Tobramycin-Containing Bone Cement and Systemic Cefazolin in a One-Stage Revision. Treatment of Infection in a Rabbit Model

Marc W. Nijhof,[1] Andre Fleer,[2] Karin Hardus,[1] H. Charles Vogely,[1] Leo M. Schouls,[3] Abraham J. Verbout,[1] Wouter J. A. Dhert[1]

[1] Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands

[2] Department of Medical Microbiology, Eijkman-Winkler Institute, University Medical Center Utrecht, Utrecht, The Netherlands

[3] Research Laboratory for Infectious Diseases, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Received 30 March 2001; accepted 29 June 2001

Abstract: The efficacy of tobramycin-containing bone cement with that of systemic cefazolin for treatment of infection in a one-stage revision model is compared. In addition, the value of detecting bacterial DNA after antibiotic treatment was investigated. An implant was inserted into the right tibia of rabbits after inoculation with *Staphylococcus aureus*. At 28 days, the implant was removed. Subsequently, either plain bone cement with or without systemic administration of cefazolin, or tobramycin-containing bone cement was injected into the medullary canal. The tibiae were cultured 14 days after revision (Day 42), and showed a significant decrease in bacterial counts for both antibiotic groups compared with the control group ($p \leq 0.05$). The rate of infection in the tobramycin-cement group was slightly higher (2/9) than in the cefazolin group (0/8), although the difference was not significant. Persistence of bacterial DNA after antibiotic treatment may be the result of delayed clearance of DNA and not a sign of active infection. This animal model shows that in a one-stage revision tobramycin-containing bone cement can reduce size and rate of infection, although systemic cefazolin may be more efficacious. Therefore, the use of antibiotic-containing bone cement combined with systemic antibiotic might provide optimal treatment. © 2001 John Wiley & Sons, Inc. J Biomed Mater Res (Appl Biomater) 58: 747–753, 2001

Keywords: one-stage revision; tobramycin-containing bone cement; arthroplasty infection; systemic antibiotic; animal model

INTRODUCTION

Infections of total joint prostheses are frequently associated with the presence of necrotic bone, devascularization, and bacteria with specific growth characteristics. The latter, that is the ability of pathogens to adapt to the presence of a foreign body and sustain capability to withstand antibiotics and host defenses, complicates treatment especially. Therefore, rigorous treatment modalities are necessary to eliminate the infection, but the best choice for treatment of an infected total joint prosthesis still remains to be clarified. Consensus exists among most orthopedic surgeons to remove the infected prosthesis if possible, because the infection is difficult to treat in the presence of foreign material covered with bacteria.[1] Usually antibiotic-loaded bone cement is used for fixation of a new prosthesis to provide a high local tissue concentration of antibiotics. Such a revision operation can be performed as either a one- or two-stage procedure. In the one-stage revision the infected implant is removed, the implant bed is debrided and lavaged, and a new prosthesis is inserted. In a two-stage revision, the insertion of the new implant is postponed until after removal of the infected implant and treatment of the infection with treated with systemic antibiotics and/or local antibiotic-loaded beads. The new prosthesis is inserted not until the infection parameters have regained normal levels. The use of antibiotic-loaded bone cement for fixation of the revision prosthesis is preferred, given the higher incidence of infection after revision in comparison with primary joint prostheses. Although most surgeons choose the two-stage procedure for exchange of an infected prosthesis, the one-stage procedure is also widely used, especially in Europe.[2,3]

No benefits of any kind will be received either directly or indirectly by the authors.

