microbiology, central laboratory TMCH .

COLLABORATING DEPARTMENT

Collaborating department for the study was department of Orthopaedics Surgery, Thanjavur Medical College.

DESIGN OF STUDY

Observational Study covering bacteriological profile of chronic osteomyelitis and their antibiogram.

MATERIALS

During the April 2011 to April 2012 study period for this study 50 patients who were diagnosed clinically and radiologically as a case of Chronic Osteomyelitis are participating in this study. The risk factors for Chronic Osteomyelitis were obtained from patient case sheets with the help of orthopaedic surgeons in the department of orthopaedic TMCH.

INCLUSION CRITERIA

All cases of chronic osteomyelitis with the following clinical features are included in this study

1. Prolonged history of disease present
2. Constitutional symptoms are absent
3. Frequent flare up of infection occurs
4. Occasionally bony spicules emerges out of the discharging sinus
5. Restricted neighbouring joint movement
6. Deformities like shortening and gross angulations of bone occurs due to bone loss

EXCLUSION CRITERIA

The below cases were excluded from this study

1. Acute osteomyelitis cases
2. Osteomyelitis due to Anaerobic organisms
3. Tuberculous osteomyelitis

EXAMINATION OF CHRONIC OSTEOMYELITIS PATIENTS

In the orthopaedic TMCH ward general examination of patients was done for below details.

- Age and Sex of the Patient
- **Patients Past history regarding**
- hyper tension,
- diabetes,
- smoking ,
- and malignancy.
- **History of present illness**
- duration of illness,
- type of Osteomyelitis,
- implantation of any prosthetic implants,
- To look for any septic focus e.g tonsil, caries tooth, any abscess and skin infections

- To identify the presence of anaemia
- To look for diabetic ulcer
- To rule out mal nutrition

SAMPLE COLLECTION

The specimen included bone aspirate and bone curretings are plated under aseptic conditions in the ortho ward or in ortho operation theatre. Surface swabs are not included in this study.

PREPRATION OF SMEAR

Once the bone aspirate is plated, the remaining pus is spread evenly on a clean slide and then it is allowed to air dry, and heat fixed by passing the slide through the flame intermittently 3-4 times. Then this smear is stained by Gram's staining and viewed under 40x and oil immersion microscope to look for the presence of pus cells and micro organisms.

BACTERIAL CULTURE[52]

The specimen is plated in Nutrient agar, MacConKey agar and blood agar and incubated for 18 hrs at 37 deg C.

The organisms isolated were identified by

- colony morphology,
- gram staining,
- Catalase test,
- Oxidase test,
- Motility test,
- Indole test,
- Methyl Red test,
- Voges Prausker test,
- Citrate test,
- Urease test,
- Triple sugar Iron Agar test,
- Coagulase test specific for S.aureus

The isolates were confirmed and speciated by adapting the standard biochemical procedures.

Colonies are preserved and maintained in nutrient agar slants.

ANTI-BACTERIAL SUSCEPTIBILITY TEST PROCEDURE

In each sterile petri plate, 15-20ml of sterilized MHA medium was poured and allowed to become solid. The bacterial test cultures were spread evenly on the media by using cotton sterile swab.

For Gram positive organisms,

antimicrobial disc like Amoxycillin, Ampicillin, Erythromycin, Gentamicin, Amikacin, Doxycycline, Cotrimoxazole, Ciprofloxacin, Cephalexin, Ceftriaxone, Cefotaxime, Cefuroxime s, Oxacillin, Cefoxitin, Vancomycin and Linezolid was used.

For Gram negative organisms,

antibiotic disc like Ampicillin, Gentamicin, Amikacin, Doxycycline, Cotrimoxazole, Ciprofloxacin, Cephalexin, Ceftriaxone, Cefotaxime, Cefuroxime , Ceftazidime, Ceftazidime +Clavulanic acid and Imipenem were tested.

On agar plates, the discs were kept firm to have complete contact with surface of agar. Discs were placed more than 25 mm apart from centre and incubated for 16 to 18 hrs at 37 deg C. Once incubation period is complete, the zone of inhibition was measured around each disc and interpreting of results is done.

MRSA TEST PROCEDURE

Cefoxitin Disc Diffusion Test:

Cefoxitin, an inducer of mecA regulatory system is used as a surrogate marker for mecA gene-mediated methicillin resistance detection.

MRSA strains with inducible resistance to methicillin grow easily in the presence of cefoxitin when compared with oxacillin, due to increased induction of PBP 2a by cefoxitin. CLSI recommends cefoxitin disc diffusion method for MRSA detection.

Interpretive Criteria for Cefoxitin Disc Diffusion Test - in mm		
	Susceptible	**Resistant**
S. aureus	≥ 22 mm	≤ 21 mm
CoNS	≥ 25 mm	≤ 24 mm

Method:

A 0.5 Mc Farland standard suspension is prepared and lawn culture is made using MHA plate. A 30 µg cefoxitin disc is placed and incubated at 37 deg C for 18 hours and zone diameter is measured in the presence of reflected light. If the zone of inhibition diameter >22mm is considered as methicillin sensitive and < 21mm is reported methicillin resistant. Latest studies indicate disc diffusion tests using cefoxitin disc superior compared to other methods. According to CLSI guidelines, the cefoxitin disc will detect MRSA with mecA gene mediated resistance.

Oxacillin Disc diffusion test:

A 0.5 McFarland standard suspension of S. aureus is prepared and plated on Mueller-Hinton agar with 2-4% NaCl. An oxacillin disc(1µg) is kept on surface and incubated at 37 deg C for 18 hours. Oxacillin disc is resistant to degradation on storage and detects heteroresistant strains. In transmitted light, the zone of inhibition is measured. Zone diameter >13mm is sensitive and <10 mm is considered resistant.

Interpretive Criteria for Oxacillin Disc Diffusion Test - in mm		
	Susceptible	Resistant
S. aureus	≥ 13 mm	≤ 10 mm
CoNS	≥ 18 mm	≤ 17 mm

READING AND INTERPRETATION OF RESULTS

After completion of 16 to 18 hrs of incubation period, each plate was examined for uniformly semi confluent of growth and circular zones of inhibition around the individual disc. Petri plate was examined to measure diameter of zones of inhibition using zone scale that was held by inverting Petri plate. The Petri plate was held a few inches above a black, non reflecting background and illuminated with reflected light.

The disc around which there is no growth of appropriate diameter when measured will indicate whether the organism is sensitive or intermediately sensitive or resistant to the drug. The sizes of the zones of inhibition were interpreted by referring to the CLSI standards and reported as susceptible, intermediate or resistant to the drugs that were tested.

ZONE SIZE INTERPRETATION CHART AS PER CLSI [4]

S.No	Antimicrobial agent	Symbol	Disc. Conc (µg)	Zone size in mm		
				Resistant	Intermediate	Sensitive
1	Amoxycillin	AMX	10	-	-	28-36
2	Cotrimoxazole	COT	1.25/23.75	10	11-15	16
3	Doxycycline	DO	30	12	13-15	16
4	Ampicillin	AMP	10	<13	14-16	>17
5	Oxacillin	OX	1	10	11-12	13
6	Cephalexin	CH	30	<14	15-17	>18
7	Cefotaxime	CTX	30	<13	14-20	>21
8	Ceftriaxone	CTR	30	<13	14-20	>21
9	Cefuroxime	CXM	30	14	15-17	18
10	Ceftazidime	CAZ	30	<14	15-17	>18
11	Ceftazidime + Clavulanic acid	CAC	30/10	-	-	27-34
12	Gentamicin	GEN	10	< 12	13-14	> 15
13	Amikacin	AK	30	<14	15-16	>17
14	Ciprofloxacin	CF	5	<15	16-20	>21
15	Imipenem	IMP	10	<13	14-15	>16
16	Vancomycin	VA	30	-	-	15
17	Linezolid	LZ	30	-	-	25-32

