CHRONIC OSTEOMYELITIS: A BACTERIOLOGICAL STUDY WITH SPECIAL REFERENCE TO STAPHYLOCOCCUS AUREUS
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ABSTRACT: Advances in the identification of infections and early diagnosis of Osteomyelitis have led to the improved management of Osteomyelitis. This study was undertaken to determine the bacteriological profile of Osteomyelitis and the antibiotic resistance pattern of various isolates obtained as it is an important cause of morbidity. A total of 50 patients of Osteomyelitis either attending the outpatient department or admitted in the wards of a teaching and tertiary care hospital in Sikkim from October 2013 to October, 2014 were included in the study. All those patients who were clinically and/or radiologically suspected of having Osteomyelitis were enrolled as cases. Pus/ pus swabs or sequestrum samples taken aseptically were cultured aerobically at 37°C for 18-24 hours in Blood and Mac Conkey agar plates. Culture isolates were identified by a series of standard biochemical reactions. Antibiotic susceptibility was tested on Mueller Hinton agar by Kirby Bauer disc diffusion method. Betalactamase production of S. aureus strains were verified by iodometric filter paper and acidometric agar plate methods. S. aureus strains were screened for methicillin resistance by using conventional microbiological methods. S. aureus turned out to be the most common organism isolated. Other organism isolated were P. Aeruginosa, Proteus spp., Klebsiella spp., E. coli, Enterobacter spp., S. epidermitis, Streptococcus pyogens and Enterococcus spp. Beta-lactamase production and methicillin resistance was seen in S. aureus strains respectively. Multidrug resistance was observed in other strains. Infection caused by Methicillin resistant S. aureus and multidrug resistant organisms are posing a major challenge in the treatment of Osteomyelitis. So, appropriate drug selected by antibiotic sensitivity testing should be used to treat Osteomyelitis.

KEYWORDS: Osteomyelitis, antibiotic resistance, aetiology.

INTRODUCTION: Osteomyelitis is inflammation of the bone caused by an infecting organism. Although bone is normally resistant to bacteria, colonization events such as trauma, surgery, presence of foreign bodies, or prosthesis may disrupt bone integrity and lead to bone infections. Osteomyelitis can result from haematogenous spread after bacteriaemia.¹ When prosthetic joints are associated with infections, microorganism typically grow in biofilm, which protects bacteria from antimicrobial treatment and host immune response.¹ Early and specific treatment is important in Osteomyelitis and identification of the causative microorganism is essential for antibiotic therapy. The major cause of bone infections is staphylococcus aureus. Infections with an open fracture or associated with joint prosthesis and trauma require a combination of antimicrobial agents and surgery.² When bio-film – microorganism are involved as in joint prosthesis, a combination of rifampicin with other antibiotics may be necessary treatment.²
MATERIALS AND METHODS: A total of 50 patients with Osteomyelitis either attending the outpatient department or admitted in the wards of a teaching and tertiary care hospital in Sikkim from October 2013 to October, 2014 were included in the study. All those patients who were clinically and / or radiologically suspected of having Osteomyelitis were taken as cases. Important parameters like age, sex, site of involvement, signs and symptoms, duration of illness, and any predisposing factors were taken into consideration. The specimens which comprised of sequestrum or pus / pus swabs taken from the depth of the wound under strict aseptic conditions were transported to the laboratory in Stuart's transport media. Direct smear examination of the samples were done. The specimens were inoculated on to Brain Heart infusion broth and Blood and MacConkey agar plates and incubated aerobically at 37°C for 18 -24 hours. The isolates were identified by culture characteristics, gram staining and series of standard biochemical reactions.

Antibiotic sensitivity was put on Mueller Hinton agar by Kirby Bauer disc diffusion method. The antibiotic disc used were –penicillin (10IU), cephalexin (30μg), cefotaxime (30μg), ciprofloxacin (5μg), erythromycin (5μg), gentamycin (10μg), amikacin (30μg), piperclllin (30μg), vancomycin (30μg), and linezolid (30μg), for gram-positive isolates and cefotaxime (30μg), ciprofloxacin (5μg), gentamycin (10μg), amikacin (30μg), piperclllin (30μg) and cefoperazone sulbactum (50μg), for gram-negative isolates. All the anti-biotic disc used were obtained from H. Media Laboratories Pvt. Ltd. Mumbai. For screening S. aureus strains for methicillin resistance, methicillin (5μg), discs were used on Mueller Hinton agar supplemented with 4% Nacl and plates were incubated at 35°C for 24 hours. All methicillin- resistant S. aureus strains were also confirmed by oxacillin screening using 6μg/ml of oxacillin in Mueller-Hinton agar supplemented with 4% Nacl. Beta-lactamase production of the S. aureus strains was studied by filter paper iodometric and acidometric agar plate method.

