DISCUSSION
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Once infection is established in bone, its eradication becomes a tedious task. Achievement of effective concentrations of an antibiotic at diseased site, is of paramount importance, but the factors hampering the desired local concentrations of suitable antibiotic produce great problem in the treatment of the condition. Role of surgery in treatment of bone infection (osteomyelitis) is well established by Buceta and Morgan (1954), Harris (1962), but surgery alone has failed to cure the disease. Topically applied antibiotic or irrigation-suction techniques give better local concentration of drug, but for shorter duration of period only, whereas for eradication of this disease higher local concentrations, of suitable antibiotic are required for a very long time.

All these problems indicate the need of an agent may give adequate local concentration of an effective antibiotic, upto the desired duration of time, to combat the bone infection. This agent should also be free from any local or general side effects, when implanted inside the body.
Incorporation of an antibiotic has been tried to prevent and treat the disease. Favorable results have been reported when it was used in cases of osteomyelitis. Gell (1975) reported low release of an antibiotic when it was mixed to the bone cement. Kenneth (1976) found amoxicillin, cephalolin and gentamicin were the most stable antibiotic in bone cement to treat the cases of osteomyelitis. Hovalius, et al. (1980) reported good results in treatment of infected arthroplasties with gentamicin-loaded bone cement and Bayzton with milner (1982) also reported good results in treatment of bone infection using gentamicin loaded bone cement. Therefore we planned to evaluate the role of antibiotic impregnated bone cement in prevention and treatment of bone infection. Our study was designed to confirm the preventive as well as therapeutic effects of a water-soluble antibiotic in the cases of bone infection, when this antibiotic was incorporated in bone cement.

We carried out this experimental study in Albino rats. Rats were selected as experimental animals, because of their easy availability, benign nature, low cost and resistance to environmental conditions. All the animals were fed on a standard diet throughout the period of experiments. All other factors were also kept same throughout the study in all the groups.
Hind limbs of animals were selected to produce bone infection, because in human beings also bone infection is more prevalent in lower limbs, as these limbs are exposed to more stress and strains (Kakad, 1962). Tibia was chosen for our study to produce osteomyelitis experimentally, as involvement of long bones is more common in this disease. E.g. Vishwakarma (1969), Sharts (1970) and MacNey with Watson (1970) reported more incidence of osteomyelitis in long bones. Gillespie (1981) showed that lesion was most commonly evident in upper tibia than in any other bone.

In present study we used Staphylococcus aureus (NCTC 5571) as causative organism to produce infection in bones. In majority of cases of osteomyelitis in human beings staphylococcus aureus has been reported to be the most common causative organism by several workers e.g. Trusta (1934), Gilmour (1962), Durward (1962), Thomas and Smith (1965), Harris and Willis (1965), Willis (1965), Vishwakarma (1969), MacNey and Watson (1970), Ferguson (1973), Alviraz et al. (1974), Mc Allister (1974), Made and associates (1975), Ring et al. (1976), Nollen and Biggott (1977), Durn bolt (1981), Cartlidge et al. (1981), Gillespie (1981) and Tuanon (1982). In their experimental studies staphylococcus aureus was also selected to produce bone infection by Mehlum (1966), Javurek (1974) and Debel with Francis (1981).
Males are more commonly affected by this
disease as reported by Kakad (1982). Trusta (1959) reported
that injury was a predisposing factor in 37% cases, Boyd
found the same predisposing factor in 54% of cases and Kakad
(1982) showed incidence of trauma in 60% of the cases studied
by him. Considering these facts we produced bone infection
giving surgical trauma as well as drilling the medullary
cavity in animals of this study. Metaphyseal end of tibia
was drilled to produce infection in the bone because pyogenic
infection in bones in human beings is also common at this site.
Elsen (1977) also used the upper half of tibia to produce
bone infection in rats.