ACTERIAL CULTURE PLATES AND ITS ANTI MICROBIAL SUSCEPTIBILITY

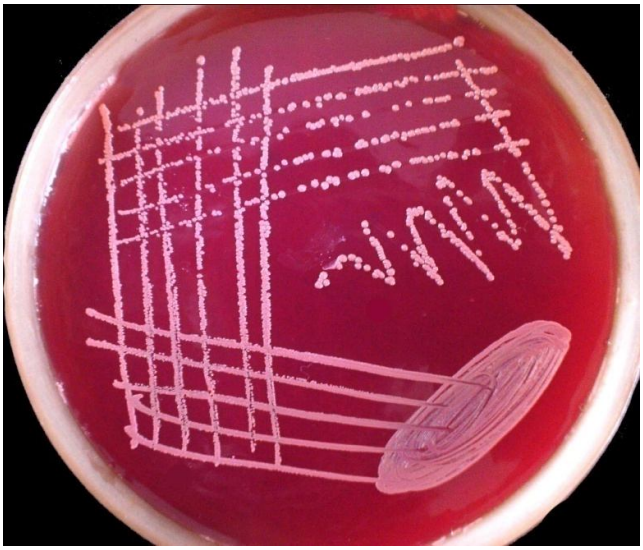


Figure 12 : Colony of Staphylococcus aureus in MacConKey Agar Plate

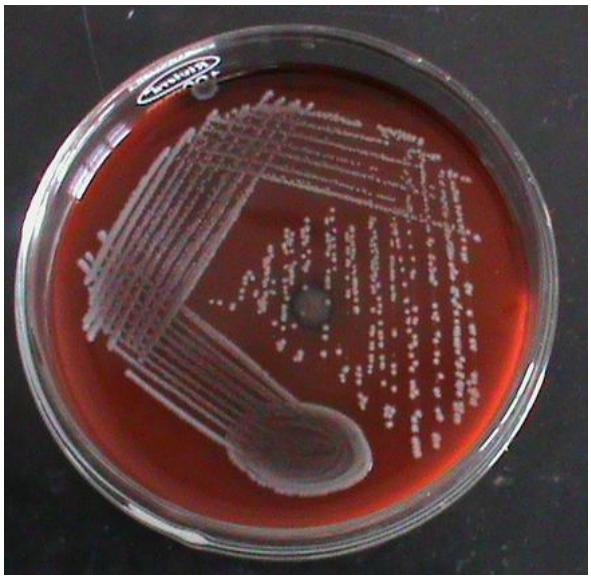


Figure 13 : Colony of Staphylococcus aureus in Blood Agar Plate

Figure 14 : Colony of Staphylococcus aureus in Mannitol salt Agar Plate

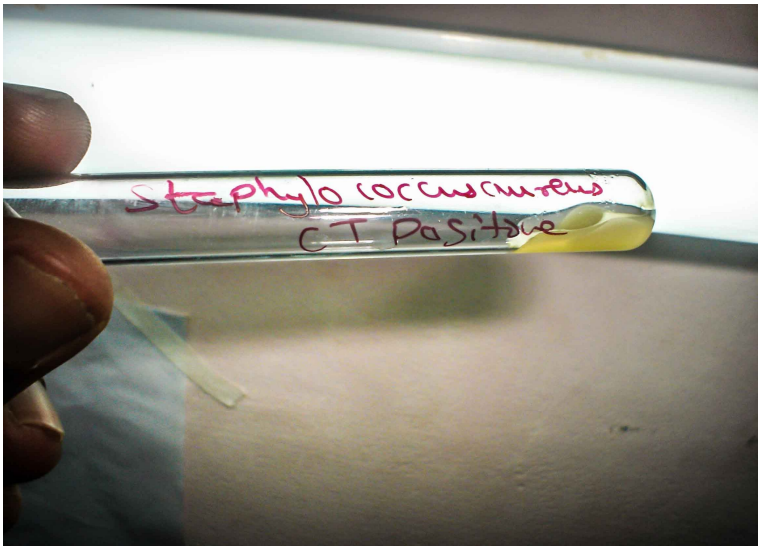


Figure 15 : Coagulase Test Positive - Staphylococcus aureus

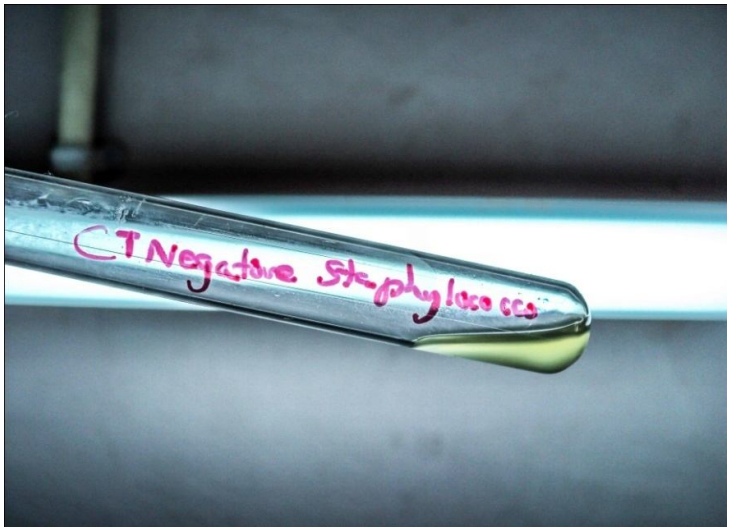


Figure 16 : Coagulase Test Negative – Staphylococcus epidermidis

Figure 17 : Antibiogram of S.aureus sensitive to oxacillin & vancomycin

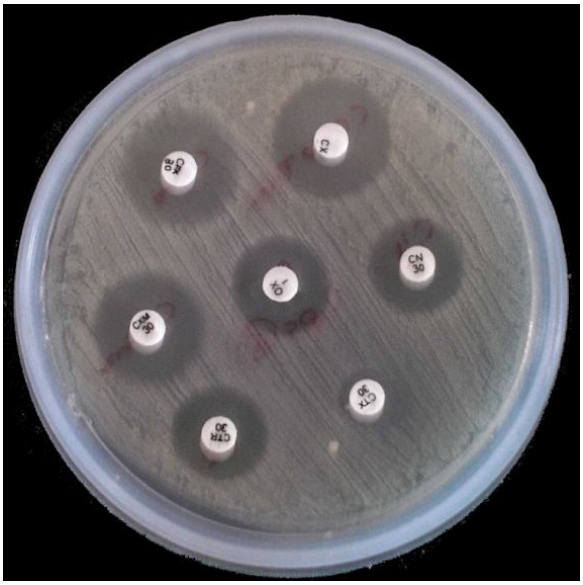


Figure 18 : Oxacillin Sensitive Staphylococcus aureus

Figure 19 : Colony of Klebsiella pneumoniae in MacConKey Agar Plate

Figure 20 : Biochemical reactions of Klebsiella pneumoniae

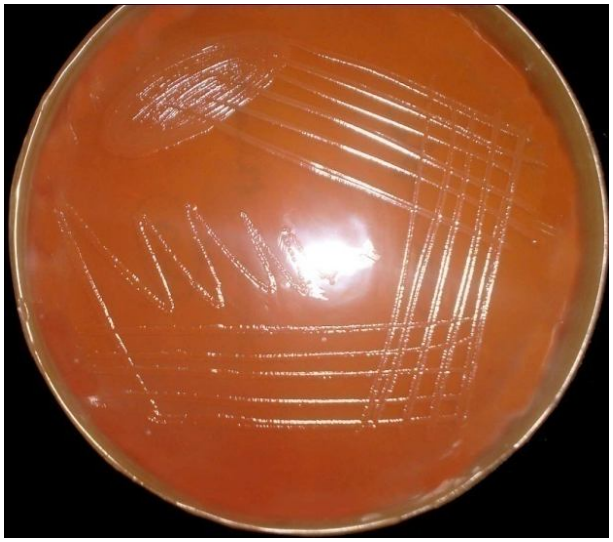


Figure 21 : Colony of Proteus vulgaris in MacConKey Agar Plate

Figure 22 : Biochemical reactions of Proteus vulgaris

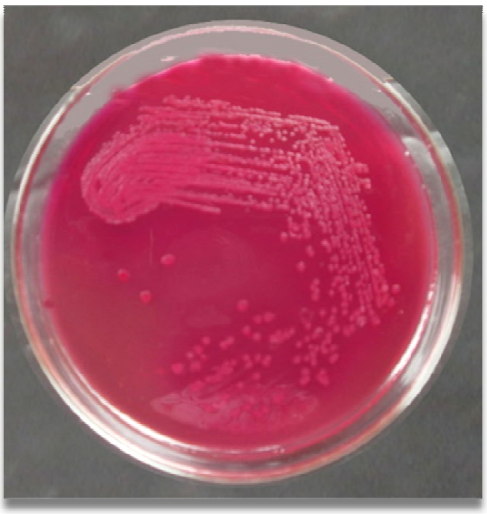


Figure 12 : Colony of Escherichia coli in MacConKey Agar Plate

Figure 233 : Biochemical reactions of Escherichia coli

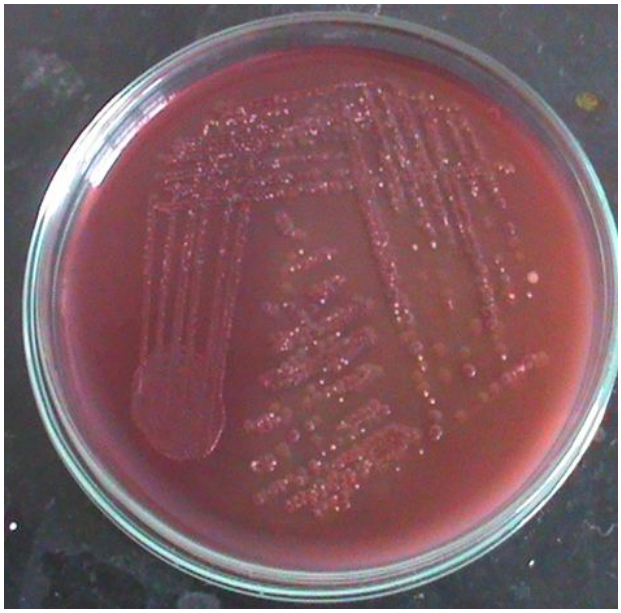