Correspondence to: W.J.A. Dhert, Department of Orthopaedics, G05.228, University Medical Center Utrecht, P. O. Box 85500, NL 3508 GA Utrecht, The Netherlands (e-mail: wdhert@chir.azu.nl)

So far, reviews of the literature on infected arthroplasties reported success rates of 82–83% for one-stage revisions, and 91–93% for two-stage revisions.[4,5]

In a previous study, it has been shown that Simplex-P tobramycin-containing bone cement can prevent *Staphylococcus aureus* and *Staphylococcus epidermidis* infections in the rabbit femur.[6] That model evaluated the development of an infection after inoculation of the medullary canal with bacteria, immediately followed by insertion of the antibiotic-containing bone cement. However, in order to treat a preexistent implant infection, one that has developed after introduction of bacteria at an earlier time point, the antibiotic-containing bone cement should also be effective against pathogens that may have become phenotypically adapted to their new habitat. In a one-stage revision procedure, when no previous surgical attempts have been undertaken to treat such an infection, this may be even more demanding.

The purpose of the present study was to compare the efficacy of tobramycin-containing bone cement with that of systemic cefazolin for treatment of infection in a one-stage revision model. In addition to conventional culture techniques a PCR hybridization assay was used in the detection of bacteria as bacterial DNA.

MATERIALS AND METHODS

Design

To establish an infection, a preformed non-antibiotic-containing cement plug was introduced in the right tibial medullary canal of 30 rabbits, after local inoculation with *S. aureus*. Four weeks after insertion of the implant, the rabbits were divided into three groups (10 rabbits each), and a one-stage revision of the implant was performed in all rabbits. After removal of the infected implant and lavage of the implant bed (without formal debridement), bone cement was injected into the medullary canal. Group 1 received tobramycin-containing bone cement, Group 2 received plain bone cement (no antibiotics) as control, and Group 3 received plain bone cement and additional systemic antibiotics. Fourteen days after the revision procedures the tibiae were excised and the cortex adjacent to the cement was cultured. The efficacy of the different treatments was compared based upon the number colony-forming units of bacteria following culture.

Bacterial Strain

Staphylococcus aureus, strain Wood-46 (ATCC 10832) was used. After culture in Mueller-Hinton broth, a stock of aliquots was frozen. The concentration of bacteria (colony-forming units per milliliter, CFU/ml) was determined by serial dilution and plating on blood agar. In a volume of 0.1 ml, a dose of either 10^5 CFU or 10^6 CFU was injected in the medullary canal of the rabbit's tibia. The first four rabbits in each group received an inoculum dose of 10^6 CFU. This dose was the same as was used in a previous study on the prevention of implant bed infection in the tibia of rabbits.[7] In an attempt to reduce loss of rabbits due to sepsis the dose was changed to 10^5 CFU in the subsequent rabbits. The latter inoculum dose has proved to establish an infection in another animal model of tibial implant infection.[8]

Animals

Healthy adult female New Zealand white rabbits (Ico:NZW, Broekman Instituut BV, Someren, The Netherlands) weighing 3000–3500 g were obtained 1 week prior to surgery to acclimatize to the housing in the Central Animal Laboratory. The animals were caged in individual cages, fed with 80–100 g antibiotic-free Hope Farms rabbit diet LKK-20 and water *ad libitum*. Postoperatively, the animals were kept in the barrier housing facility of the Central Animal Laboratory until they were killed.

The guidelines of the Dutch act on animal experiments (1985) were observed.

Surgery

The anesthesia protocol was the same for both operations (induction of infection and revision of implant). Surgery was performed under strict aseptic conditions and under general inhalation anesthesia. Preoperatively the rabbits were weighed. The anesthesia was prepared by an intramuscular injection of 4 mg methadone, 4 mg acepromazinemaleate, and 0.5 mg atropine. A pressure line was introduced into the auricular artery for measuring blood pressure. Subsequently the anesthesia was induced by an intravenous injection of etomidate (8–12 mg). An endotracheal tube (No. 3) was introduced, through which the anesthesia was maintained by a 1:1 mixture of nitrous oxide, oxygen, and halothane 1%. The skin of the right leg was clipped and the rabbit was placed with its left side on the table. The operative area was disinfected with povidone–iodine and isolated by sterile drapes. Postoperatively, pain relief was provided by 3 mg nalbufine i.m. immediately postoperative and subsequently 0.3 mg buprenorfine i.m. If necessary, buprenorfine injection was repeated postoperatively.