Establishment of infection in bones is
uncertain in experimental studies if necrotic or foreign
material is not there (Elsen, 1977). Norden (1970) and
Vinkersma et al. (1974) used sodium naphthalate to create a
necrotic focus to perpetuate or potentiate the infection in
the bones of rabbits. Andriole, Nagel and Southwick (1973)
used steel pins in tibia of rabbit as foreign body (Elsen, 1977).
In our study bone cement bead was used as foreign body which
probably helped in induction of bone infection. Implantation
of foreign substance is contraindicated inside the infected
bone as reported by Beer (1923), but we placed bone cement
bead in which antibiotic was incorporated and found that when
it was implanted into infected bone, favourable results were obtained.

Rat is quite resistant animal, tolerating high dosage of staphylococci, when used to produce bone infection experimentally (Klom et al., 1977). We used one hundred thousand staphylococci in 0.01 ml of suspension poured into the hole made in tibia (2 mm in diameter) the same concentrations (10⁷ bacteria in each ml) and amount (0.01 ml of suspension was also used by Klom and co-workers (1977) to produce bone infection in rats. The size of hole in their study was also the same (2 mm)."

Our study was based on the following investigating procedures, which were carried out in all the animals.

(1) Radiological examination
(2) Necropsic examination
(3) Bacteriological examination
(4) Histopathological examination

**Radiological Examination**

In their study Klom and Watson (1978) used the following criteria to diagnose infection in a bone.

(1) Periosteal reaction
(2) Erosion of bone
(3) Cavititation
(4) Sequestration
In our study also, criteria to diagnose bone infection were very close to those established by Meekay and Watson (1970). We observed erosion of bone, periosteal reaction and changed appearance of bone in considerable number of cases showing other evidences of bone infection. Sequestration is also an important feature of bone infection (Robert, 1962). Ilsa A. et al (1977) reported that radiological features were not evident most of the time, when they produced bone infection in rats experimentally and examined their cases, after two weeks of bacterial inoculation. Beed and Francis (1981) in their experimental study in rabbits, failed to find radiological evidences of bone infection. In our study pathological fractures were seen, probably because of weakening of bone due to infection itself as well as due to drilling the cortex. John and Thomas (1933) recommended rest and immobilisation in cases of bone infection. Orr (1933) also applied the principles of John and Thomas. Trueta (1958-59) also used plaster cast immobilisation, for prolonged period. But we did not provide any type of external splintage or support to infected limb, this may also be some of the cause of pathological fractures observed radiologically in our study.

Maximum sclerotic changes were observed around the drill hole of tibia, where pyogenic organisms were poured in the
beginning of experiment, associated soft tissue changes were also noticed in roentgenographs, in the cases showing infection of bones. In our study reversion of bony changes was not remarkable radiologically after 14 days of treatment, in cases of second test group, probably because of the short duration of treatment (14 days only). On the other hand Wahlig et al (1978) used antibiotic loaded bone cement bead in experimental osteomyelitis, which they produced in femur of dogs, they reported that response of their treatment was remarkable, they also showed radiological changes in bones prior to and after the treatment with antibiotic impregnated cement bead, they showed remarkable improvement radiologically in infected bones after treatment, but the duration of follow up was 9 to 6 months in their study which is much longer than the follow up of animals in our study.