Figure 14 : Polymicrobial Growth

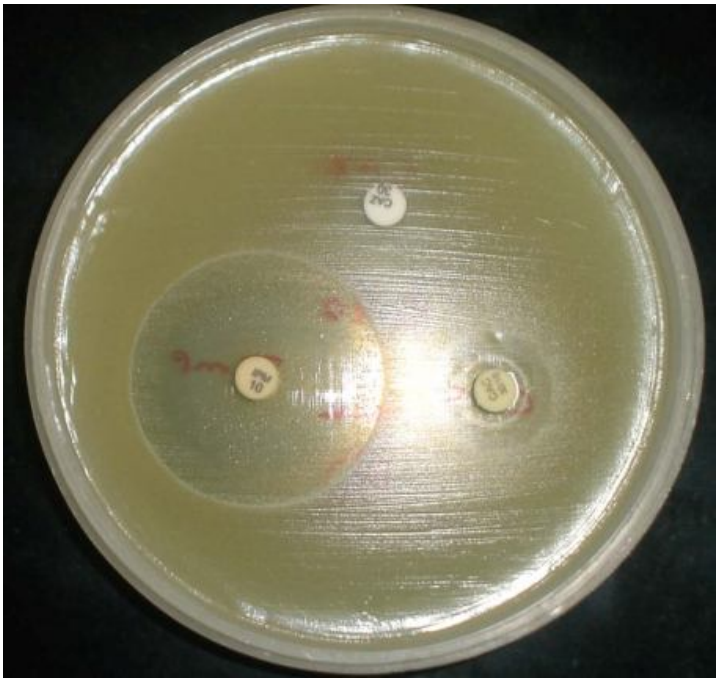


Figure 245 : Antimicrobial Susceptibility Plate - Pseudomonas colony sensitive to Imipenem

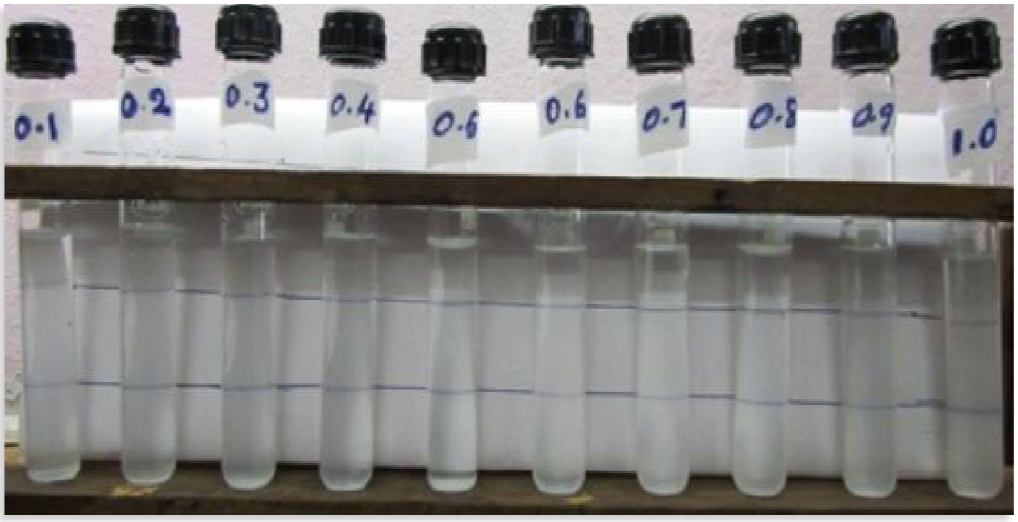


Figure 16 : McFarland's turbidity standard

RESULTS

In this study, the total number of cases Chronic Osteomyelitis considered was 50.

1. Following Haematogenous Osteomyelitis – 3 cases
2. Trauma – 30 cases
    a. Trauma patients without diabetes – 24
    b. Trauma patients with diabetes as risk factor– 6
3. Postoperative Osteomyelitis – 17 cases
    a. Postoperative patients without implants – 10
    b. Postoperative patients with prosthetic implants – 7

Table 1: Percentage of Bones Involved in Chronic Osteomyelitis

S.No	***Bones Involved***	***Number of cases***
1	*Femur*	*23*
2	*Tibia*	*15*
3	*Femur + Tibia*	*2*
4	*Tibia + Fibula*	*3*
5	*Radius + Ulna*	*4*
6	*Humerus*	*2*
7	*Acetabulum*	*1*
	Total	*50*

Bones involvement in Chronic Osteomyelitis

The involvement of long bones in Chronic Osteomyelitis is as follows

1. Femur – 46%
2. Tibia – 30%
3. Femur + Tibia – 4%
4. Tibia + Fibula – 6%
5. Radius+ Ulna – 8%
6. Humerus – 4%
7. Acetabulum – 2%

Chart 1 : Percentage of Bones Involved in Chronic Osteomyelitis

(Bar Chart 3D View)

