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Bacteriological Profile of Osteomyelitis in a Tertiary Care Hospital

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Abstract

Background: Widespread use of antibiotics has changed the etiological pattern and antibiotic susceptibility pattern of Osteomyelitis. Continuous monitoring in a particular geographical area will indicate the current resistance patterns, which would help in the initiation of appropriate prophylactic antibiotic until the culture reports, are available.

Objectives: 1. To identify Aerobic bacteria isolated from pus and tissue samples of patients suspected with Osteomyelitis. 2. To study the Antibiotic susceptibility pattern of the isolates.

Methods: 104 cases clinically diagnosed as Osteomyelitis were included in the study. Aspirated pus / swabs or tissue samples were collected from the patients maintaining strict asepsis. Gram's stain was performed from the samples and inoculated onto MacConkey Agar, Blood Agar, Nutrient agar and incubated aerobically at 37^0 C for 18-24hours and was observed for growth. The bacterial colony isolated was identified by colony morphology, cultural characteristics and biochemical reactions according to the standard techniques. Antibiotic sensitivity testing was performed according to CLSI guidelines by Kirby – Bauer disk diffusion method in Mueller Hinton agar plates.

Results: *Staphylococcus aureus* (32%) was the most common organism isolated followed by P*seudomonas aeruginosa*(15.6%), *Citrobacter freundii* (11.5%), *Proteus mirabilis* and *Klebsiella pneumoniae* (9%each), *Escherichia coli*(5.7%), *Acinetobacter baumannii*(4.9%),*Citrobacter koseri*(4.1%), *Coagulase negative Staphylococci* (3.3%), *Enterobacter aerogenes* (2.5%), *Enterococcus faecalis* (1.6%), *Proteus vulgaris* (0.8%).

For *Staphylococcus aureus* , Vancomycin (100%), Teicoplanin (100%), Linezolid (97.4%) and Doxycycline (79.5%) were the most sensitive antibiotics .For the Gram negative bacilli (Fermenters) , the most sensitive antibiotics were – Cefoxitin (76.7%) and Imipenem(65.4%).Amongst the Gram negative bacilli (Non fermenters) – Tigecycline (100%) , Meropenem , Aztreonam and Amikacin (68% each) were the most sensitive antibiotics.

Conclusion: Proper selection of an antibiotic preceded by a bacterial culture and sensitivity is very important to prevent emergence of drug resistance in an organism and decrease indiscriminate use of unnecessary antibiotics.

Keywords: Osteomyelitis, Pus, Swab, Tissue, Staphylococcus aureus.

Introduction

Osteomyelitis refers to an infection of bone and its marrow irrespective of the aetiology of the infection which can range from pyogenic bacteria, mycobacteria , fungi ,viruses and even presence of a foreign body.[1] Osteomyelitis has long been one of the most difficult and challenging problems faced by orthopaedic surgeons. [2] According to the duration of symptoms, it can be classified into acute or chronic

osteomyelitis.[1] Aetiology of osteomyelitis can be polymicrobial or monomicrobial [2]. Adults are normally resistant to the infection but the presence of devitalized bone,soft tissue or a foreign body increases the chances of developing the infection.

Microorganisms reach the bone or the adjacent muscle via blood (Haematogenous) or open wound contamination (Exogenous) [3]. Signs and symptoms of the infection include - purulent discharge, bone pain, fever, malaise, fatigue, weight loss & tenderness on the affected area [4].Successful management is aided by early diagnosis with appropriate antimicrobial and surgical treatment [2] .

Widespread use of antibiotics has changed the etiological pattern and antibiotic

Susceptibility pattern of these infections. Hence, continuous monitoring in a particular geographical area will indicate the current resistance patterns[5] .The resistance pattern of bacterial isolates in a particular geographical setting for osteomyelitis must be known which would help in the initiation of appropriate prophylactic antibiotic until the culture reports are available. This is crucial, as it should be safe yet effective and active against the most common organisms known to cause the infections. Proper antibiotic usage would inhibit the emergence of drug resistant strains and would prevent morbidity and mortality [6,7].