**Macroscopic examination**

In present study we mainly relied upon changes occurring in bone macroscopically, to diagnose bone infection, associated soft tissue changes were also observed but were not given much consideration. Redleg reported (1939) that constant feature of osteomyelitis was discharging sinus in chronic stage, but in our study sinus was not seen in much cases of bone infection, this might be because, duration was shorter (only 14 days) in our cases for establishment
of infection. On the contrary Griffith (1962), reported higher incidences of sinus formation after drilling the cortex, he also reported that incidences of sequestra formation were increased by drilling the bone. In our study also sequestra were also not found in much of the infected bones, Gillespie (1982), reported that the dead bone may be responsible for chronicity of the disease. Nallan and Piggot (1977), in their study found that the formation of subperiosteal abscess was there even in absence of clinical signs, we also observed that the presence of subperiosteal abscess was not related to formation of pus beneath the skin producing cystic swelling over the inoculated leg and in certain cases no clinical sign was present, but subperiosteal abscess was drained. Nallan and Piggot (1977), also described that loss of function in infected limb was an important feature, but we did not observe loss of function in case of infected bones. Desai and Francis (1981), observed that in infected cases the tibia of animals appeared larger macroscopically and there was gross swelling of limb and abscess formation in their untreated cases. We also observed that macroscopic changes were evident in considerable number of infected bone the changes such as thickening, roughening or destroyed bone. Swelling of legs were also observed in many of the infected cases in our study, but abscess formation was not evident in our cases.
The other important macroscopic findings which were observed in our cases are brittle character of infected bone and thick periosteum. In some of the cases of bone infection cement plug which was used to plugged the hole was loosened, but in most of the time it was neither tight nor loose. We also observed that formation of granulation tissue was more in the cases of first test group and in cases of second test group, on 28th day (when antibiotic loaded cement bead was used for 14 days) than those found in control group of animals. The presence of granulation was probably due to control of infection in these cases, in our study 5 out of 9 (55.55%) sinuses were healed after the used of antibiotic loaded bone cement bead in second test group of animals while Carrel and Woods Worth (1930) in their clinical study, achieved healing in 62% cases of osteomyelitis treated with sequestration and primary closures.

**Bacteriological Examination**

Bacteriological evidences of bone infection were considered as the most sensitive and confirmatory index to find out the exact number of infected bones in our study. It was the culture of bone piece (removed from the area adjacent to drill hole) which gave the true incidence of bone infection on the 14th day after inoculation of pathogens.
In our study we observed that microbiologically all (100%) cases were infected on 14th day in second test group (when plain bone cement was used) and after the use of antibiotic incorporated bone cement bead (for 14 days), bacteriological evidences were limited to 19 (38%) cases only on 28th day in this group. This shows that 62% cases were treated in 14 days with the use of antibiotic impregnated bone cement bead in infected bones. Klaen with Jephcott and M. Geachie (1977) in a similar type of experimental study found that only 37% cases of their study were sterile at the end of two weeks. In their study rats were used, the size of tibial hole and concentration of staphylococci was same, but they incorporated 1 gram fusidin to 40 grams of bone cement. In the study conducted by Klaen with Jephcott and M. Geachie also, all the bone pieces (100%) showed positive culture of pathogens after two weeks. These findings are also similar to ours, observed in this experimental study on rats.

In the present study, we found that the culture of discharge from ains showed growth of the inoculated organisms (staphylococci) as well as Bacillus subtilis, as contaminating organism in all the cases (100%) in which ains was present (table-3). This presence of Bacillus subtilis as contaminants shows that this contamination probably occurred from the external environment where the animals were operated or were kept post operatively, because-

1) Contamination did not reach bone in 14 days and was
limited superficially in discharge of sinus, as no
contaminants were observed in culture of bone piece,
adjacent to drill hole.

(ii) Contaminants were seen in all the cases, while animals
were operated in different groups at different times,
using separate set of autoclaved instruments, every time.

(iii) No contaminant was grown in culture of sub periosteal
abscess also.

However *Bacillus subtilis* is non pathogenic
in rats (Wilson and Milen, 1966) and hence did not affect the
result in our study.

The culture of plain bone cement bead before
implantation in body of animals showed that all the cultures
were sterile, but all the plain bone cement bead from control
group and second test group revealed growth of the inoculated
organisms (*staphylococci*) on 14th day after the removal of
beads from tibial hole. These findings prove that plain bone
cement beads do not have any antibacterial activity.
Picknell et al (1977) also reported that plain bone cement
does not show any antibacterial activity. But when bone
cement bead of first test group and also of second test group
at 20th day (in which penicillin was incorporated) were
removed after 14 days of implanting them inside the rats
tibia with pathogens, culture of all these beads (1986) was
sterile. This proves that antibacterial activity of antibiotic
impregnated bone cement was still evident after 14 days of
its insertion in infected tibia. Dall et al (1976) also reported that antibiotic is found in effective concentration for prolonged periods of mixed with bone cement. Steven Heff et al (1981) reported sustained release of an antibiotic for prolonged period from antibiotic impregnated bone cement and similar were the observations of Alson et al (1977). Picknell et al (1977) in their experimental study observed that incorporated antibiotic is having antibacterial activity for at least two weeks, they observed this fact in their vive and vitro experiments.