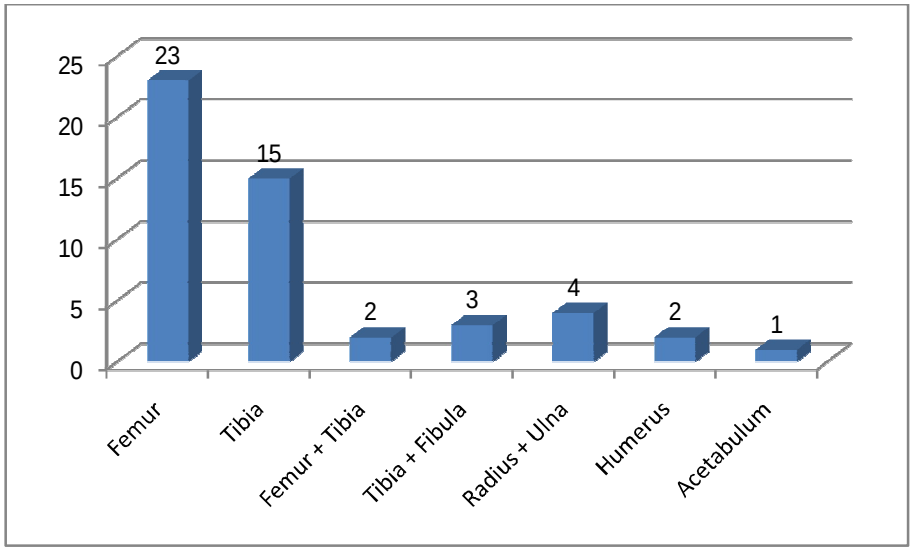
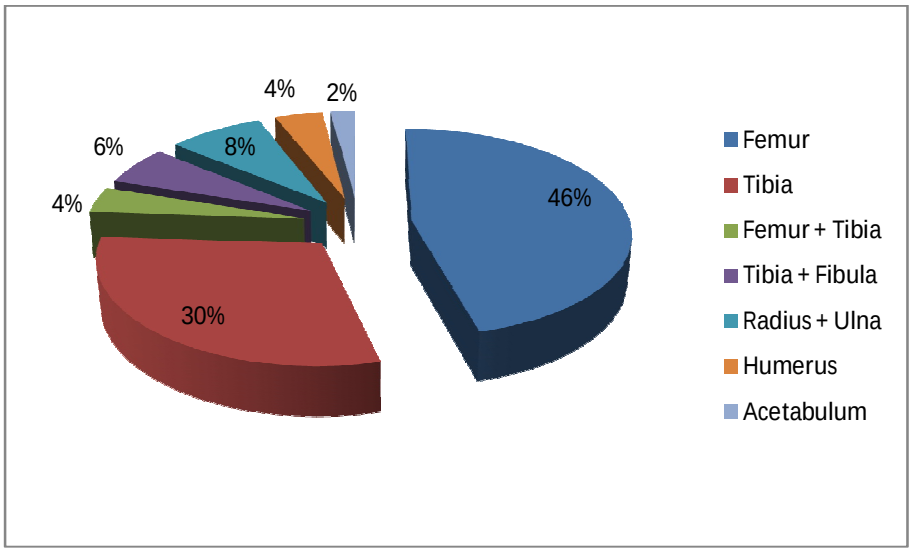


Chart 2 : Percentage of Bones Involved in Chronic Osteomyelitis (PIE Exploded 3D View)



The incidence of Osteomyelitis in males is 84% and female is16%. And the male to female ratio is 5.25:1

Age & Sex distribution of Chronic Osteomyelitis

The incidence of Osteomyelitis in different age group is as follows

1. 1-20 years– 20%
2. 21-40 years – 40%
3. 40-65 years – 40%

Table 2: Age & Sex Distribution of Chronic Osteomyelitis

S.No	***Age Group***	***Male***	***Female***
1	*1-20 Years*	*7*	*3*
2	*21-40 Years*	*19*	*1*
3	*41-65 Years*	*16*	*4*
	Total No of Cases	***42***	***8***

Chart 3 : Age & Sex Distribution of Chronic Osteomyelitis (Bar Chart 3D View)

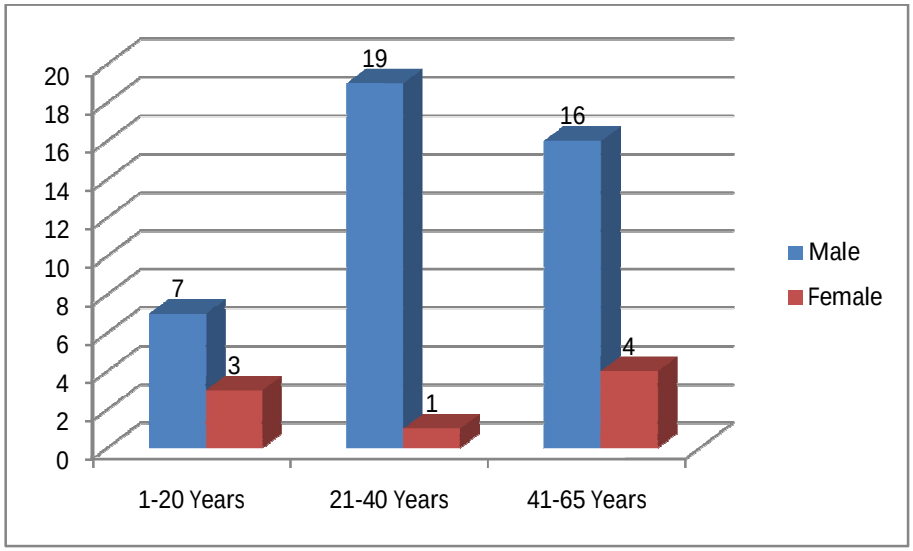
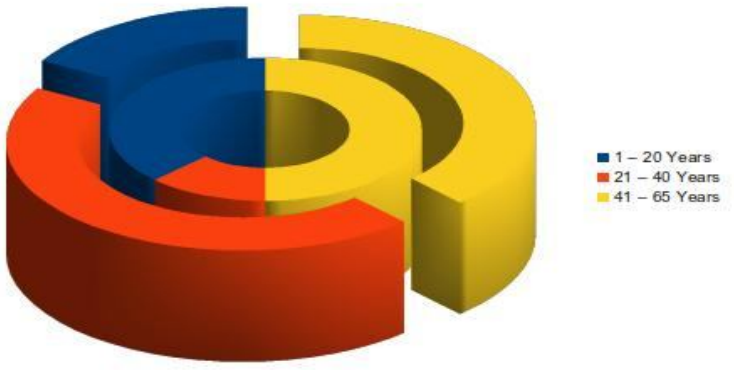


Chart 4 : Age & Sex Distribution of Chronic Osteomyelitis (PIE EXPLODED - 3D View)

Inner Ring – Females

Outer Ring - Males



Bacterial culture results

The Bacterial culture results is given below

1. No of samples with positive cultures – 45 cases
    a. Monomicrobial growth – 40 cases
    b. Polymicrobial growth – 5 cases
2. No growth was obtained – 5 cases

Table 3: Culture Results

S.No	***Type of Growth***	***Number***
1	*Monomicrobial growth*	*40*
2	*Polymicrobial growth*	*5*
3	*Negative growth*	*5*
	Total	*50*

Chart 5 : Culture Results (Bar Chart 3D View)

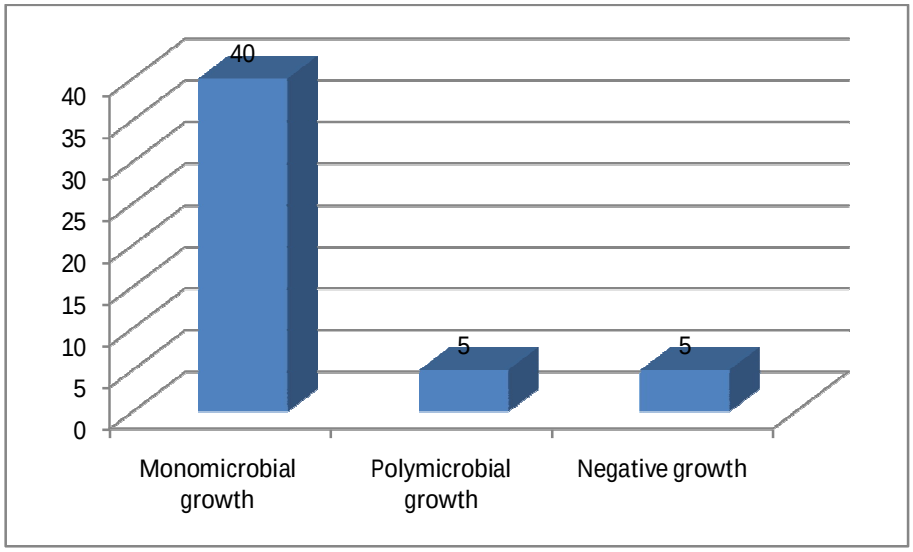
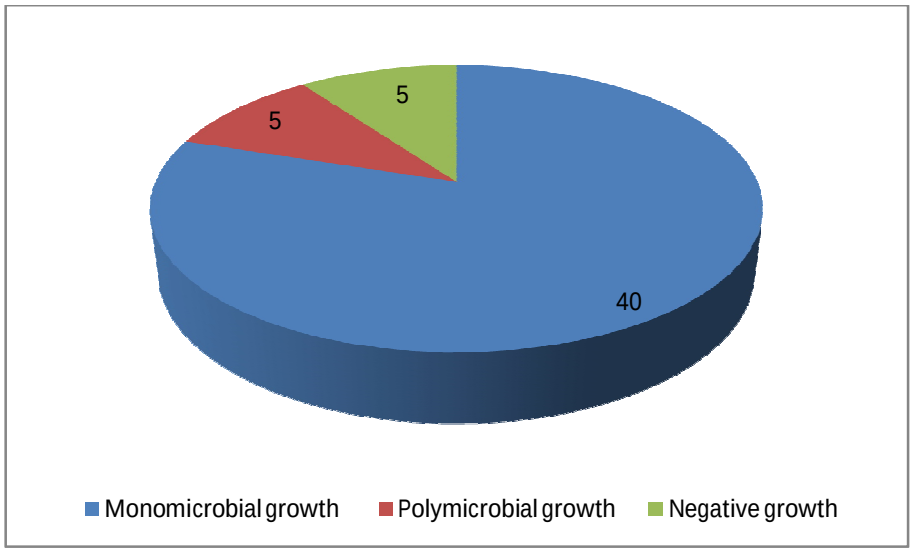