Materials and Methods

The study was conducted in the Department of Microbiology, M.S. Ramaiah Medical College and Hospital, Bangalore from January 2017 to December 2017

Inclusion Criteria

Cases of all age groups and both sexes, clinically diagnosed as Osteomyelitis either admitted to IPD or attending OPD or referred to Orthopaedic department were included in the study.

Exclusion criteria

Cases diagnosed or clinically suspected to be suffering from Tubercular bone infection were excluded from the study.

Recording History

A validated proforma was filled for each patient.

Samples collected-

1. Aspirated Pus / Swabs
2. Tissue

Samples were collected from patients diagnosed with Osteomyelitis under strict aseptic precautions by following measures-All care was taken to avoid surface contamination

Closed wounds and aspirates - Area of skin was disinfected with 70% alcohol followed by an iodine solution (10% solution of P.I.). Iodine was removed with alcohol wipes before collecting the specimen.

Open wounds – Wounds were debrided thoroughly, followed by thorough rinsing with sterile saline changing sponges with each application. All superficial exudates and debris were removed with the help of scalpel /sponges.[8]

Collection & Transport

Swabs -*Two swabs* were collected – One for Gram's stain and one for culture. Swabs were gently rolled over the

surface of the wound approximately *five times*[6] .Samples were immediately transported to the microbiology laboratory and processed within 30 minutes to optimize best recovery. Cotton tipped swabs were used and they were moistened with sterile normal saline prior collection to prevent drying of swabs [8] .

Aspirates-After the skin preparation, aspiration was done from the deepest portion of the suspected site. The contents of the syringe were emptied into sterile wide mouth bottles to prevent injury due to needle during transport / handling of specimens [8].

Tissue-Tissue biopsy samples were collected from areas within and adjacent to the area of infection (3 to 4 mm size biopsy samples) [6]. Necrotic areas were avoided while taking the tissue samples. Tissue samples were mostly taken intraoperatively which were also processed within 30 minutes of collection. All tissue samples were transported to the laboratory in sterile wide mouthed containers and moistened with sterile normal saline to help retain organism viability.[8]

Sample Processing- All samples were processed under BSC2 (Biosafety cabinet). All samples were subjected to Gram's Stain and Aerobic Culture.

Swabs **-** Each swab was *rolled over* a clean microscopic glass slide to retain cellular morphology and bacterial organisation in the specimen for Gram's stain. ***Aspirates*** – After inoculation onto the agar plates, Gram stain was done from the rest of the specimen. Samples were spread over a large area[9] approximately (1.8 cm) [10] on the slide to form a thin smear. Excessively purulent samples were diluted with normal saline on the slide to avoid making a thick smear . [8]

Tissue-Touch preparation was used to process tissue samples. Larger tissue samples were minced in a sterile petri dish with the help of sterile surgical blade. With the help of sterile forceps small pieces of tissue were held and different sides were touched on the glass slide .All the slides were air dried inside the biosafety cabinet. Then the air dried slides were passed 2-3 times through a flame for heat fixation. Slides were cooled before staining [8].

Aerobic Culture-All samples were inoculated onto the following media -Nutrient agar, MacConkey's agar, Blood agar (5%sheep BA), Thioglycollate broth

All plates and tubes were incubated aerobically at 37^{o}C and observed at 24 and 48 hours.

The bacterial colonies isolated was identified by colony morphology, cultural characteristics and biochemical reactions according to the standard techniques.[6] Antibiotic sensitivity testing was performed according to CLSI guidelines by Kirby – Bauer disk diffusion method in Mueller Hinton agar plates**.** [5]

Thioglycollate broth subculture

If no growth was observed in primary culture media. Thioglycollate broth was checked for turbidity . If it was found turbid then a subculture was made on MacConkey's and Blood agar. The Thioglycollate broth if not turbid, was further incubated for 4 days at 37^{o} C and examined daily for any turbidity. Organisms that were isolated from the samples were identified using standard techniques based on colony characteristics, morphology on Gram's stain and biochemical reactions.