After incision and drainage 14 cases of second group showed discharge on 14th day and in all these cases (100%) only staphylococcus was grown on culture (table-3), but when antibiotic impregnated bone cement was implanted for 14 days in these cases, only 4 out of 6 (66.66%) cultures were positive for staphylococci after incision and drainage showing (33.33%) became sterile on 20th day. Hughes (1979) also showed that risks of infection in hematomas are reduced when antibiotic impregnated bone cement is used.

HISTOPATHOLOGICAL EXAMINATION:

Histopathological and radiological appearances of infected tibiae in rabbits or in rats resemble those of human osteomyelitis. (Greenfield, 1969; Woods, 1972; Murray and Jacobsen, 1977). Periosteal reaction, bone resorption and formation of sequestra, all are observed in these animals and human beings (Dukel and Francis 1981). But the period of appearing the different stages of osteomyelitis may vary in animals than those in man (Greenfield, 1969).
In this study bone infection was diagnosed histopathologically when changes in bone architecture were present and soft tissue changes were given little consideration. Dhal and Francis (1981) also reported that they observed loss of bone architecture in osteomyelitis, which they produced experimentally in rabbits. Among the bony changes loss of trabecular striations and bone necrosis was observed in our experimental study. We also noticed presence of inflammatory exudates and polymorphonuclear cell infiltration as important histopathological features. Examination of marrow showed shrinkage of fat cells in cases of bone infection, in our study it was seen in more number of infected cases of group two. Dhal and Francis (1981) also found that the marrow of infected bones was not normal in experimentally produced osteomyelitis.

In the present study, the number of animals showing bone infection histopathologically was 42 (84%) on 14th day when plain bone cement bead was used, but on 20th day, after the use of antibiotic impregnated bone cement bead for 14 days the number of infected cases was reduced to 18 (36%) only. This difference of infection in bone on the 14th and 20th day, they showed that bone infection was cured histopathologically in 97.14% cases in animals studied in second test group. But in their clinical study Dhal et al.
with Rotteger and others (1979) observed cure of 71% cases treating deep infection in hip arthroplasties.

In the present study we observed that in control group bone infection was evident radiologically in 34 (68%) cases, but macroscopic and also microscopic evidences of bone infection were present in 44 (88%) case of this group and all (100%) cases were infected bacteriologically on the 14th day. These findings proves that infection was present in bones, though radiological signs were not positive in all the infected cases. Dekel and Fransis (1981) also reported that radiological findings were not evident in all the infected cases of their experimental study on dogs, at the end of two weeks. Elsan et al (1977) were also unable to correlate the radiological findings of infection after two weeks to macroscopic and histopathological features in experimentally bone infection produced experimentally in rats.