Chart 6 : Culture Results (PIE 3D View)



Monomicrobial growth

For Monomicrobial growth (40 isolates),

organisms isolated is as follows

1. Staphylococcus aureus – 25 isolates
2. Coagulase negative staphylococci – 7 isolates
3. Escherichia coli – 2 isolates
4. Klebsiella – 2 isolates
5. Pseudomonas – 2 isolates
6. Proteus – 1 isolate
7. Enterococci – 1 isolate

Table 4: Monomicrobial Growth

S.No	***Pathogen***	***Number***
1	*Staphylococcus aureus*	*25*
2	*Coagulase Negative Staphylococci*	*7*
3	*Escherichia coli*	*2*
4	*Klebsiella*	*2*
5	*Pseudomonas*	*2*
6	*Proteus*	*1*
7	*Enterococci*	*1*
	Total	*40*

Chart 7 : Monomicrobial Growth (Bar Chart 3D View)

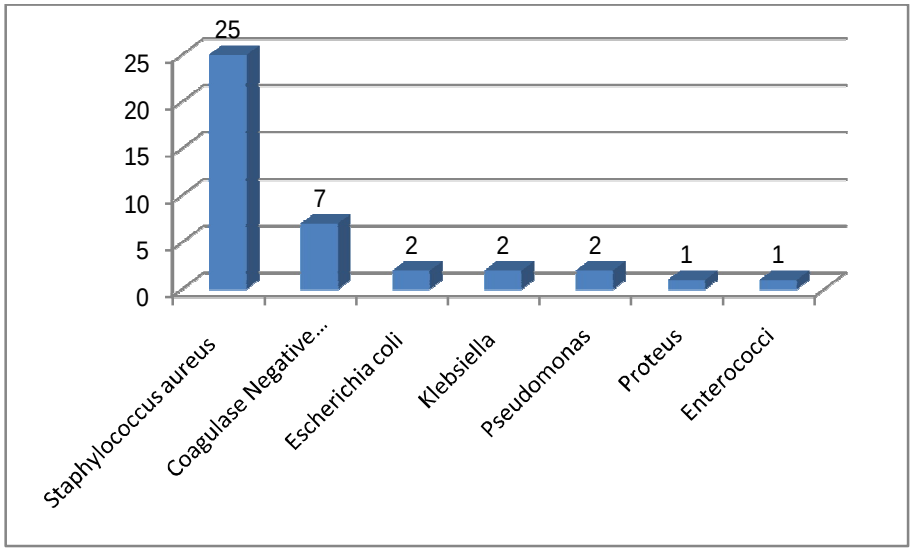
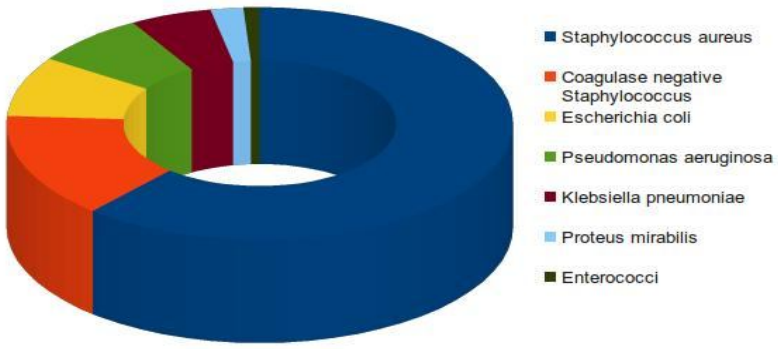


Chart 8 : Monomicrobial Growth (PIE DONUT 3D View)



Polymicrobial growth

For Polymicrobial growth (5 isolates),

organisms isolated is as follows

1. Staphylococcus aureus+pseudomonas – 2 isolates
2. Staphylococcus aureus + E.coli – 1 isolates
3. Staphylococcus aureus + Proteus – 1 isolate
4. Klebsiella + Pseudomonas – 1 isolate

Table 5: Polymicrobial Growth

S.No	***Pathogen***	***Number***
1	*S.aureus + Pseudomonas*	*2*
2	*S.aureus +E.coli*	*1*
3	*S.aureus + Proteus*	*1*
4	*Klebsiella + Pseudomonas*	*1*
	Total	*5*

Chart 9 : Polymicrobial Growth (Bar Chart 3D View)

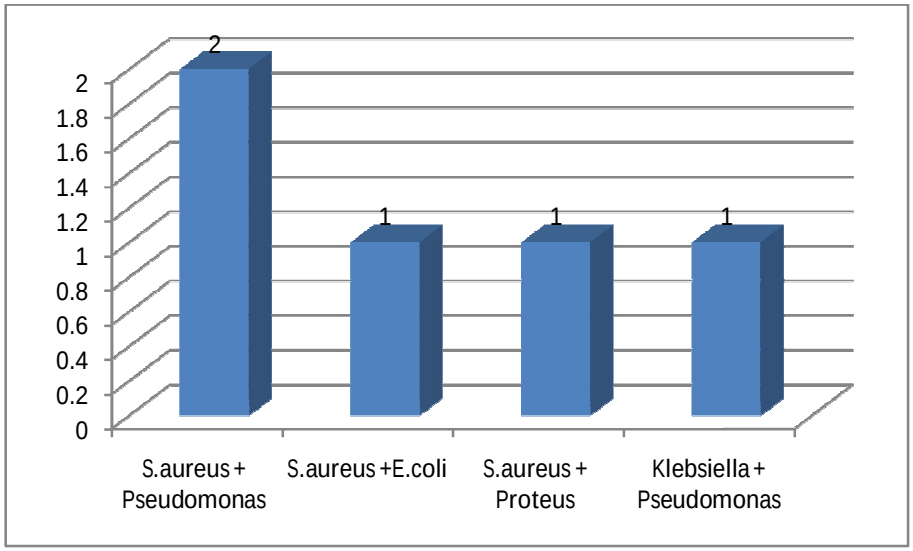
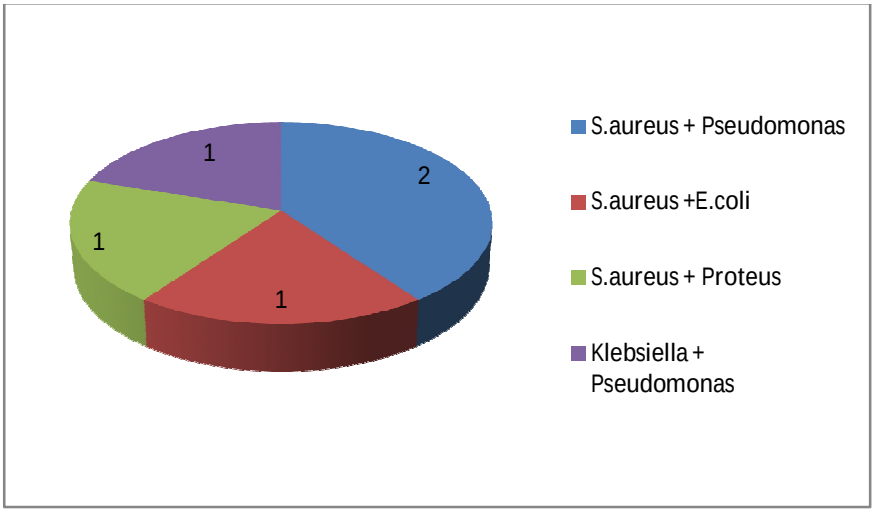