Infection of Primary Implant

At the first operation (establishment of infection), the right knee joint was opened via a parapatellar incision. Anterior to the insertion of the anterior cruciate ligament on the tibia, the medullary canal was opened. With the use of an air-pressured AO minidrill the cortex was penetrated by a small drill (diameter 1.2 mm), and the medullary canal was reamed with drills and fraises up to a length of at least 25 mm and a width of 3.9 mm. The content of the medullary canal was suctioned and flushed with saline. Prior to insertion of the implant, the bacterial suspension was introduced in the tibial canal. Subsequently, the implant (preformed cement on a central metal wire, 25 mm in length, 3.9 mm in diameter, Figure 1) was press-fit inserted in the medullary canal. The joint capsule and skin were closed in layers with Vicryl 3-0.

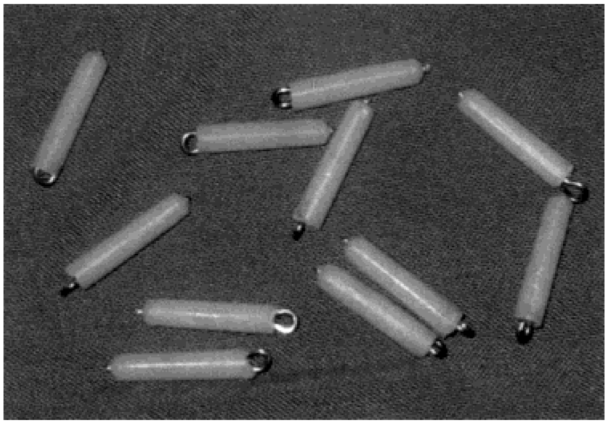

Figure 1. Example of implants used to create an infection.

Revision of Implant

The implant was exchanged 28 days after the first operation. Through the parapatellar scar the knee joint was opened. The present implant was removed from the right tibial canal, inoculated on blood agar plates, and incubated for 24 h at 37 °C. In addition, medullary tissue samples were obtained for culture. Subsequently, the canal was debrided and washed with sterile antibiotic-free physiologic saline. In Group 1, tobramycin-containing bone cement (Simplex-P bone cement, premixed with 1.0 g of tobramycin as a sulphate in 40 g powder, Stryker-Howmedica-Osteonics, Rutherford, NJ) was inserted in the right tibial medullary canal. In the same manner, plain Simplex-P bone cement was used in Groups 2 and 3. The animals in Group 3 received also systemic antibiotics (cefazolin, 30 mg/kg, injected subcutaneously every 8 h for 14 days total, from Day 28 through Day 42). The rabbits in Group 2 did not receive any form of antibiotic treatment. The shelf life of premixed tobramycin-containing bone cement is 2 years. In this study all cement was used within 18 months after manufacture and stored at room temperature until use. The bone cement (precooled at 4 °C) was vacuum-mixed for 60 s (tobramycin-containing bone cement) or 100 s (plain bone cement) on the surgical table. Approximately 1.2 ml cement was injected gently into the medullary canal, while the syringe was slowly being retracted. The exact amount of injected cement was determined by weighing the syringe containing the cement. After polymerization of the cement and wound drainage with saline, the joint capsule and skin were closed in layers with Vicryl 3-0.

Follow-Up

The follow-up period after revision surgery was 14 days (42 days after the first operation). Routine AP and lateral x-rays of the right femur were obtained after the first operation and before and after revision surgery on Day 28. Body weight and body temperature were recorded on a regular basis. Blood samples from the auricular vein on erythrocyte sedimentation rate (ESR) and white blood cell counts (WBC) were taken prior to the first operation and at Days 1, 7, 14, 21, 28 (prior to revision), 35, and 42 postoperatively. The animals were killed with an intravenous overdose of pentobarbital sodium.