RESULTS: The highest incidence of Osteomyelitis was seen in the 16-30 years age group followed in order by age group 31-45 years, 0-15 years and >46 years. Male patients outnumbered the female patients by a ratio of 3.3:1. The commonest predisposing factor was compound fractures followed by post-operative and prosthetic infections.

| Sl. No. | Predisposing factors                                                                 | No. of cases |
|--------|--------------------------------------------------------------------------------------|--------------|
| 1      | Compound fractures due to road side and other accidents.                             | 21           |
| 2      | Post-operative / Prosthetic infections                                                | 8            |
| 3      | Diabetes mellitus                                                                     | 7            |
| 4      | Soft tissue injuries & injections                                                     | 6            |
| 5      | Blunt trauma                                                                          | 4            |
| 6      | Others-otitis media intra-abdominal abscess sinusitis, tonsillitis, peritonsillar abscess, dental abscess or no significant predisposing factor. | 4            |

Table 1: Predisposing factors in 50 cases of Osteomyelitis
Long bones of the lower extremity were involved in most of the cases followed by upper extremity. The bones involved in rest of the cases included short bones of hands and feet, pelvic bones (8%), vertebral bones (4%) and bones of face (4%). Tibia was the most commonly involved bone (32%) followed by femur (23%). In all the pediatric patients, bones involved were long bones and bones of lower extremity were affected.

Patients mostly in the pediatric age group (15yrs), presented with acute Osteomyelitis with fever, lethargy, tenderness at the affected site, and decreased range of motion. Adults mostly presented with sub-acute or chronic form of Osteomyelitis with deformity, instability and draining sinus tracts.

| Sl. No. | Organism                  | No. of isolates |
|---------|---------------------------|-----------------|
| 1       | S. aureus                 | 23              |
| 2       | Pseudomonas aeruginosa    | 5               |
| 3       | Proteus spp.              | 3               |
| 4       | Klebsiella spp.           | 2               |
| 5       | E. coli                  | 6               |
| 6       | S. epidermidis            | 2               |
| 7       | Enterobacter spp.        | 1               |
| 8       | Streptococcus pyogenes    | 2               |
| 9       | Enterococcus spp.        | 1               |

Table 2: Total number and percentages of various isolates obtained in 50 cultures

| Worker                | Year | Culture +vity | S. aureus | E. coli | Proteus spp. | P. aeruginosa | Enterobacter spp. |
|-----------------------|------|---------------|-----------|---------|--------------|---------------|-------------------|
| Waldvoge              | 1970 | 95.3%         | 59.4%     | *       | *            | *             | *                 |
| Bhattacharya et al    | 1974 | 95.2%         | 48%       | 3.4%    | 3.7%         | 8.7%          | 15%               | 3.2%              |
| Dich                  | 1975 | 85%           | 59%       | *       | *            | *             | *                 |
| Arora                 | 1977 | 95%           | 42%       | *       | *            | *             | 27.3%             |
| Henry et al           | 1990 | *             | 42.2%     | *       | 6.9%         | 3.5%          | 17.25%            | 11.8%             |
| Perry et al           | 1991 | *             | 3.3%      | *       | 8.5%         | 20%           | 5%                |
| Dormans and Drumond   | 1994 | 85.90%        | *         | *       | *            | *             | *                 |
| Present study         | 2014 | 90%           | 51%       | 14%     | 5%           | 7%            | 10%               | 3%                |

Table 3: Organisms isolated by various workers from cases of Osteomyelitis
Out of 50 cultures put up, growth or organisms was obtained in 90% of cases, 10% of cultures were sterile, S. aureus was the most common organism isolated 51%. Age wise distribution of organism showed S. aureus to be the commonest aetiological agent in children, but in higher age group gram negative organisms was predominantly obtained. Beta lactamase production was seen in 19 (38%) of S. aureus stains and almost half of the S. aureus strains were MRSA. All MRSA strains were found to produce beta lactamase and were multidrug resistant. There was a mark difference in the antibiotic sensitivity pattern of MRSA vs MSSA isolates. High level of resistance was also observed in gram negative organisms with majority of the isolates showing multi-drug resistance.
DISCUSSION: Osteomyelitis remains a vexing illness despite major advances made in surgery and antimicrobial therapy. The advent of prosthetic joints has added new dimensions to the challenges of septic arthritis and Osteomyelitis as these are prone to become infected by a wide range of organisms including low grade pathogens.\(^1\) Widespread use of antibiotics has altered aetiological pattern of infections and antibiotic susceptibility.