Chart 10 : Poly microbial Growth (PIE 3D View)



Pathogens associated with risk factors

1. Postoperative cases with Prosthetic Implants – 7 cases.

   Pathogens isolated is as given below

   a) Staphylococcus aureus – 2 isolates
   b) Coagulase negative staphylococci – 3 isolates
   c) Escherichia coli – 1 isolate
   d) Enterococci – 1 isolate

2. In trauma patients with Diabetes – 6 cases.

   Pathogens isolated is as given below

   a. Staphylococcus aureus – 3 isolates
   b. Staphylococcus aureus + E.coli – 1 isolates
   c. Staphylococcus aureus + Pseudomonas – 1 isolates
   d. Klebsiella – 1 isolate

Antimicrobial susceptibility of Staphylococcus aureus – 29 cases

   a. MSSA- 17
   b. MRSA-12

Antimicrobial susceptibility of MSSA

MSSA Antibiogram

Drug	***Cases***	***% Sensitive***	***% Resistance***
amoxycillin	*0*	*0*	*100*
ampicillin	*0*	*0*	*100*
cotrimoxale	*13*	*76*	*24*
doxycycline	*15*	*88*	*12*
erythromycin	*13*	*76*	*24*
amikacin	*15*	*88*	*12*
gentamicin	*13*	*76*	*24*
ciprofloxacin	*11*	*65*	*35*
cephalexin	*10*	*59*	*41*
Oxacillin	*17*	*100*	*0*
cefotaxime	*10*	*59*	*41*
cefuroxime	*13*	*76*	*24*
ceftriaxone	*13*	*76*	*24*
vancomycin	*17*	*100*	*0*

The sensitivity of Methicillin sensitive Staphylococcus aureus for the following drugs is as follows

amoxycillin - 0%, ampicillin - 0%, cotrimoxale - 76%, doxycycline - 88%, erythromycin - 76%, amikacin - 88%, gentamicin - 76%, ciprofloxacin - 65%, cephalexin - 59%, oxacillin - 100%, cefotaxime - 59%, cefuroxime - 76%, ceftriaxone - 76%, vancomycin - 100%.

Antimicrobial susceptibility of MRSA

MRSA Antibiogram

Drug	***Cases***	***% Sensitive***	***% Resistance***
Amoxicillin	0	0	100
Ampicillin	0	0	100
Cotrimoxale	10	83	17
Doxycycline	6	50	50
erythromycin	7	58	42
Amikacin	3	25	75
Gentamicin	2	17	83
ciprofloxacin	0	0	100
Cephalexin	0	0	100
Oxacillin	0	0	100
Cefotaxime	0	0	100
Cefuroxime	1	8	92
Ceftriaxone	2	17	83
Vancomycin	12	100	0
Linezolid	9	75	25

The sensitivity of Methicillin resistant Staphylococcus aureus for the following drugs is as follows

amoxycillin - 0%, ampicillin - 0%, cotrimoxale - 83%, doxycycline - 50%, erythromycin - 58%, amikacin - 25%, gentamicin - 17%, ciprofloxacin - 0%, cephalexin - 0%, oxacillin - 0%, cefotaxime - 0%, cefuroxime - 8%, ceftriaxone - 17%, vancomycin - 100%, linezolid - 75%.

Antimicrobial susceptibility of Coagulase negative Staphylococcus

The sensitivity of Coagulase negative Staphylococcus for the following drugs is as follows

amoxycillin - 29%, ampicillin - 43%, cotrimoxale - 57%, doxycycline - 71%, erythromycin - 57%, amikacin - 71%, gentamicin - 57%, ciprofloxacin - 43%, cephalexin - 57%, oxacillin - 100%, cefotaxime - 86%, cefuroxime - 71%, ceftriaxone - 86%

Drug	***Cases***	***% Sensitive***	***% Resistance***
amoxycillin	*2*	*29*	*71*
Ampicillin	*3*	*43*	*57*
cotrimoxale	*4*	*57*	*43*
doxycycline	*5*	*71*	*29*
erythromycin	*4*	*57*	*43*
Amikacin	*5*	*71*	*29*
gentamicin	*4*	*57*	*43*
ciprofloxacin	*3*	*43*	*57*
Cephalexin	*4*	*57*	*43*
Oxacillin	*7*	*100*	*0*
Cefotaxime	*6*	*86*	*14*
Cefuroxime	*5*	*71*	*29*
ceftriaxone	*6*	*86*	*14*

Antimicrobial susceptibility of Enterococci

The sensitivity of Enterococci is studied with 1 case.

And it is found to be resistant to all drugs except vancomycin.

Antimicrobial susceptibility of Escherichia coli

The sensitivity of Escherichia coli is studied with 3 cases.

And its sensitivity pattern for the following drugs is as follows:

ampicillin - 0%, cotrimoxale - 33%, doxycycline - 67%, amikacin - 67%, gentamicin - 67%, ciprofloxacin - 67%, cephalexin - 33%, cefotaxime - 67%, cefuroxime - 33%, ceftriaxone - 167%, ceftazidime - 67%, ceftazidime - clavulanic acid - 67%, imipenem - 100%

Antimicrobial susceptibility of Pseudomonas aeruginosa

The sensitivity of Pseudomonas aeruginosa is studied with 5 cases.

And its sensitivity pattern for the following drugs is as follows:

ampicillin - 0%, cotrimoxale - 0%, doxycycline - 0%, amikacin - 40%, gentamicin - 20%, ciprofloxacin - 40%, cephalexin - 20%, cefotaxime - 20%, cefuroxime - 20%, ceftriaxone - 40%, ceftazidime - 60%, ceftazidime - clavulanic acid - 80%, imipenem - 100%

Antimicrobial susceptibility of Klebsiella Pneumoniae

The sensitivity of Klebsiella Pneumoniae is studied with 3 cases.

And its sensitivity pattern for the following drugs is as follows:

ampicillin - 0%, cotrimoxale - 33%, doxycycline - 33%, amikacin - 67%, gentamicin - 33%, ciprofloxacin - 67%, cephalexin - 33%, cefotaxime - 67%, cefuroxime - 33%, ceftriaxone - 67%, ceftazidime - 67%, ceftazidime - clavulanic acid - 100%, imipenem - 100%

Antimicrobial susceptibility of Proteus vulgaris

The sensitivity of Proteus vulgaris is studied with 2 cases.

And its sensitivity pattern for the following drugs is as follows:

ampicillin - 0%, cotrimoxale - 0%, doxycycline - 0%, amikacin - 100%, gentamicin - 100%, ciprofloxacin - 100%,cephalexin - 100%, cefotaxime - 100%, cefuroxime - 50%, ceftriaxone - 100%, ceftazidime - 100%, ceftazidime - clavulanic acid - 100%, imipenem - 100%,

Antimicrobial susceptibility pattern of Gram negative bacilli

Drug	**Pseudomonas n-5**	**E.coli n-3**	**Klebsiella n-3**	**Proteus n-2**
Ampicillin	0%(0)	0%(0)	0%(0)	0%(0)
Cotrimoxale	0%(0)	33%(1)	33%(1)	0%(0)
Doxycycline	0%(0)	67%(2)	33%(1)	0%(0)
Amikacin	40%(2)	67%(2)	67%(2)	100%(2)
Gentamicin	20%(1)	67%(2)	33%(1)	100%(2)
ciprofloxacin	40%(2)	67%(2)	67%(2)	100%(2)
Cephalexin	20%(1)	33%(1)	33%(1)	100%(2)
Cefotaxime	20%(1)	67%(2)	67%(2)	100%(2)
Cefuroxime	20%(1)	33%(1)	33%(1)	50%(1)
Ceftriaxone	40%(2)	167%(5)	67%(2)	100%(2)
Ceftazidime	60%(3)	67%(2)	67%(2)	100%(2)
ceftazidime - clavulanic acid	80%(4)	67%(2)	100%(3)	100%(2)
Imipenem	100%(5)	100%(3)	100%(3)	100%(2)

DISCUSSION

In this study occurrence of haematogenous osteomyelitis is only 6%.

