Aerobic Microbiological Profile Of Contigious Focus Osteomyelitis

Dr Umesh Hassani*, Dr SV Jalgaonkar, Dr Gopal Agrawal*****

*Lecturer, in NKP Salve Institute Of Medical Sciences,, Second Author is **EX Prof & Head, Indira Gandhi Govt Medical College & Hospital Nagpur,*** Lecturer Indira Gandhi Medical College Nagpur,

Abstracts: Background: Bone and Joint infections are painful for patients and frustrating for them and their doctors. Eradication of infection from bone is difficult since there is fibrosis and low vascularity due to chronic inflammation where antibiotics are difficult to reach. Such infections tend to persist until the infected biomaterial and the adjacent tissues are removed. Osteomyelitis is also a disease in transition with ongoing changes in predisposing factors, causative organism and treatment. The relative frequency of haemetogenous osteomyelitis and relapsing osteomyelitis continues to decline, conversely the incidence of bone infections related to joint replacements, complex surgical interventions and wound infections are increasing. Factors involved in choosing the appropriate antibiotics include infection type, infecting organism, sensitivity results and antibiotic characteristics. **Objective:** To know microbiological etiology in different types of osteomyelitis in our region. **Material & Methods:** Specimens were collected from clinically and radiologically diagnosed 97 patients of Contiguous focus osteomyelitis, In cases of contiguous focus osteomyelitis presenting with sinus tract, deeper material from sinus tract was collected, wherein surgical intervention was required operative biopsy from bone or scrapping from prostheis was collected. Tissue biopsy/ sequestrum obtained were homogenized with sterile mortar and pestle with little sterile broth**,** for microscopic examination and inoculation**.** Pus, aspirate, sinus swab were directly used for microscopic examination and Inoculation. All the samples were inoculated on sheep blood agar and MacConkey agar. The plates were examined for growth after 24 hrs. Any growth was identified by colony characters & standard biochemical tests. **Results:** Staphylococcus aureus was etiological agent (42.05%) in majority of contiguous focus osteomyelitis. Enterobacteriaceae organisms and Pseudomonas aeruginosa were also found to be common agents (11.36% each).Poly-microbial etiology was evident in 15 (17.04%) cases In all 9 cases of contiguous focus osteomyelitis secondary to vascular insufficiency poly-microbial flora was seen. **Conclusion:** Bone infections that originate from contiguous soft tissue infections can have various etiologies and may even be due to mixture of bacteria from multiple genera Staphylococcus aureus is most common etiology [Hassani U NJIRM 2014; 5(3) :102-107]

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Author for correspondence: DR Umesh Hassani Dept Of Microbiology, NKP Salve Institute Of Medical Sciences, Digdoh hills, Hingna MIDC Road, Email - drumeshhassani@yahoo.com

Introduction: Bone and Joint infections are painful for patients and frustrating for them and their doctors.[1] Bone infection, osteomyelitis is characterized by a progressive infectious process resulting in inflammatory destruction of bone, bone necrosis and new bone formation.[2] The eradication of infection from bone is difficult since there is fibrosis and low vascularity due to chronic inflammation where antibiotics are difficult to reach. Such infections tend to persist until the infected biomaterial and the adjacent tissues are removed.[3] Osteomyelitis is also a disease in transition with ongoing changes in predisposing factors, causative organism and treatment. The relative frequency of haemetogenous osteomyelitis and relapsing osteomyelitis continues to decline, conversely the incidence of bone infections related to joint replacements, complex surgical interventions and wound infections are increasing.[4]

Factors involved in choosing the appropriate antibiotics include infection type, infecting organism, sensitivity results and antibiotic characteristics.[5] Because of the changes in the manifestations, epidemiology, and etiological agents, it is important to make a precise microbiological diagnosis. It is important to know microbiological etiology in different types of osteomyelitis in our region.

Material And Methods: After receiving approval from Institutional ethics committee, Specimens were collected from clinically and radiologically diagnosed 97 patients of Contiguous focus osteomyelitis, attending out patient department and/or admitted to wards of our hospital. The

cases were classified as contiguous focus osteomyelitis with or without vascular insufficiency.[6]

1. **Contiguous focus osteomyelitis**: The diagnosis of osteomyelitis secondary to a contiguous focus of infection was established if clinically and radiologically diagnosed cases were associated with penetrating injuries, surgical procedures or by direct extension of infection from adjacent tissue.

