

Development of resistant strains of *Staphylococcus epidermidis* on gentamicin-loaded bone cement in vivo

B. Thornes, P. Murray, D. Bouchier-Hayes

From Beaumont Hospital, Dublin, Ireland

We have compared the rates of infection and resistance in an animal model of an orthopaedic procedure which was contaminated with a low-dose inoculum of *Staphylococcus epidermidis*. We randomised 44 Sprague-Dawley rats to have bone cement implanted subcutaneously containing either gentamicin or saline (control). The wound was inoculated with a dilute solution of gentamicin-sensitive *Staphylococcus epidermidis*. At two weeks the cement was retrieved and microbiologically tested. A lower overall rate of infection was seen in the gentamicin-loaded cement group, but there was a significantly higher rate of gentamicin-resistant infection in this group (Fisher's exact test, $p < 0.01$). Antibiotic-impregnated cement has an optimum surface for colonisation and prolonged exposure to antibiotic allows mutational resistance to occur. Gentamicin-loaded cement may not be appropriate for revision surgery if it has been used already in previous surgery.

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Staphylococcus epidermidis is the most commonly implicated pathogen in infections of orthopaedic implants.^{1,2} It is a normal skin commensal of low virulence, but has the ability to adhere to, and colonise, implanted material. It can produce a biofilm which protects against parenteral antibiotic therapy as well as the host response. This infection of an implant may take weeks or months to become clinically apparent.

The use of antibiotic-loaded cement has been successful in reducing rates of infection³ and is now used widely in orthopaedic surgery. It allows a high concentration of antibiotic to be delivered to the site of the operation while

reducing systemic side-effects. However, the problem of emerging resistant strains of bacteria has provoked controversy regarding its routine use. There is in vitro evidence that bacteria, including *Staphylococcus epidermidis*, can alter their resistance profiles after adherence to antibiotic-loaded cement.⁴⁻⁶

We have therefore compared in an animal model the rates of infection and profiles of antibiotic resistance in gentamicin-loaded bone cement with those in cement without antibiotic.

Methods and Materials

We used 44 adult male Sprague-Dawley rats weighing 350 to 500 g in a blinded, randomised control trial. Cement containing gentamicin was implanted in 22 and cement containing normal saline only in the other 22. The latter formed the control group. Syringes of additive were drawn and labelled by an independent third person, in order to blind the main operator. Plain Palacos bone cement (Schering-Plough, Brussels, Belgium) was prepared under aseptic conditions with either 2 ml (80 mg) of gentamicin or 2 ml of normal saline added to a half mix (20 g) of cement at the time of mixing. Pellets of equal cylindrical proportions were prepared using a 20 ml syringe as a mould, giving a size of pellet of approximately 10 mm in height and 15 mm in diameter. A standard dilute solution (10^3 /ml) of *Staphylococcus epidermidis* was prepared from a known gentamicin-sensitive colony.

The rats were weighed, labelled and anaesthetised using inhalational halothane. An area of skin on the back was prepared by shaving and painting with Betadine antiseptic solution. An incision 4 cm long was made and a subcutaneous pocket developed into which the pellets were inserted, two similar pellets per rat. The pocket was inoculated with 2 ml of the standard solution of *Staphylococcus epidermidis*, and the wound closed with nylon sutures.

After 14 days the rats were re-anaesthetised, the skin painted with Betadine, the wound reopened and the pellets retrieved. We gently poured 10 ml of saline over the retrieved pellet to remove blood and debris, without disturbing any adherent biofilm. The pellets were then immediately placed in a vial containing 10 ml of nutrient broth. A culture swab was taken from the depth of the wound.

B. Thornes, FRCS I, Orthopaedic Registrar
P. Murray, FRCS Ed (Orth), Consultant Orthopaedic Surgeon
D. Bouchier-Hayes, FRCS, Professor of Surgery
Department of Surgical Research, The Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland.