The development of Osteomyelitis depends on the host and microbial factors. The host factors include destruction of cartilage, resorption of bone. Microorganisms play dominant role in the development of Osteomyelitis.

In this study commonest pathogen in haematogeneous osteomyelitis is S.aureus 66% (2/3 cases),

it coincides with the findings of Lipsky et al. [54]. In developed countries, Lipsky says haematogenous osteomyelitis is completely wiped out.

In a study by Haider Abdul-Lateef Mousa et al, in haematogenous osteomyelitis the most causative agent was Staphylococcus aureus (45.2%). [53]

In this study, incidence of Osteomyelitis in males is 84% and females 16% , male female ratio is 5.25:1 , where as it is 1.9:1 according to Haider Abdul-Lateef Mousa et al. [53]

In this study occurrence of Staphylococcus aureus is 58% and Coagulase negative Staphylococci is 14%.

This study correlates with the study of the following people,

According to Mader et.al the occurrence of osteomyelitis is due to Staphylococcus aureus and Coagulase negative Staphylococci is 75% followed, by gram negative organisms and anaerobes. [3]

In a study by Saurabh Agarwal, Mohd Zahid, Mohd K.A et al. Staphylococcus aureus is the most common organism followed by Streptococcus, Pseudomonas, Proteus, E.coli and Klebsiella. [55]

R.D. Char, N.S. Brara, K.D.Khare et al.(1975), in their study 19 out of 27 patients had positive culture of Staphylococcus aureus 70.37%.

Augsburg J (1991) found Staphylococcus aureus was the commonest organism in a study in the pathogens and their antibiogram conducted with 79 osteomyelitis patients.

According to the study by Kaur J , Gulati VL , Aggarwal A, Gupta v (2008) et al. on 100 patients in North India hospitals found Staphylococcus aureus in 43%.

According to Sheehy SH, Atkins BA, Bejon P (2010) et al. on 166 patients in Oxford U.K. observed Staphylococcus aureus in 32%..

According to Mita D. Wadekar (2010) et al. [56] observed, Staphylococcus aureus in 43% followed by Pseudomonas 10%, Proteus

species 6%, Klebsiella 5%, E.coli 5%, Staphylococcus epidermidis 4%, Enterobacter 3% and Enterococci 2% .

This study doesn't correlate with the study of the following people,

In 2008, Alok.C.Agrawal et al, found Staphylococcus aureus in 21 out of 111 cases in India. [29]

A.K. Ako-Nai , I.C Ikem ,A.Aziba et al.(2003) conducted a study on bacteriological examination of chronic osteomyelitis in southwestern Nigeria concluded Staphylococcus aureus 20.5%, Coagulase negative Staphylococci is 12.8%

In this study, Coagulase negative Staphylococci is the second commonest pathogen with 14% occurrence .

This study correlates with the study of the following people,

A.K. Ako-Nai , I.C Ikem ,A.Aziba et al.(2003) found Coagulase negative Staphylococci in 12.8% of their cases under study.

According to Waldvogel et al. Staphylococcus aureus + Coagulase negative Staphylococci in 75%.

In this study, the occurrence of Enterococci is 2% .

This study correlates with the study of the following people,

According to Kaur J , Gulati VL , Aggarwal A, Gupta v (2008) et al. the incidence of Enterococci is 2% in their study.

In this study, the occurrence of gram negative bacilli is E.coli 6%, Klebsiella 6%, Pseudomonas 10%, Proteus 4%.

This study correlates with the study of the following people,

Dr Mita D. Wadekar (2010) et al [56], observed the occurrence of Pseudomonas 10%, Proteus species 6%, Klebsiella 5%, E.coli 5%, Staphylococcus epidermidis 4%, Enterobacter 3% and Enterococci 2% .

This study doesn't correlate with the study of the following people,

In 2008, Alok.C.Agrawal et al, In India found, Pseudomonas 29%, E.coli 38%, Klebsiella 9%, Proteus species 7%. [29]

In 2010, Haider Abdul-Lateef Mousa et al, in post operative osteomyelitis observed Pseudomonas 25%, Proteus species 12.9% [53]

In this study, 5 out of 50 cases showed no growth. The absence of growth may be due to anaerobic organism.

According to Haider Abdul-Lateef Mousa et al, incidence of osteomyelitis due to anaerobic bacteria is significant, because anaerobes multiply easily in dead tissue due to low oxygen tension.

Chronic osteomyelitis associated with prosthetic implants as risk factor.

In this study, no of cases with prosthetic implants is 7.

- Coagulase-negative staphylococci 43% (3 cases) ,
- Staphylococcus aureus 28% (2)
- Enterococci 14% (1)
- E.coli 14% (1)

Coagulase-negative staphylococci is more prevalent than Staphylococcus aureus in prosthetic joint infections. [57]

Enterococci and Streptococcus viridans also cause prosthetic joint infections. [58]

Enterococci and Gram negative bacilli from the gastrointestinal tract casues prosthetic joint infections.[61]

Staphylococcus aureus and Staphylococcus epidermidis together cause about 65% of Prosthetic joint infections. [59, 60]

Chronic osteomyelitis associated with diabetes.

In this study, number of cases of osteomyelitis patient with diabetes is 6.

- Monomicrobial growth - 4
  - ❖ Staphylococcus aureus - 3
  - ❖ Klebsiella - 1
- Polymicrobial growth - 2
  - ❖ Staphylococcus aureus + E.coli - 1
  - ❖ Staphylococcus aureus + Pseudomonas - 1

The percentage of occurrence of pathogens is listed below

- Staphylococcus aureus - 63%
- E.coli - 12%
- Klebsiella - 12%
- Pseudomonas - 12%

This study correlates with the study of the following people,

Diabetic osteomyelitis are caused by single micro-organism. Staphylococcus aureus along with coagulase negative staphylococci accounts for 70-80% of such cases. [62, 63]

This study doesn't correlate with the study of the following people,

According' to Eric Senneville et al, Occurrence of micro-organisms in bone samples were staphylococci (52%) and gram-negative bacilli (18.4%). [31]

According to the study by Asha Konipparambil Pappu , Aprana Sinha, Aravind Johnson et al, Occurrence of Pathogens is Staphylococcus aureus 21%, Pseudomonas 23%, Proteus mirabilis (15%), Klebsiella (17%), E. coli (12%). [30]

Discussion on Anti Microbial Susceptibility of Staphylococcus aureus

Of the 29 isolates of Staphylococcus aureus, MSSA - 58%, MRSA 42%.

This study correlates with the study of the following people,

In 2008, Ethan Rubinstein et. al, found 20%–40% of MRSA patients. All isolates are resistant to erythromycin and β-lactams, 2.Success rate was 66% for patients on linezolid treatment and 68% for patients on vancomycin. [29]

According to Fatholahzadeh et. al, MRSA is 36% . All MRSA isolates were susceptible to vancomycin, linezolid. Complete resistance to beta-lactam drugs and high resistance (> 95%) to tetracycline, erythromycin, gentamicin, and ciprofloxacin is observed. [14]

According to Marcinak et al, Trimethoprim-sulfamethoxazole is used for the treatment of methicillin-resistant Staphylococcus aureus in children, and emerging option for treatment is linezolid. [16]

According to S Anupurba et al, In eastern Uttar Pradesh., MRSA is (54.85%). MRSA resistance to penicillin, cotrimoxazole, ciprofloxacin, gentamicin, erythromycin, tetracycline is >80% and 60.5% to amikacin. No vancomycin resistance was appreciated. [17]

In According to Adebayo O Shittu et al, 26.9% were MRSA and all strains were susceptible to teicoplanin, vancomycin. [18]

According to Viudes A et al, MRSA is 42.85%. All MSSA were susceptible to vancomycin, gentamicin, teicoplanin, ciprofloxacin and linezolid. 90% were erythromycin susceptible. All the MRSA were susceptible to vancomycin and linezolid, 92% to gentamicin. [19]

According to Rohani MY et al, MRSA 39.7%, Resistance to penicillin was 94.1%, ciprofloxacin 29.2%, erythromycin 45.9%, gentamicin 40.5%, tetracycline 47.2%, co-trimoxazole 38.5%. All isolates are vancomycin sensitive. Erythromycin, gentamicin, tetracycline and ciprofloxacin are least susceptible to MRSA. [20]

In this study in antimicrobial susceptibility test, the sensitivity for different drugs are vancomycin (100%), linezolid (75%), cotrimoxale (83%), doxycycline (50%) and erythromycin (58%).