2. **Contiguous focus osteomyelitis secondary to vascular insufficiency:** The diagnosis of contiguous focus osteomyelitis secondary to vascular insufficiency was established in clinically / radiologically diagnosed cases associated with peripheral vascular disease/diabetes mellitus and there was involvement of small bones of feet.

Collection & Processing of Specimen: All the samples were collected preferably before start of antibiotics. If patient was already on antibiotics, samples were collected just prior to next dose of antibiotics. In cases of contiguous focus osteomyelitis presenting with sinus tract, deeper material from sinus tract was collected. The sinus orifice was cleaned with cotton bud loaded in tincture of iodine. Deeper material was then collected in syringe by inserting the nozzle without needle into the tract and aspirating while simultaneously applying deep pressure. This was applied preferably by the patient, who had often learnt, how to produce large amount of discharge from the sinus by voluntary muscle contraction, pressure or adopting certain postures. Only when sinus was relatively dry, a specimen was taken with cotton tipped swab. From multiple sinuses, most active sinus was used.[7]

In cases of contiguous focus osteomyelitis wherein surgical intervention was required operative biopsy from bone or scrapping from prostheis was collected. The specimen was obtained during surgical debridment. A large amount of infected tissue or sequestrum was obtained and brought to the laboratory immediately in sterile container. In case of infected prosthesis, scrapping from prosthesis was collected.[8]

In contiguous focus osteomyelitis associated with vascular insufficiency all the patients had undergone surgical debridement and operative biopsy sample were collected.

Tissue biopsy/ sequestrum obtained were homogenized with sterile mortar and pestle with little sterile broth[9], for microscopic examination and inoculation**.** Pus, aspirate, sinus swab were directly used for microscopic examination and Inoculation

All the samples were inoculated on sheep blood agar and MacConkey agar. Sheep blood agar plate was incubated in candle jar at 37^{o}C with 5-10% carbon dioxide. MacConkey agar plate was incubated aerobically. The plates were examined for growth after 24 hrs. Any growth was identified by colony characters & standard biochemical tests.

Antimicrobial susceptibility testing was performed as per the NCCLS guidelines (2002) by modified Kirby Bauer method.[10]

Results: Total of 97 cases of osteomyelitis were included in this study. 88 (80.73%) of cases belonged to contiguous focus osteomyelitis, 9 (8.25%) cases were Osteomyelitis secondary to vascular insufficiency.

The mean age of the patients suffering from osteomyelitis was 41 years. The range in which osteomyelitis occurred was from 1year to 60 years. Male predominance was seen with the male:female ratio of 1.95:1.

Staphylococcus aureus was the predominant organism in 37 (42.05%) cases, followed by organisms of Enterobacteriaceae in 10 (11.36%) cases and Pseudomonas aeruginosa in 10 (11.36%) cases. Polymicrobial etiology was evident in as many as 15 (17.04%) cases. Four strains of Staphylococcus epidermidis isolated from sinuses were excluded as etiological agents and 7 samples yielded no growth. Thus etiology was undecided in 11 (12.5%) cases.

Table 1: Shows Aetiology Of Contiguous Focus Osteomyelitis

Organism	No. Of Cases (%)
Staph.aureus	37 (42.05)
Staph. epidermidis	4 (4.54)
E. faecalis	1 (1.13)
Enterobacteriaceae	10 (11.36)
E. coli	1 (1.13)
Pr.mirabilis	4 (4.54)
Pr.vulgaris	1 (1.13)
K. pneumoniae	1 (1.13)
Ser marcescens	1 (1.13)
Sal. typhi	1 (1.13)
Enterobacter spp.	1 (1.13)
Ps. aeruginosa	10 (11.36)
Polymicrobial	15 (17.04)
No significant organism	11 (15.90)
Total	88 (100)

In all 9 cases of contiguous focus osteomyelitis secondary to vascular insufficiency poly-microbial flora was seen.