Results

Out of total 104 study subjects, 92 (88.46%) were male and 12 (11.54%) were female. Chronic osteomyelitis was the commonest, which was present in 97.1% of the patients. Acute osteomyelitis was found in only 2.9% of the study subjects.

Most of the specimen was swab (64%) followed by pus aspirates (26%) and tissue (10%). Tissues were mostly sequestrum and few bone chips taken during surgery.

100 samples (96%) had aerobic bacterial growth, 3 specimens showed no growth in aerobic bacterial culture

.1 specimen – Showed growth of *Mycobacterium tuberculosis*, so it was excluded from the study . So total bacterial isolations were 122 out of total 104 samples.

Staphylococcus aureus was the most common organism found (n=39), followed by *Pseudomonas aeruginosa* (n= 19). *Citrobacter freundii* was the next common organism isolated (n=14) followed by *Proteus mirabilis* and *Klebsiella pneumoniae* (n=11 each). 7 samples showed growth of *Escherichia coli* followed by *Acinetobacter baumannii* (n=6), *Citrobacter koseri* (n=5), *Coagulase negative Staphylococci* (n=4), *Enterobacter aerogenes* (n=3), *Enterococcus faecalis* (n=2) and *Proteus vulgaris* (n=1). *Pseudomonas aeruginosa* and *Coagulase negative Staphylococci* was found mostly as repeated isolates from the respective patients.

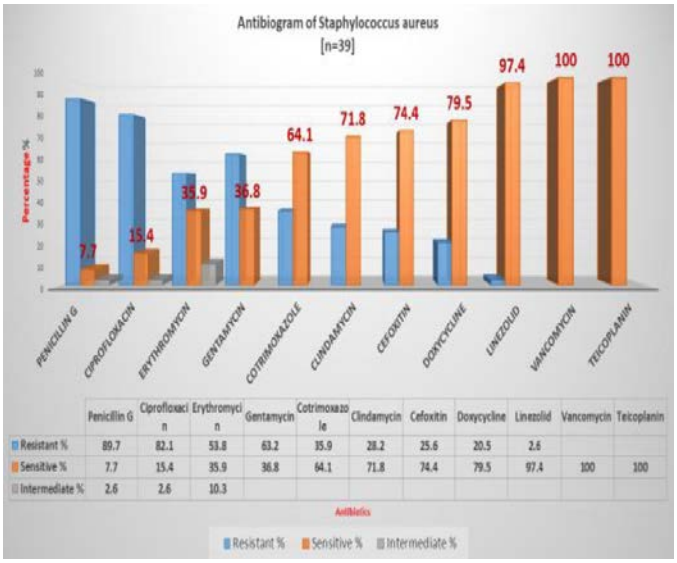

Fig-1: Antibiogram of *Staphylococcus aureus*

All the Staphylococci were sensitive to Vancomycin and Teicoplanin (100%). No VRSA was reported in our study. Among the other sensitive drugs, Linezolid was the most sensitive (97.4%) followed by Doxycycline (79.5%) as indicated in Fig-1 .

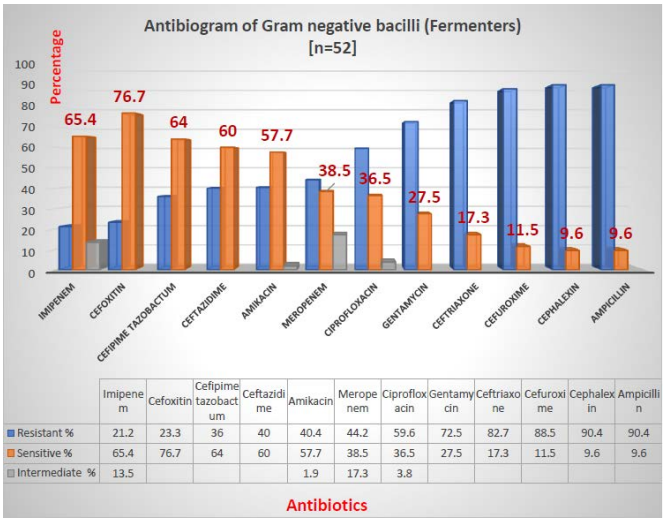

Fig-2: Antibiogram of Gram negative bacilli (Fermenters)