SUMMARY

This study on Chronic Osteomyelitis was conducted at Central Lab TMCH, from April 2011 to April 2012 in 50 patients.

- Staphylococcus aureus (58%) is the dominant pathogen followed by Coagulase negative Staphylococcus (14%) causing Chronic Osteomyelitis.
- Gram negative bacilli E.coli, Klebsiella, Pseudomonas and Proteus constitute 26% infections.
- 80% of Osteomyelitis occurs in 20-65 years age group.
- In Monomicrobial growth, the commonest organism is Staphylococcus aureus, followed by CoNS, E. coli, Klebsiella and Pseudomonas
- In Polymicrobial growth,

  the commonest organism is Staphylococcus aureus followed by Pseudomonas, E. coli and Proteus.
- In post operative patients with prosthetic implants,

  commonest organism isolated is Coagulase negative staphylococci (42%) followed by Staphylococcus aureus (28%), Enterococci (14%) and E.coli (14%).

- In trauma patients with diabetes, the commonest organism is S.aureus (63%) followed by E.coli (12%), Klebsiella (12%) and Pseudomonas (12%).
- Out of 29 cases Staphylococcus aureus, 17 cases were MSSA (58%) and 12 were MRSA (42%).
- In antimicrobial susceptibility test, the sensitivity for different drugs are vancomycin (100%), linezolid (75%), cotrimoxale (83%), doxycycline (50%) and erythromycin (58%).

CONCLUSION

Chronic Osteomyelitis is a chronic disease most commonly occurring in adults with the involvement of long bones especially femur and tibia. Due to the advent of antibiotics and high vascular metaphysis of growing bones in children, the occurrence of haematogenous Osteomyelitis is coming down. In this study staphylococcus aureus is the commonest organism causing Chronic Osteomyelitis .The Methicillin Resistant Staphylococcus aureus is sensitive vancomycin and linezolid.Cotrimoxazole , doxycyline , erythromycin can also be used for the treatment of MRSA.The injudicious use of antibiotics has led to development of MRSA and resistance to betalactam drugs. As a routine the orthopaedician should ask for bacterial cultural sensitivity for Chronic Osteomyelitis. As anaerobes and gram negative bacilli constitute a major proportion of Chronic Osteomyelitis , culture and sensitivity should be done for both the organisms. Patients' hospital stay duration should be minimised and out-patient treatment with oral drugs should be encouraged. Prevention is better than cure. Strict asepsis should be maintained during any operative procedures.

The spread of MRSA from fomites and gram negative bacilli from cheatle forceps, hospital environment can be prevented. The use of towels, handkerchiefs between patients and their attenders should be discouraged. The therapeutic approach directed towards organisms forming biofilm will bring down incidence of Chronic Osteomyelitis to a large extent in the near future.

Scope for further research

Continued surveillance for incidence of drug resistance among the microorganisms causing chronic osteomyelitis should be done in our medical college.

Updating the antimicrobial policies based on the sensitivity pattern should also be done.

ANNEXURES

PROFORMA

NAME: **SERIAL NO:**

AGE: **LAB NO:**

SEX: **OP/IP NO:**

ADDRESS: **DATE OF Sample collection:**

OCCUPATION:

INCOME:

Chief Complaints:

- **Pain:**
- **Discharging sinus:**
- **Any restricted joint movements:**

H/O Present illness:

- **Duration of the disease**
- **Presence of Diabetic Ulcer**
- **Lymphedema**
- **Presence of cellulitis**

Past History:

- **Road traffic accidents**
- **Trauma**
- **History of Hypertension**
- **Smoking**
- **Diabetes**

Personal history:

General Examination:

- **Pulse rate**
- **Heart rate**
- **Temperature**
- **Anaemia**
- **Presence of malnutrition**

Systemic examination:

- **CVS**
- **RS**
- **CNS**
- **P/A**

Clinical diagnosis:

- **X-ray**

WORKSHEET

Specimen:

Bone Aspirate (or) Bone Curretings:

FOR ISOLATION OF BACTERIA:

Culture:

- **MacConkey Agar**
- **Nutrient agar**
- **Blood Agar**

Biochemical reactions:

- **Catalase**
- **Oxidase**
- **Motility**
- **IMViC**
- **Urease**
- **TSI**
- **LAO**
- **OF Test**
- **Coagulase**
- **Sugar fermentation tests**

Culture Report:

Antimicrobial Susceptibility:

GRAM STAINING

The gram stain was prepared as follows:

PRIMARY DYE:

Crystal violet - 10g

Ammonium oxalate - 4.25g

Absolute alcohol - 50ml

Distilled water -500ml

The methyl violet dye was dissolved in 50 ml absolute alcohol and mixed thoroughly. Then ammonium oxalate 4.25 g was dissolved in 100 ml of distilled water and this mixture was added to the violet stain and finally distilled water was added to make 500 ml. The total mixture was filtered before use.

Gram's iodine solution consists of the following

Iodine - 25g

KI - 50G

DW - 500ml

Fifty grams of KI was dissolved in 500 ml of water and then 25 grams of iodine was added to that. When iodine is dissolved, the solution was made up to 500ml with distilled water.

Counter stain used in grams stain was dilute carbol fuschin. It consists of the following:

Basic fuschin - 5g

Phenol -25g

Absolute alcohol -50 ml

The basic fuschin powder was added to alcohol at intervals until it was dissolved. Then phenol too was dissolved in distilled water. Both the solution was mixed in a separate container.

CATALASE TEST:

Done by both slide & tube methods.

Tube method:

A small amount of the culture was picked up from the nutrient agar plate with a clean, sterile glass rod and inserted into a tube of 3% hydrogen peroxide; there was no effervescence or bubble formation.

Slide method:

Pure growth of the organism from the agar was transferred to a clean slide with a sterile glass rod. Immediately 2 to 3 drops of 3% hydrogen peroxide was added to the growth, observed for the release of the bubbles.

MEDIA PREPARATION

1. **Peptone water:**

Peptone	1 g	
Sodium chloride	0.5 g	
Distilled water	100 ml	PH – 7.4

Sterilise by autoclaving at 121d C for 15 minutes.

2. **Nutrient broth :**

Peptone water	100ml
Beef extract	1 g
Ph	7.4

Sterilise by autoclaving at 121dC for 15 minutes.

3. **Nutrient agar :**

To the nutrient broth, add required amount of agar. Steam to dissolve agar, filter, and adjust ph to 7.4. Sterilise by autoclaving at 121dC for 15 min.

4. **Blood agar :**

To the 100 ml of nutrient agar, in water bath at 50dC, add 5% (5ml) of Sheep blood.

5. **Mac conkey agar**

Peptone	20 g
Sodium chloride	5 g
Sodium taurocholate	5 g
Lactose	10g
Neutral red	10 ml
Agar	15 g
Distilled water	1000 ml

Sterilise by autoclaving at 121dC for 15 minutes.

6. Muller Hinton media:

Beef infusion	300 g/l
Casein acid hydrolysate	17.5 g
Starch	1.5 g
Agar	17 g
Distilled water	1000 ml

Sterilise by autoclaving at 121dC for 15 minutes.

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