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Bacteriological Study of Chronic Osteomyelitis

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Chronic osteomyelitis is a persistent disease difficult to treat or eradicate completely. The prognosis depends heavily on proper identification and treatment of the bone-infecting organism. Change in the bacterial flora and indiscriminate use of antimicrobial agents has led to the emergence of multidrug resistant strains. One hundred chronic osteomyelitis cases attending Orthopaedic OPD and those admitted in Orthopaedic wards of K R hospital, Mysore medical college, Mysore were studied. Under proper aseptic conditions suitable specimens were collected, processed and isolates were identified using standard laboratory techniques. There were 74 were males and 26 females. Majority of the patients were in the age group of 21-50 years (66%). Femur (49%) was commonly affected. Monomicrobial flora was 63% and polymicrobial flora 19%. A total of 110 organisms were isolated, 101 (91.82%) were aerobes and 9 (8.18%) anaerobes. The most commonly isolated aerobes were *Staphylococcus aureus* (41.82%) and *Pseudomonas Spp*. (21.82%) and anaerobes were *Peptostreptococcus Spp*.(4.54%)and *Bacteroides spp*.(3.64%) Trauma was the most common predisposing factor. In the present study *S. aureus* was the most common cause of chronic osteomyelitis followed by *Pseudomonas Spp.* Trauma was the most common predisposing factor.

Key words: Chronic osteomyelitis; Microbial flora; Predisposing factors; Aerobes.

Osteomyelitis is an inflammation of bone caused by an infecting organism. It may remain localised or it may spread through the bone to involve the marrow, cortex, periosteum and soft tissue surrounding the bone. Chronic osteomyelitis is a long-standing infection of bone by microorganisms that evolves over months or even years, characterised by low-grade inflammation, presence of dead bone, reactive new bone formation and sinus tracts discharging pus or dead bone.

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Chronic osteomyelitis is found most commonly in adults and usually involves long bones, vertebral bodies and small bones of the feet. Chronic osteomyelitis in adults is most frequently the sequelae of trauma and occasionally recurrent infection following acute haematogenous osteomyelitis during childhood.

MATERIAL AND METHODS

The present study was done in the Department of Microbiology, Government Medical College, Mysore. A total of one hundred chronic osteomyelitis patients attending Orthopaedic outpatient department and those admitted in Orthopaedic wards of K.R. Hospital, Government Medical College, Mysore were studied.

Inclusion criteria

One hundred clinically diagnosed patients of chronic osteomyelitis of all age groups and both sexes attending the Orthopaedic outpatient department and those admitted in Orthopaedic wards.

History taking and examination

A proforma was filled for each patient documenting age, sex, address and clinical information including chief complaints, duration of symptoms, predisposing factors and any history of previous treatment.

Collection of specimen

The sinus orifice and surrounding skin was first cleaned with Povidone-Iodine solution. The superficial discharge of the sinus was squeezed out gently and discarded. The deeper material exudate, sequestrum and granules were collected aseptically into a 3 separate sterile container for gram stain, aerobic culture and anaerobic culture while simultaneously applying deep pressure surrounding sinus track. In cases of multiple sinuses, the most active one was considered. For anaerobic bacteria study specimens were immediately transferred into thioglycollate broth at the point of specimen collection and sealed tightly, transported immediately to laboratory. Specimens were subjected to Gram staining and direct microscopy, aerobic culture and anaerobic culture. The dead bone fragments and granules were grinded into very small fine pieces in a sterile mortar and pestle.

Processing of specimen

Direct microscopic examination

Gram stain: Smear was prepared on clean glass slide, air dried and fixed with one or two drops of methanol and was left on the smear for a minimum of two minutes or until the methanol evaporated. Gram stain was done for the smear and examined under oil immersion objective for the presence of pus cells and bacteria.

Culture

Aerobic culture

The specimen was inoculated onto nutrient agar, blood agar and MacConkey agar. All plates were incubated aerobically at 37°C and observed for growth at 24, 48 and 72 hours. The organisms isolated were identified using standard techniques based on the colony characteristics, morphology in Gram staining of culture smear and biochemical properties.

Anaerobic culture

Growth on thioglycollate medium

Gramstaining was done for the smears made from broth showing turbidity after incubating for 24, 48 and 72 hours following which subcultures were made onto neomycin blood agar and further processing was done.

The specimen was inoculated onto neomycin blood agar and then transferred to thioglycollate medium. After inoculation of the sample on neomycin blood agar, a metronidazole disc was placed at the inoculation site. The inoculated media were immediately placed in Dyanox anaerobic jar and incubated at 37°C for 48 hours. Anaerobiasis was achieved using cold catalyst, Dyanox charge (J:I Model). The charge was activated by pouring 60 ml of 25% sulphuric acid and lid of the jar was clamped tightly with silicon jelly and outer tube of the jar was 2/3rd filled with saturated sodium carbonate solution. Anaerobiasis was monitored by observing immediate vigorous bubbling from the outer tube of the jar. Change in the colour of the freshly prepared methylene blue solution indicator and the growth of strict aerobe *Pseudomonas aeruginosa* were used as control for ensuring the maintenance of anaerobiasis.