Autopsy and Sample Acquisition

After the animals were killed, the skin of both legs was clipped, disinfected with povidone–iodine, and isolated by sterile drapes. The right and left (not operated on) tibia were excised and cleaned from soft tissue debris. First, bone samples were taken from the left tibia from a region corresponding to the right tibia samples. Second, the external surface of the right tibia was notched circumferentially at each end of the shaft and longitudinally on two sides, posterior and anterior. An osteotome was used to break off each metaphysis and then to free the medial half of the bone from the lateral half. Care was taken not to damage the cement.

Bacteriological Culture

Both the medial bone half of the right tibia adjacent to the cement plug and bone from the corresponding region of the left tibia were submitted for quantification of bacteria. The bone samples were homogenized in a sterile phosphate-buffered saline solution (pH 7.4) with the use of a Polytron tissue grinder (Kinetica, Best, The Netherlands) and the number of bacteria per gram of bone was determined by dilution and plating techniques.

PCR Hybridization Assay

A part of the medial half of the right tibial cortex adjacent to the cement plug was collected for molecular biological analysis for the presence of bacterial DNA. These samples were incubated for 18 h at 60 °C in 1.5 ml digestion buffer [500-mM Tris (pH 9), 20-mM EDTA, 10-mM NaCl, 1% SDS, 0.5 mg/ml proteinase K] to release total DNA. A volume of 200 μl of the extracted DNA was used for DNA isolation the QIAamp Tissue Kit (Qiagen, Hilden, Germany). The last step in the isolation of the DNA included the elution of DNA in an end-volume of 400 μl. Subsequently, 2.5 μl of the dissolved DNA was amplified by the technique described by Wilbrink et al.[9] Broad-range biotin-labeled primers, targeting conserved regions of the gene for the 16S subunit of ribosomal RNA (16S-rRNA), were used to set up an eubacteria-specific polymerase chain reaction (PCR). An internal spike was added to screen for possible inhibition of PCR and to reduce the amplification of contaminating DNA. The presence of *S. aureus* DNA was determined by reverse line blot hybridization. The reverse line blot hybridization technique, as described by Kaufhold et al.[10] was used. For this purpose, a genus-specific staphylococcal oligonucleotide probe (5'-AACCTACCTATAAGACTGG-3') and a species-specific *S. aureus* oligonucleotide probe (5′-TCAAAAGTGAAA-GACGGTC-3'), which were covalently linked to a membrane (Biodyne C, Pall Biosupport, Portsmouth, UK), were used. Ten microliters of PCR products were hybridized to the oligonucleotide probes on the membrane for 1 h at 42 °C, with the use of a miniblotter system (MN45, Immunetics, Cambridge, MA). Subsequently, nonspecific DNA was washed of the membrane at 55 °C and the membrane was incubated at 42 °C with Streptavidin-peroxidase (Boehringer Mannheim Biochemica, Mannheim, Germany). Finally, the presence of *S. aureus* DNA could be visualized on a film (Hyperfilm ECL) with the use of an enhanced chemoluminescent detection system (ECL, Amersham International, Little Chalfont, England).

Statistics

The probability of a positive culture was compared between the three groups of rabbits with the use of Fisher's exact test. Furthermore, to account for inoculum dose, also a more sophisticated analysis was also performed with the use of a stratified two by two chi-squared test. One-sided tests were performed for comparison of each antibiotic group (tobramycin bone cement and systemic cefazolin) with the control group. Two-sided tests were performed for comparison of the two antibiotic groups. A P value of less than 0.05 was considered significant. Exact P values have been computed with the use of the statistical program StatXact 4.

Figure 2. Lateral radiographs of the right tibia of the same rabbit at Day 28, before (left) and after (right) revision surgery. Periostal reactive bone formation as a sign of local response to infection is seen in the proximal half of the tibia. At the time of revision surgery, the implant was removed and bone cement was injected into the tibial canal after debridement.