Elsan et al (1977) also found that bacterial were present adjacent to bone after two weeks although macroscopic or microscopic changes were not seen in these bones. Our findings were comparable to those of Elsan et al (1977) and bacteria were failed to produce macroscopic or histopathological changes of bone infection (osteomyelitis) in the observed animals of our study, probably due to the body resistance of animals or because of short duration of action (14 days only), however further studies are required to confirm this hypothesis.
The important part in treatment of bone infection is, achievement of proper concentration, of a suitable antibiotic locally, for prolonged period. The various methods, described to get the effective concentration of an antibiotic at diseased site, having their own risks and limitations. Local instillation of an antibiotic provides better concentration of an antibiotic but it is affected temporarily. Lautenschlager (1960) observed that local instillation of an antibiotic is less effective than the use of loaded bone cement. Local irrigation suction techniques are also not safe as antibiotic in it may get absorbed in the body and because of their higher amount in irrigation solution, the toxic manifestations may appear, its failure and complications are also reported. Reker (1974) when used irrigation suction techniques, he observed recurrence in more than 50% cases of osteomyelitis treated with this method. Buda (1974) also showed failure in 44% cases after irrigation. Plugging or leaking of irrigating tube is one of the commonest problem of this technique (Lotte and Wung, 1975). All these risks and complications compelled the scientists to find our safe and effective method of treatment, which can provide the desired, local concentration of an antibiotic for prolonged period to prevent and treat the cases of bone infection (osteomyelitis).
We selected the use of antibiotic impregnated bone cement to prevent and treat the cases of bone infection because:

(i) Its use is safe (Mc Kee, 1970)

(ii) provides higher, local concentrations of incorporated antibiotic, even more that found after parenteral administrations (Steven Haff et al, 1981).

(iii) Most of the antibiotics are stable when incorporated in bone cement (Kenneth et al, 1976).

(iv) It provides bactericidal concentrations of incorporated antibiotic in cancellous tissue, for prolonged period even for months (Steven Haff et al, 1981, and Endler and Weselka, 1980) and thus act as antibiotic depot causing slow release of the incorporated antibiotic (Levin, 1975).

Uthkeff and Geleberg (1980) reported good results of antibiotic impregnated bone cement in treatment of bone infection, they found it more effective then local instillation of an antibiotic, but Joyce Hill et al (1977) said that instant killing of organism is the main requirement during the operation in bone of human being, while incorporated antibiotic takes time to release from bone cement, they therefore recommended the use of systemic or topical antibiotic. In his study Joyce Hill et al (1977)
were not worried about prolonged activity of an antibiotic locally which is one of the important requirements to prevent or treat the bone infection. Elson et al. (1977) also recommended the use of antibiotic impregnated bone cement and said that released antibiotic from cement depot can permeate through the dense cortical bone. In the present study we did not find any adverse effect of bone cement in rats within 14 days of its insertion inside the body.

Ramsey (1970) also reported firm contact between bone and bone cement and absence of any reaction, similarly Dekal and Francis (1981) found that bone cement can be used safely inside the body, he observed formation of bone normally around the bone cement beads which were placed inside the infected bones and left there for months. But Crawford described pathological reaction of bone cement at the site of insertion in human bones, when he observed the cases for long duration. This study was done in 1970.

Most of the adverse effects of bone cement are due to absorption of excess liquid monomer, into the circulation, just after the setting of the bone cement, inside the body (Kittshar and Ser, 1978). In our study we used cement which was allowed to set outside the body, prior to its implantation in the bones of animals. In this way we probably eliminated the possibilities of adverse reactions
of bone cement, during or just after its setting inside the body. Freeman et al (1982), observed a radiolucent line at bone-cement interface due to death of adjacent osteocytes during polymerization of cement inside the bone. We did not observe any radiological evidence of bone reaction to implanted cement in the duration of 14 days, probably because of using set cement in our animals, outside their bodies.

Benzyl penicillin was incorporated in the bone cement in our study because of its water solubility, low cost, easy availability and sensitivity of organisms to this antibiotic. Steven Haff et al (1981) reported the sustained release of penicillin from bone cement, they also observed that heat of polymerization does not inactivate the Beta lactem antibiotics such as penicillin. In incorporation of an antibiotic does not change the physical properties of the bone cement up to a certain limit (Levin, 1975). Although we were not concerned, but used the antibiotic within the limit of safety and which does not affect the physical properties of bone cement (Knight, 1977) we also observed that the penicillin came out of bone cement in active form and thus it could produce antibacterial action upon the inoculated pathogens. Hughes et al (1977) reported that antibiotic leach out of the bone cement rapidly for first two days and
then slowly for six weeks, we observed in our experimental study that antibiotic came out from the bone cement at least for 14 days, as all the antibiotic impregnated cement beads were sterile when examined microbiologically on 14th day of implantation.