Table 2: Shows Etiology Of Contiguous Focus Osteomyelitis Secondary To Vascular Insufficiency

Organism	No. of Cases (%)
Staph.aureus	37 (42.05)
Staph. epidermidis	4 (4.54)
E. faecalis	1 (1.13)
Enterobacteriaceae	10 (11.36)
E. coli	1 (1.13)
Pr.mirabilis	4 (4.54)
Pr.vulgaris	1 (1.13)
K. pneumoniae	1 (1.13)
Ser marcescens	1 (1.13)
Sal. typhi	1 (1.13)
Enterobacter spp.	1 (1.13)
Ps. aeruginosa	10 (11.36)
Polymicrobial	15 (17.04)
No significant organism	11 (15.90)
Total	88 (100)

Discussion: Osteomyelitis is a complex disease that is often associated with high morbidity and considerable health care costs. Despite continued progress towards understanding its pathophysiology and optimal management, many patients with osteomyelitis fail aggressive medical and surgical therapy. The presence of poorly vascularized tissues, the adherence to bone structures and implants, and a slow bacterial replication rate are recognized as important factors for the persistence of infection.[11]

Because the etiology of osteomyelitis is no longer almost certain, identification of the infecting organism has become necessary for appropriate antibiotic treatment.[6]

Identification of the etiological agents responsible for bone and joint infection requires either hard to obtain specimens that are easy to interpret or easy to obtain specimen of difficult interpretation. Isolates obtained by blood cultures, arthrocentesis, or trephine or surgical biopsy are usually clinically significant. In contrast, samples obtained from sinus tract or through open wounds are difficult to interpret.[12] Isolation of the causative agent is essential since appropriate antibiotic therapy is required to cure these infections.

In post-traumatic osteomyelitis and postoperative osteomyelitis contamination of traumatic wounds with soil and water results in infections due to diverse group of micro-organisms, gram negative bacilli in particular. Pseudomonas aeruginosa, have been isolated in more than half the patients, and even fungi are occasionally isolated in post traumatic Osteomyelitis.[13]

In the present study, postoperative / post-traumatic osteomyelitis accounted for 71 (80.68%) cases of contiguous focus osteomyelitis. These cases included posttraumatic, postoperative, and cases with foot puncture.

Staphylococcus aureus was isolated in maximum number of cases i.e 31 (43.66%). Out of 10 (14.08%) enterobacteriaceae isolates, Proteus mirabilis was the predominant organism in 4 (5.63%) cases. As many as 9 (12.67%) strains of Pseudomonas aeruginosa were isolated; 3 (4.05%) were isolated from cases of foot puncture. Poly-microbial growth was seen in 9 (12.67%) cases .Seven (9.85%) samples yielded no growth. (Table 2)

In a study of cases of post surgical and post traumatic wounds by Kelly et al [14], the infecting organism was Staphylococcus aureus in all 9 cases.

In a study by Perry et al [15], the etiological agents recovered from cases of post surgical and post traumatic osteomyelitis were Staphylococcus aureus (42%), Pseudomonas aeruginosa (13%), Enterobacter cloacae (6.5%), Proteus mirabilis (5.5%), Streptococcus group A (5.5%,) and group B (4.5%), E coli in (3%)and Corynebacterium, Morganella and Salmonella spp.were isolated in 1% case each. Anaerobes were found in 3% cases, polymicrobial infection was seen in 41.42% cases.

Bone infections that originate from contiguous soft tissue infections can have various etiologies and may even be due to mixture of bacteria from multiple genera Staphylococcus aureus is most common etiology, but various gram negative bacteria can cause osteomyelitis in this clinical setting. In the present study, 14 (15.90%) cases were due to contiguous source of infection. Out of these 3 cases were of decubitus ulcer, 2 cases of odantogenic infection and 9 cases of mastoiditis.

The organisms isolated in these cases were Staphylococcus aureus 4 (28.57%), Staphylococcus epidermidis 3 (21.42%), Pseudomonas aerginousa 1 (7.14%). Poly-microbial etiology was seen in majority of cases 6 (42.85%)

In study of osteomyelitis associated with pressure sores by Darouiche et al16, the organisms isolated were poly-microbial in 66.66% cases. In mono-microbial cases the etiological agent was Staphylococcus aureus (33.33%). The poly-microbial organisms were Staphylococcus aureus and Streptococcus in 16.66% cases and three or more organisms with mixture of aerobic and anaerobic organisms in 50% cases. In a study of anaerobic osteomyelitis in children with contiguous source, the predominant organisms isolated were anaerobes in 89.18%, Staphylococcus aureus in 4.05% and streptococci in 6.75%.[17]

Elective procedures without the implantation of foreign bodies and procedures performed on children have low infection rate (0.5%)[18]**.** In surgery after fracture and dislocation, especially with an open injury, the infection rate is 1 to 2 percent. Osteomyelitis frequently develops from infection after surgery that involves a foreign body a plate, screws, a rod or a total joint arthoplasty[19]**.** Most infections are due to gram positive bacteria acquired in operating rooms.[13]

The implants are colonized by air borne, skin and surgeon related bacteria during surgery despite being operated in closely respected operating regime.[20]

In the present study, Staphylococcus aureus was recovered from scrapping of infected prosthesis in 2 (66.66%) cases and Staphylococcus epidermidis in 1 (33.33%) case.