TABLE I. Erythrocyte Sedimentation Rate and White Blood Cell Count

Group of Rabbits	ESR (mm/h, mean ± SD)			
	Day 0	Day 7	Day 28	Day 42
Tobramycin	1.3 ± 0.5	42.9 ± 34.7	0.9 ± 0.3	1.3 ± 0.5
Cefazolin	1.1 ± 0.4	35.3 ± 27.2	1.0 ± 0.0	1.1 ± 0.4
Control	1.5 ± 0.5	41.9 ± 26.5	1.6 ± 0.5	1.7 ± 0.7
	WBC ($\times 10^9$/l, mean ± SD)			
	Day 0	Day 7	Day 28	Day 42
Tobramycin	5.5 ± 1.6	13.2 ± 3.5	4.7 ± 0.7	6.7 ± 1.7
Cefazolin	4.2 ± 1.1	10.4 ± 1.2	6.5 ± 2.0	5.8 ± 1.8
Control	4.3 ± 0.7	10.8 ± 1.9	5.8 ± 2.4	6.8 ± 2.3

ESR = erythrocyte sedimentation rate; WBC = white blood cell count.

RESULTS

General

Three rabbits were lost before the time of revision. Two of these rabbits died in the first week after the initial operation, the third showed signs of severe sepsis and was killed in the third postoperative week. In all three rabbits the culture revealed an overwhelming *S. aureus* infection at the site of the implant. All other rabbits had a good recovery from both operations. The inserted cement (mean ± standard deviation) weighed 0.97±0.17 g in Group 1 (tobramycin cement), 1.03±0.13 in Group 2 (systemic antibiotic), and 1.02±0.16 g in Group 3 (control).

The development of an infection in all rabbits was confirmed at the time of revision by the presence of pus macroscopically and/or by positive cultures of debrided tissue on the revised implant. The x-rays of all rabbits taken on Day 28 all showed clear signs of reactive bone tissue, predominantly at the proximal half of the right tibia, indicating a response to the presence of a local fulminant infection at the site of the implant (Figure 2).

No signs of side effects of systemic cefazolin, such as diarrhea caused by pseudomembraneous colitis, were seen in rabbits treated with cefazolin.

No clear differences between the three treatment groups were seen in body temperature and loss of body weight, ESR, and WBC. Table I shows the results of ESR and WBC.

Bacteriological Culture

The outcome of cultures is presented in Table II. Results of cultures of the cortex of the right tibia adjacent to the cement

TABLE II. Outcome of Cultures

Group of Rabbits	Infection Rate	Culture (mean ± SD, ^{10}log CFU/g)
Tobramycin	2/9	1.1 ± 2.2
Control	10/10	5.7 ± 1.4
Cefazolin	0/8	0

CFU/g = colony forming units per gram of bone.

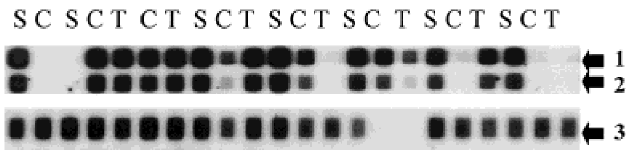

Figure 3. Details of the film with the results of the reverse line blot hybridization assay. PCR products of the right tibiae of the rabbits in the three different treatment groups are oriented in vertical lanes (T = tobramycin group, C = control group, S = systemic cefazolin group). The oligonucleotides are oriented in horizontal lanes (1 = staphylococci probe, 2 = *S. aureus* probe, 3 = internal spike probe). An internal spike was added to all samples to exclude possible inhibition: The lower lane shows no inhibition in samples that were negative for staphylococci or *S. aureus*.