Correspondence should be sent to Mr B. Thornes at 25 Haddon Court, Clontarf, Dublin 3, Ireland.

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Microbiological analysis. The wound swab was immediately plated onto Columbia blood agar, incubated at 37°C for 24 hours and then read. The broth containing the harvested pellets was vortexed for one minute to dislodge any colonised bacteria into the broth, incubated at 37°C for 24 hours, and plated on to Columbia blood agar. A 10 µg gentamicin sensitivity disc was placed in the centre of the plate. These plates were incubated for a further 24 hours and then read. Negative and positive controls were used from uncontaminated broth and from a broth sample of the original inoculum. A positive growth was defined if the bacterial count of the broth exceeded 10⁶/ml. Resistance was defined as a positive growth up to the edge of the sensitivity disc. Sensitivity was recorded if a zone of inhibition of at least 3 mm was present around the disc. No intermediate cases were seen.

Results

Both groups of rats were similar in terms of age and weight. None died during the experiment and there was no evidence of gross infection in either group.

The rates of wound infection as determined by the wound swabs were 27% (6/22) and 32% (7/22) for the gentamicin and control groups, respectively. The rates of pellet infection were 41% (9/22) and 73% (16/22) for the gentamicin and control groups, respectively (Fisher's exact test, $p < 0.07$; Fig. 1). The relative risk is 0.56 (95% confidence interval (CI) 0.32 to 0.99).

A larger number of gentamicin-resistant infections was seen in the cement group containing gentamicin; 78% (7/9) of infected cases were gentamicin-resistant compared with only 19% (3/16) in the control group (Figs 2 and 3). This was statistically significant (Fisher's exact test, $p < 0.01$). The relative risk of gentamicin resistance among those which developed pellet infections was 4.1 (95% CI 1.4 to 12.2).

Taking gentamicin-resistant infection as a primary endpoint, seven and three animals developed it in each group, respectively. The relative risk of a gentamicin-resistant infection is 2.3 (95% CI 0.69 to 7.8). The small number of events means that this relative risk is not distinguishable from 1 (no effect), but in view of these results, reflects the small number of incident cases of gentamicin resistance, rather than suggesting no effect.

Discussion

Our animal model was designed to resemble the clinical situation of an orthopaedic procedure which becomes contaminated with a low-dose inoculum of *Staphylococcus epidermidis* and the resulting colonisation of the implant.

Detection of infection on implants by low-virulence bacteria can be difficult. Tunney et al⁷ described a method in which suspected implants were immediately transferred into a culture medium after extraction, followed by ultra-

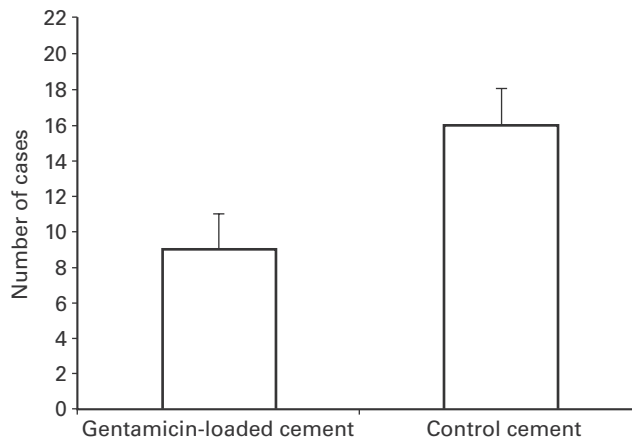


Fig. 1

Total pellet infection rates in both groups.