The organisms were identified to genus level by Colony morphology, Gram's stain of the colony, Pigmentation, Pitting of agar, Haemolysis, Sensitivity to Metronidazole disc, Aero tolerance test, Resistance to Kanamycin, Vancomycin and Colistin.

RESULTS

Out of 100 cases studied, 74 (74%) were males and 26 (26%) were females, 24 (24%) were of age group 21-30 years. Chronic osteomyelitis was most commonly seen in 3rd, 4th and 5th decades (66%) and in males (74%). Predisposing factors :Trauma was seen in 59 (59%) cases, followed by post-operative infections and multifactorial each in 9 (9%) cases, diabetes mellitus 8 (8%) cases, orthopaedic implants 6 (6%) cases, contiguous infection 5 (5%) and haematogenous infections in 4 (4%) cases. The most common predisposing factor for chronic osteomyelitis was trauma (59%). Bones affected; Femur was involved in 49 (49%)

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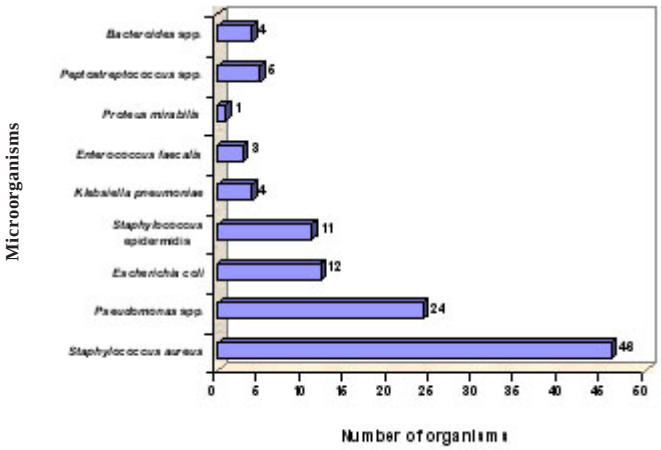

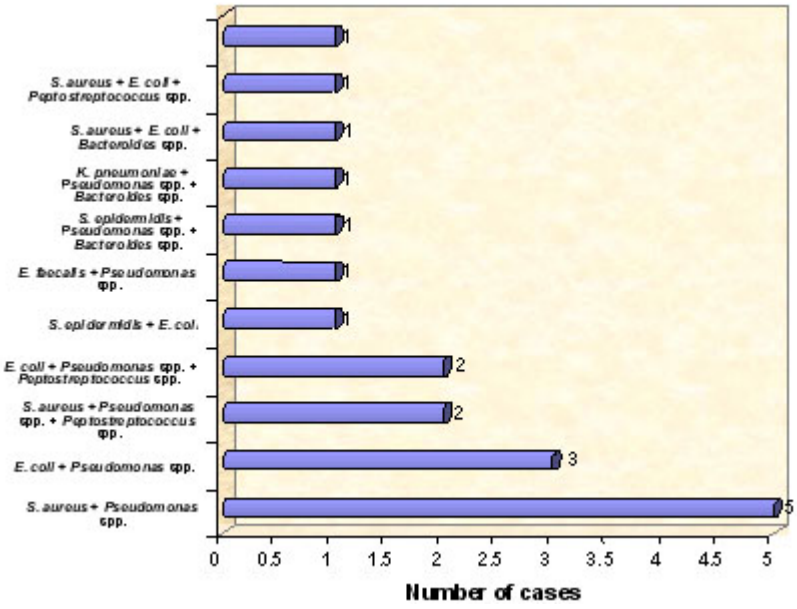

Fig. 2.