(weight 1.03 ±0.03 g, mean ±SD) showed a decrease for both antibiotic groups (tobramycin cement and systemic cefazolin) in comparison with the control group. Because in both antibiotic groups most rabbits (seven out of nine and eight out of eight, respectively) showed negative culture results, no statistical analysis was performed on the mean culture results to point out the difference. The rate of infection in both antibiotic groups was significantly lower than in the control group, $p<0.01$. The rate of infection in the tobramycin-cement group was slightly higher (2/9) but not significantly different from the systemic cefazolin group (0/8); $p = 0.47$. When the inoculum dose (10^5 or 10^6 CFU) was taken into account, the statistical analysis also showed significant differences between both antibiotic groups and the control group ($p < 0.01$), but no significant difference between the two antibiotic treatment groups ($p = 0.5$).

Reverse Line Blot Hybridization Assay

No PCR sample at 42 days follow-up was obtained in 6 out of the 27 rabbits for which culture results were available at that time. Figure 3 shows a detail of the film on which the results of the reverse line blot hybridization assay on samples (weight 0.34 ±0.12 g, mean ±SD) of the right tibia of the other 21 rabbits are visualized. Table III relates the results of the reverse line blot hybridization assay on these samples to the subsequent culture results.

DISCUSSION

Based on the results of bacteriological culture, the present investigation demonstrated that both tobramycin-containing bone cement and systemic cefazolin reduce size and rate of infection in the treatment of an infected prosthesis in a one-stage revision procedure in rabbits. This model was designed to mimic a one-stage revision procedure for an infected joint arthroplasty, so that a high efficacy of an antibiotic treatment would be required to clear the infection. A two-stage revision model was not incorporated into this study, because the main objective was to test a specific antibiotic-containing cement. The temporary treatment between removal of the implant and insertion of the cement at a subsequent operation, may be a confounding factor for this purpose. Furthermore, this model differs from previous efficacy studies, in that the treatment did not start immediately after inoculation, but after a prolonged time period. This delay of treatment was introduced to create an established infection with the subsequent inflammatory responses and alterations in microcirculation and bone morphology. Therefore, in comparison with the direct-infection models, this model is more similar to the clinical situation in which antibiotic treatment is started only after the infection has settled itself.

Only a few studies have addressed an animal model to evaluate the option of treating an established prosthesis-related infection with antibiotic-containing bone cement. Fitzgerald showed that gentamicin-containing bone cement could effectively prevent, but not treat, *Staphylococcus aureus* infections around injected PMMA in tibiae of dogs.[11] In three out of five infections, the one-stage revision failed as a treatment. Gerhart et al. showed that gentamicin- and/or vancomycin-loaded bone cement had some, but no absolute efficacy in the treatment of *Staphylococcus aureus* infections in 34 rat tibiae, based upon the number of colony-forming units.[12] The number of failed treatments, however, was not mentioned. The use of preformed cement to create an infection in this model could have made the removal of the cement easier as compared to the model of Fitzgerald, who inserted the initial cement as dough.

In the present study, systemic cefazolin was effective in treating the infection in rabbits after revision of the implant. Saleh Mghir et al. studied the treatment with either systemic vancomycin or teicoplanin of a tibia implant infection in rabbits.[13] Neither of these antibiotics could fully clear the infection in the majority of rabbits. In contrast with the present study, these rabbits were inoculated with methicillin-resistant *S. aureus* and treated only for 1 week. Notably, also in contrast with the present study, the implants in these rabbits were not revised before starting treatment, stressing the need for revising the prosthesis once it becomes infected. In the present study, systemic antibiotic treatment was not significantly superior to local antibiotic treatment, although higher numbers of animals may have revealed such a difference. In another animal model, tobramycin-containing bone

TABLE III. Outcome of Reverse Line Blot Hybridization

	Culture −		Culture +	
RLB −	Tobramycin	2	Tobramycin	0
	Control	0	Control	3
	Cefazolin	1	Cefazolin	0
RLB +	Tobramycin	4	Tobramycin	1
	Control	0	Control	5
	Cefazolin	5	Cefazolin	0