Amongst the Gram negative fermenters , Cefoxitin was the most sensitive antibiotic (76.7%) followed by Imipenem (65.4%) and Cefipime tazobactum (64%). Ampicillin and cephalexin were the most resistant drug as indicated in Fig 2 .

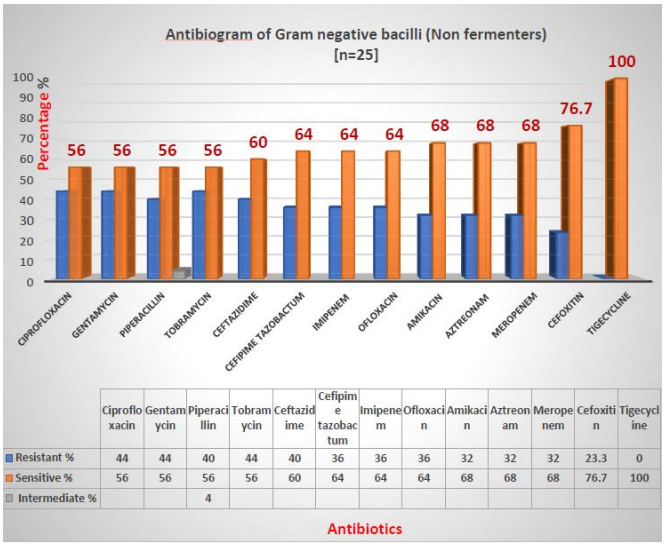

Fig 3: Antibiogram of Gram negative bacilli (Non-Fermenters)

Tigecycline was only tested for Acinetobacter baumanii which showed 100 % sensitivity. Cefoxitin showed 76.7% sensitivity in overall non-fermenters followed by Meropenem, Aztreonam and Amikacin (68% each) as shown in Fig 3 .

Discussion

The results were compared with similar studies done by different authors around the globe . In the present study 88.46% of the study subjects were males and 11.54% were females. Similar findings were also noted in a study by Zuluaga et al [11] (2006) in which males were 87.10%. Hilal Maradit Kremers [12](2009) noted a higher proportion of females (42%) in their study. Sheehy et al [13](2010) in their study showed males to be 72.89%. Vladimir Cordeiro de Carvalho [14] (2011) and Faaiz ali shah [15] (2012) also reported the majority of their patients to be males (63.40% and 68% respectively).

In the present study Staphylococcus aureus was the predominant isolate (32%) which is similar to other studies done by authors previously . Pseudomonas aeruginosa had 15.6% isolation rate in our study which is similar to a study done by Mita D Wadekar [5] et al in 2014. Citrobacter was present at a higher incidence of 15.6% which was not reported before . Ako nai et al [16] has reported Citrobacter freundii (2.5%) in 82 samples from Osteomyelitis patients.

In this study Staphylococcus aureus was mostly sensitive to Teicoplanin (100%),Vancomycin (100%), Linezolid (97.4%), Doxycycline (79.5%), Cefoxitin (74.4%),Clindamycin (71.8%).Similar findings was reported in a study by G.Suguneswari et al[6] (2013) for Staphylococcus aureus, the most sensitive drug reported was Vancomycin (100%) . Clindamycin was reported to be 67.39%. Another study by Kaur J et al[17] (2003) showed Staphylococcus aureus was most sensitive to – Vancomycin (100%) and Linezolid (97.7%).Similar results were also shown by MD Ali et al[3](2014). Staphylococcus aureus was mostly sensitive to – Vancomycin , Linezolid (100%) and Doxycycline (88%).