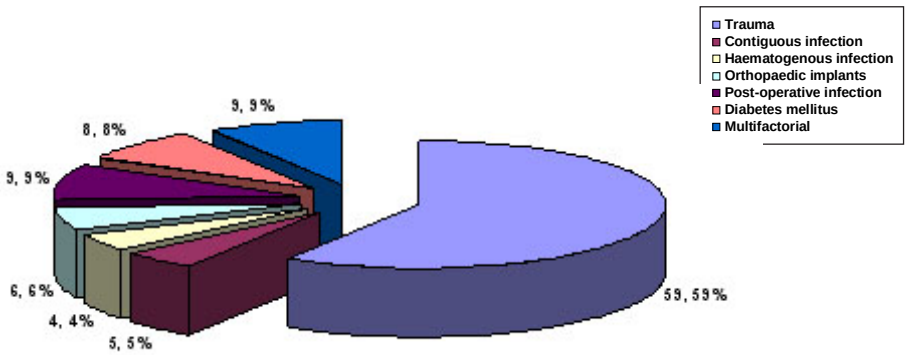

Fig. 3. Showing predisposing factors for chronic

cases, tibia 31 (31%) cases, calcaneum 6 (6%) cases, humerus, metatarsals and ulna each in 3 (3%) cases, fibula 2 (2%) cases, clavicle, radius and metacarpals each in 1 (1%) case. The most common bone involved in chronic osteomyelitis was femur (49%) followed by tibia (31%). 15(15%) of specimens were culture negative. Total numbers of organisms isolated were 110. Of which 101 (91.82%) were aerobes and 9(8.18%) were anaerobes. The most common cause of chronic osteomyelitis was aerobes (91.82%). Monomicrobial flora was seen in 63 (63%) cases and polymicrobial flora was seen in 19 cases and no growth in 18 (18%) cases. The most common microbial flora in chronic osteomyelitis was Monomicrobial (63%) flora compared to polymicrobial flora (19%). Among aerobes, *Staphylococcus aureus* 46 (41.82%), followed by *Pseudomonas Spp.* 24 (21.82%), *Escherichia coli* 12 (10.91%), *S. epidermidis* 11 (10%), *K. pneumoniae* 4 (3.64%), *E. faecalis* 3 (2.72%) and *P. mirabilis* 1 (0.91%) were isolated. Among anaerobes, *Peptostreptococcus* spp. 5 (4.54%) and *Bacteroides* spp. 4 (3.64%) were isolated. Among Polymicrobial aerobic flora, combination of S.*aureus* and *Pseudomonas* spp. in (26.35%), *E.coli* and *Pseudomonas* spp. in (15.79%) were seen. Mixed Aerobic and Anaerobic flora seen in 5%. Most common cause of chronic osteomyelitis was *S. aureus* (41.82%) followed by *Pseudomonas* spp. (21.82%).

DISCUSSION

One hundred patients with clinically diagnosed chronic osteomyelitis were studied in the Department of Microbiology, Government Medical College, Mysore to know the Bacteriology. Age of the patients ranged from 6 to 90 years. 21-50 years was the most common age group affected (66%). Studies of Green *et al.*,[5], Mader *et al.*,[6] and Simpson *et al.*,[7] showed that number of cases between 21-50 years were 78.57%, 88.51% and 44% respectively. Majority of patients were males (74%) than females (26%), Mader *et al.*,[6], Ali *et al.*,[8] (73.07%, 73.50%) reported that chronic osteomyelitis was seen commonly in males and in 3rd, 4th,5thdecades because males are more mobile and vulnerable to injuries. Trauma was the most common predisposing factor (59%), bone infections most commonly follow after injuries like road traffic accidents, crush injuries, post operative wound infections, implant infections, studies of Simpson *et al.*,[7] and Zuluaga *et al.*,[9] showed trauma as major predisposing factor in 63% and 64% of cases. The most common bone affected was femur (49%), studies of Zuluaga *et al.*,[9] showed that most common bone affected was femur as this is the long bone most commonly affected in injuries. Out of 100 specimens studied, 82% were culture positive and 18% culture negative as this can be attributed to prior antibiotic administration, the similar finding was observed in studies of Sugandhi *et al.*,[10], Zuluaga *et al.*,[9] with 80.49% and 86% for culture positive and 19.51% and 14% for culture negatives respectively. Monomicrobial flora was (63%) and polymicrobial flora was (19%), studies of Perry *et al.*,[11] and Simpson AHRW *et al.*,[7] showed 68.33% and 31.66%, 62% and 19% respectively monomicrobial and polymicrobial flora.

Gram-positive organisms (59.09%) were more common than Gram-negative organisms (40.91%), studies of Waldvogel *et al.*,[12] (56% and 38%), Jindal *et al.*,[13] (48% and 42%) respectively showed similar observations as gram positive organisms are more commonly infecting bone and isolated from bone infections. Aerobes (91.82%) were more commonly isolated than anaerobes (8.18%), studies of Ali *et al.*,[8] (95.45% aerobes and 4.55% anaerobes) and Jindal *et al.*,[13](86.40% aerobes and 13.5% anaerobes) showed similar observations with more aerobes isolated. The most common organisms isolated were *Staphylococcus aureus* (41.82%), *Pseudomonas* spp. (21.82%) *E.coli* (10.91%), studies of Simpson *et al.*,[7] (40%), Zuluaga *et al.*,[9](40%) and Jindal *et al.*,[13] (48.3%) showed isolation of *S.aureus*, *Pseudomonas* spp. as the most common organisms isolated. The other organisms were *E. coli* (10.91%), *Staphylococcus epidermidis* (10%), *Peptostreptococcus* spp. (4.54%), *Bacteroides* spp. (3.64%), *Klebsiella pneumoniae* (3.64%), *Enterococcus faecalis* (2.72%) and *Proteus mirabilis* (0.91%). The similar pattern of organisms were isolated in studies of Simpson *et al.*,[7], Zuluaga *et al.*,[9] and Jindal *et al.*,[13].

CONCLUSION

The bacteriological study of chronic osteomyelitis showed *Staphylococcus aureus* as the most common agent followed by *Pseudomonas* spp., and *E. coli*. Chronic osteomyelitis is the common form of osteomyelitis in male adults and is usually the squeal of trauma. Isolation of causal organism is critical in the treatment of patients.

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