The highest incidence of Osteomyelitis was observed in the 16-30 years age group, which is attributable to the greater likelihood of trauma and compound fractures at this age, this turned out to be the commonest predisposing factor in our study (Table 1) Trauma results in hemorrhage and cell destruction in the region of epiphyseal cartilage followed by diminished tissue resistance postoperative and prosthesis related infections, which formed the second most important predisposing factor in our study, arise from exogenous and endogenous sources. The patient’s skin is the most common source of contamination even though sporadic outbreaks traced to the operating have been reported. Metastatic source of infection may be involved as well e.g. percutaneous sutures, suction drains, intravenous catheters and indrawing urinary catheters.\(^6\)

Our study contrasts with Waldvogel\(^7\) and Okoroma\(^8\) who reported maximum incidence of Osteomyelitis in the age group of 3-15 years but is in accordance with Esperson\(^9\) who described a fall in the incidence of infection in pediatric age group. The decreased incidence observed among children could be due to improved standards of living and early administration of board-spectrum antibiotics.

In children bones of lower extremely are more often affected and of these upper end of tibia and lower end of femur are more liable to infection because of greater amount of growing bones in these areas. Detailed studies by Hobo\(^10\) of the vasculature adjacent to the metaphyseal side of growth plate have provided the most satisfactory explanation for the involvement of growing end of long bones. Adult Osteomyelitis may occur at any site. On our study long bones were more commonly involved, particularly those of the lower extremely.

In children, S aureus was the commonest organism isolated because in them the infection is mostly hematogenous while in adults, gram negative organisms are commonly isolated because infection is commonly secondary to contiguous focus of infection. Male patients outnumbered the females. This has been attributed to more exposure to trauma in males.

Although bone and joint infections caused by gram-negative organisms have significantly increased but S. aureus remains the most common cause of Osteomyelitis till date (Table 3) and MRSA strains is further complicating the situation. Osteomyelitis caused by MRSA often follows multiple traumas and is usually hospital acquired. There was a marked difference in the antibiotic sensitivity pattern of MRSA vs MSSA isolates Beta-lactam antibiotics like penicillin and cephalaxin were not effective against MRSA. A high resistance to quinolones (75%) was seen which correlates with studies from other parts of the country.\(^11,12\) An increased of gentamycin resistance among MRSA strains from 0% prior to 80% after 1996 has been reported.\(^13\) In our study MRSA showed high degree of resistance to gentamycin. Beta-lactamase production was seen in 19(38%) of S. aureus strains. All MRSA strains were found to produce Beta- lactamase and were multi drug resistant. Multi drug resistance among MRSA strains has serious implications as far as treatment of MRSA infections is concerned. It also highlights the need to test newer
group of antibiotics routinely like Linezolid and Vancomycin. Linezolid and Vancomycin showed 91.7% and 100% sensitivity to MRSA respectively. Alternative drugs like teicoplanin and quinupristin-dalfopristin have also shown promising results.\(^{(14)}\)

For gram negative organisms, cefoperazone-sulbactum combination was the most effective drug followed by amikacin. Ampicillin was found to be highly ineffective drug against gram negative infections with a wide range of resistance in Klebsiella spp and E. coli. Gentamycin once found to be most effective drug for the treatment of Osteomyelitis has now become a resistant drug in our study.\(^{(15)}\) gentamycin showed only a minimal sensitivity among gram negative isolates.

P. aeruginosa was found to be highly resistant gram-negative organism with high resistance to gentamycin, ciprofloxacin and pipercillin, as high as respectively. In various studies investigating the resistance of P. aeruginosa to ciprofloxacin, the resistance was reported to be 0 to 89%.\(^{(16)}\)

Multi-drug resistance among pathogenic organisms poses a major challenge in the treatment of infections and increase the morbidity and mortality associated with these infections. A finding of greater concern is the progressively developing resistance to cefoperazone-sulbactum (beta-lactam + beta-lactamase inhibitor) combination among gram negative isolates since antibiotic of choice in the treatment of infections is very much limited.

**CONCLUSION:** Thus we get know that for coverage of gram negative bacteria, betalactam and betalactamase inhibitor combination would be more useful. Use of mono drug therapy needs to be guided by sensitivity report. Continuous monitoring of susceptibility pattern needs to be carried out in individual setting to prevent antibiotic resistance among organisms.

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