Coagulase negative Staphylococcus is reported in many Osteomyelitis studies but mostly Staphylococcus epidermidis was reported. Case reports of Staphylococcus lugdunensis as a pathogen in Osteomyelitis cases has been previously reported in literature .[18,19,20]

The Gram negative bacilli (Fermenters) in our study were mostly sensitive to – Cefoxitin (76.7%) , Imipenem (65.4%), Ceftazidime (60%), Amikacin (57.7%) and Meropenem (38.5%). Ruchi V et al[20] (2017) reported Escherichia coli to be sensitive to Amikacin (75%), Imipenem (25%) and Klebsiella spp sensitive to – Amikacin (30.76%), Imipenem (30.76%). MD Ali reported that – Escherichia coli and Klebsiella spp were sensitive to Imipenem (100%), Cefuroxime (33%)[3].Amongst the Gram negative bacilli , Klebsiella pneumoniae was shown sensitive to – Imipenem 80% , Cefepime 60% ,Amikacin 60% , Gentamicin 40% in another study by Suguneswari G et al (2013)[6]. Kaur J et al (2008) reported that Escherichia coli was mostly sensitive to Amikacin (80%),Cefotaxime (40%), Cefoperazone sulbactum (20%) [17].

In this study the Gram negative bacilli (Non Fermenters) – were mostly sensitive to–Tigecycline (100% - for Acinetobacter baumannii) , Meropenem (68%), Aztreonam (68%), Amikacin (68%), Ofloxacin (64%) , Imipenem (64%), Piperacillin , Gentamicin , Tobramycin (56% each). Mita D Wadekar (2014) reported Pseudomonas aeruginosa to be mostly sensitive to – Imipenem (76.4%), Amikacin (58.8%) , Gentamicin (23.5%) [5]. Ako nai (2003) reported Pseudomonas aeruginosa mostly sensitive to –

Gentamicin 55% [16].Ruchi V et al (2017)[20] reported Pseudomonas aeruginosa to be sensitive to Amikacin (53.84%); Imipenem and Gentamicin were reported to have a lower sensitivity of 38.43% and 15.38% respectively. MD Ali (2014) reported that Pseudomonas was sensitive to – Imipenem (100%), Ceftazidime (60%), Ciprofloxacin (40%), Amikacin (40%) [3]. Suguneswari G

et al (2013) in their study has shown Pseudomonas aeruginosa to be sensitive to – Piperacillin tazobactum, Aztreonam, Levofloxacin (89% each), Amikacin (78%) , Piperacillin , Tobramycin ,Gentamicin (67%), Imipenem (54%) [6].

Conclusion

A total of 104 cases , clinically diagnosed as Osteomyelitis were studied from January 2017 to December 2017. The bacteriological profile of Osteomyelitis showed Staphylococcus aureus as the commonest pathogen followed by Pseudomonas aeruginosa and Citrobacter freundii. According to the antibiotic sensitivity pattern; Gram positive organisms were most sensitive to Vancomycin, Teicoplanin , Linezolid and Doxycycline . Gram negative organisms (Fermenters) were sensitive to Cefoxitin and Imipenem, Non fermenting Gram negative bacilli were sensitive to Tigecycline (for Acinetobacter baumannii), Meropenem , Aztreonam and Amikacin.

Osteomyelitis is a bone infection, which has the potential to cause significant morbidity. The emergence of antibiotic resistant etiological agents makes it important to study current patterns of organism profile. Proper selection of an antibiotic preceded by a bacterial culture and sensitivity is very important to prevent emergence of drug resistance in an organism and decrease indiscriminate use of unnecessary antibiotics.

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Ethical approval – Study was approved by Ethics Committee of M.S.Ramaiah Medical college .

Conflict of Interest – None

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