Infection of bone was prevented in most of the cases of first test group, when plain bone cement bead was used all the cases (100%) showed bone infection, but infection was limited to only 6 (16%) cases of first test group, when antibiotic incorporated bone cement bead was used for 14 days, showing that infection was prevented in 42 (84%) cases of first test group. Elson and Mc Geachie (1976) also observed beneficial effects of antibiotic impregnated bone cement to prevent bone infection in hip arthroplasties.

In our study all the cases (100%) of second test group showed evidences of bone infection on 14th day, when plain bone cement bead was used, but on 26th day when antibiotic impregnated bone cement bead was used, number of infected bone reduced to 31 (62%) only. These differences on 14th and 26th day show, that established bone infection was cured in 52% cases of second test group. Eshke (1970), in his clinical study reported healing in 70% of his infected cases of traumatic osteomyelitis, infective arthritis and infections of soft tissue. Results in our study are not comparable with those of Eshke (1970)
because his study was based on clinical evaluation and
he also included healing of soft tissues in his results.
Klemm (1979) in his clinical study found that 91.4% cases
of chronic osteomyelitis were subsided completely, when
he used gentamicin incorporated in bone cement. Results
obtained by Klemm (1979) and those in our study are also
not comparable because Klemm (1979) performed conventional
surgery of osteomyelitis to remove scarred tissue, debris
and sequestrated bone etc., and then implanted antibiotic
impregnated bone cement in the infected bones. But in our
study we did not perform any surgery as a treatment of
osteomyelitis, neither sequestrum nor debris were removed
from infected bones in our cases before implantation of
antibiotic impregnated bone cement head and this might be
the cause that we could not get comparably good results.
Klemm (1979) himself explained that failure in his 8.6% cases
were there, because the sequestrum was overlooked in his
non treated cases, at the time of surgery. Wahlig et al (1978)
reported that recovery was evident in infected bones, when
they used antibiotic impregnated bone cement head, they
noticed cure of bone infection clinically, histopathological-
cally as well as bacteriologically, though their reports
and observations are very much same as we observed in our
study, but total duration of follow up in their experimental
study was longer than ours and they incorporated gentamicin
in bone cement.
Rotteger et al (1979) reported success rate of 71% when they treated infected arthroplasties with antibiotic impregnated bone cement. Their study is also not comparable with our study because, their study is based on infected arthroplasties in human beings, they relied upon clinical and radiological evidences only which they observed, after healing of the infected cases and they also incorporated higher concentrations of antibiotic (i.e. 5 gram or more in 40 grams of bone cement powder), the period of follow up was also more in their study than ours. Elsom (1979) reported that antibiotic retains its potency for several years if it is incorporated in bone cement and this may be the cause that the patients studied by Rotteger et al (1979) received effective local concentration for longer periods than those of our test animals.

In our study we could not find that what exactly is the area around bone cement bed in which effective antibiotic concentrations are found. We observed that haematoma adjacent to bone most of the time, were sterile, but discharge distant or superficial to implanted bed showed culture of pathogens. Hughes (1979) in his clinical study reported that antibiotic impregnated bone cement was not able to prevent superficial wound infections.
He said that because of presence of antibiotic in drained fluids, risk of haematoma infections is reduced. This explanation seems quite acceptable but needs further studies. Eason with Jephcott and others (1977) reported that antibiotic which came out from bone cement can penetrate through dense cortical bone: they also did not mention what is the exact extent of penetration of effective concentrations of incorporated antibiotic. There is no such study which can show that what is the minimum or maximum amount of an antibiotic to achieve optimum results without added risks of toxicity. We are also interested to know that what is the exact duration of effective antibacterial activity of an incorporated antibiotic in bone cement and what is the fate of bone cement itself, if it is left inside the body, whether its removal from body is necessary or not and if removal is necessary what is the best suitable time to remove it from the body. All these queries require further studies.