In a study of prosthetic infections by Fitzgerald et al [21]**,** Staphylococcus epidermidis was found in 32.39%, Staphylococcus aureus in 26.76%, streptococcus in 11.97%, Bacillus spp. in 3.52%, Pseudomonas in 6.33%, anaerobes in 7.74%, E.coli in 2.8%, Proteus mirabilis in 2.81%, Enterobacter cloacae in 2.11% and others in 2.11%.

In the present study, Staphylococcus aureus was responsible for majority 37 (42.05%) cases of contiguous focus osteomyelitis followed by enterobacteriaceae 10 (11.36%) cases and Pseudomonas aeruginosa 10 (11.36%) cases. Poly-microbial growth was seen 15 (17.04%) cases. In enterobacteriaceae, Proteins mirabilis was the commonest organism seen in 4.54% cases, E.coli, Proteus vulgaris, Klebsiella pneumoniae, Serratia marcescens, Enterobacter spp. Salmonella typhi were seen in 1 (1.13%) case each.

In cases of contiguous focus osteomyelitis, Waldvogel et al [6] had reported 48.9% Staphylococcus aureus, 2.18% coagulase negative staphylococci, 9.48% streptococcus, 23.35% gram negative bacilli and 1.46% anaerobes.

Mackowick et al [22] found 39% Staphylococcus aureus, 34.75% enterobacteriaceae and 26.25% pseudomonas in contiguous focus osteomyelitis.
Dubey et al [23] found 39% Staphylococcus aureus, 8.7% coagulase negative staphylococci, 8.7% E.coli, 2.8% Klebsiella spp, 4.5% Proteus spp., 2.8% Enterobacter spp., 8.4% Pseudomonas aeruginosa and others 4.5%

Cierny et al [24] reported 26% cases of Staphylococcus aureus, 10% coagulase negative

staphylococcus, 8% Streptococcus, 10% Enterococcus, 15% Pseudomonas aeruginosa and 13.4% others. Poly-microbial infection was seen in 76% cases.

Etiology in contiguous focus osteomyelitis secondary to vascular insufficiency: In osteomyelitis secondary to vascular insufficiency, blunting of local tissue response due to inadequate tissue perfusion predisposes the patient to infection with multiple organisms. Organisms are coagulase positive and negative staphylococci, Streptococcus spp., Enterococcus spp., gram negative bacilli, and anaerobes. Aerobic gram negative bacilli are usually a part of mixed infection. [25]

In the present study, etiology of osteomyelitis associated with vascular insufficiency was poly-microbial in nature in all 9 cases. More than 2 organisms were seen in 1 (11.11%) case. Two organisms were seen in remaining 8 cases (Table II).

Staphylococcus aureus along with Enterobacteriaceae group of organisms was most commonly isolated in 3 (33.33%) cases, Staphylococcus aureus and Streptococcus pyogenes were isolated in 2 (22.22%) cases. Pseudomonas in combination with Enterobacteriaceae was seen in 2 (22.22%) cases. In two cases (22.22%), E.coli and Klebsiella aerogens in combination were causative organisms.

In a study by Waldvogel et al [6], most of the cultures obtained from surgical specimens or from wound, showed two or three different types of organisms. The association most frequently found included Staphylococci and Streptococci or Staphylococci, Streptococci and Enterobacteriaceae. These two combinations accounted for more than half the cultures obtained from bones or wounds.

In a study of 20 diabetic patients with osteomyelitis of foot, multiple organisms were isolated in 12 (60%), Staphylococcus aureus in 17.02%, Enterococci in 12.76%, Streptococcus group B in 10.60%, Klebsiella spp in 8.51%, Proteus spp in 6.38%, Morganella morganii in 6.58%, Corynebacterium, Staphylococcus epidermidis, E.coli in 4.25% cases each. Citrobacter, Enterobacter, Serratia marcescens and Pseudomonas in 2.12% cases each. Anaerobes were isolated from 13% cases.[26]

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