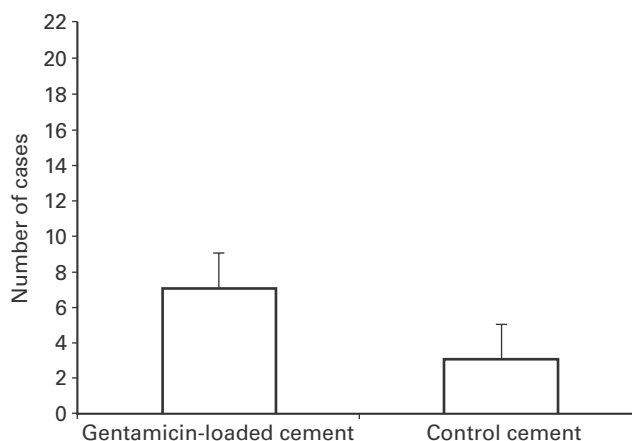


Fig. 2

Gentamicin-resistant infection rates in both groups.

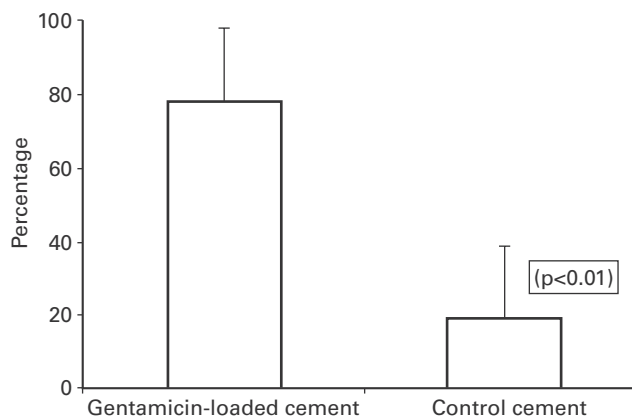


Fig. 3

Percentage of gentamicin-resistant infection.

sonication to help to dislodge colonised bacteria. This resulted in an increased rate of detection, particularly of low-virulence and anaerobic bacteria. Our method was similar and shows a higher rate of detection of pellet infection when the pellet/broth suspension is vortexed compared with the rates of infection in wound swabs.

Gentamicin-loaded cement is successful in reducing primary rates of infection and our study supports this. However, the problem of resistance to antibiotics has been noted. Resistant strains of *Staphylococcus epidermidis* were seen in both groups, but at a significantly higher rate in the gentamicin group. Resistance can occur by a variety of different processes.⁸ Mutational resistance can occur with various antibiotic/organism combinations. Translocation of resistance genes to mobile DNA is rarer, but can cause 'strain epidemics'. Also, existing clones of resistant bacteria may simply be selected out in an antibiotic-rich environment.

In our study we believe that mutational resistance has occurred. If existing clones of resistant bacteria were being selected, a similar absolute number of resistant cases would be seen in both groups. The relative risk of developing a gentamicin-resistant infection was 2.3 times higher (95% CI 0.69 to 7.8) in the gentamicin-loaded cement group.

Elution of antibiotic is related to the surface area of the cement^{9,10} and is highest in the first 24 hours after implantation, decreasing gradually with time. Therapeutic levels are maintained for 8 to 14 days.¹⁰⁻¹² However, sub-therapeutic levels of antibiotic may be present for some years afterwards. Resistant coagulase-negative staphylococci have been noted on the skin after the prolonged prophylactic use of antibiotics.¹³ Antibiotic-impregnated cement provides an excellent environment for the development of resistant strains of *Staphylococcus epidermidis*. It gives an optimum surface for colonisation and prolonged exposure to antibiotic allows mutational resistance to occur.

In revision arthroplasty, a different antibiotic should be added to the cement from that used in the primary cement, since resistant bacteria may be present, despite a lack of symptoms. This should be combined with appropriately selected systematically administered perioperative antibiotics. The emergence of gentamicin resistance among strains of *Staphylococcus epidermidis* in this infection model emphasises the potential for the development of resistance among low-grade pathogenic bacteria which

have an affinity for implanted materials. Vigilance of the use of antibiotics in bone cement is needed to reduce the development of multiresistant strains of *Staphylococcus epidermidis*.

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