The results (in number of rabbits) of the reverse line blot hybridization compared by culture. RLB = reverse line blot hybridization assay; − = negative result; + = positive result.

cement prevented all infections.[7] In the present study, the same cement could not fully treat two out of nine infected rabbits. This can be explained by the more extensive spread of infection at the time of revision; that is, the infection was not restricted only to the local area around the bone cement. Destruction of the cortex might be severe, and remaining necrotic tissue some distance from the antibiotic-containing bone cement may cause treatment failure.[11] In such a case, it is doubtful whether an antibiotic course of more than 2 weeks would have been more successful, although a previous study has shown that antibiotic elution from tobramycin-containing bone cement could be detected up to 4 weeks *in vivo*.[14] Clinically, in patients with such an extensive implant infection, few surgeons will opt for only local antibiotics. For this matter, a combination of both antibiotic-containing bone cement and systemic antibiotic might be optimal: Systemic antibiotics for the wound problems and bacteria outside the operative area, and antibiotic-containing bone cement for local, high release of antibiotics. Indeed, this combination of antibiotic administration was predominantly used in many series reporting on one-stage revision, although an important early study of Buchholz et al. reported success rates of 77–90% in one-stage revisions mainly without administration of systemic antibiotics.[2,15–21]

In addition to culture, the presence of staphylococcal DNA in the samples of the tibial cortex of the rabbits was determined by means of a reverse line blot hybridization assay. PCR-based assays like RLB might become valuable complements of conventional microbiological techniques, or even improve diagnostic accuracy.[22–25] In 9 out of 12 rabbits with negative culture results, reverse line blot hybridization showed the presence of *S. aureus* DNA in the right tibia, 14 days after treatment with antibiotics (either systemically or with antibiotic-containing bone cement). Do these reverse line blot hybridization results demonstrate the sensitivity of DNA-based detection methods, or should these findings be interpreted as false-positive results based upon the outcome of culture? The sensitivity of a PCR-based detection method, with the use of a broad range of bacterial primers, is at the same time an Achilles' heel: PCR does not only amplify DNA of viable bacteria present at the site of the implant, but also contaminating DNA or small quantities of DNA that can still be present shortly after antimicrobial killing of the bacteria. Strict policies in handling the samples should be and have been obtained in this study to exclude false-positive results due to contamination, either from contaminated reagents or from previously amplified bacterial DNA products. The presence of *S. aureus* DNA as confirmed by the reverse line blot hybridization in rabbits with negative culture can be explained to a large extent by nonviable bacteria after antibiotic treatment. Clearance of all bacterial DNA after antibiotic treatment may be species specific, but van der Heijden et al. have shown that it can take up to 26 days after initiation of therapy for the PCR of nonstaphylococcal DNA to become negative in septic joints.[26] A previous animal model studied the infection prophylaxis with systemic cefazolin or tobramycin-containing bone cement after inoculation of the rabbit tibia with *S. aureus*.[7] *S. aureus* DNA could not be detected 7 days after the procedure. These findings may implicate that the clearance of DNA after antibiotic treatment is dependent on bacterial load, because in the present model, the therapy was started only after a full-blown infection had developed. Further studies should be employed to address the persistence of bacterial DNA after antibiotic treatment, because DNA-based diagnosis will become increasingly important in the near future.[27] Important clinical decisions based on this type of diagnosis regarding the continuation of antibiotic therapy, whether systemically or locally via antibiotic-containing bone cement or beads, will benefit from more insight on this matter. Furthermore, the viability of microorganisms can be studied with the use of molecular techniques targeting RNA. Reverse transcriptase PCR and nucleic-acid sequence amplification (NASBA) have been used for this purpose.[28,29] Both ribosomal RNA (rRNA) and messenger RNA (mRNA) can be targeted, but rRNA has been shown to persist longer than mRNA in mycobacteria after chemotherapy.[30] *In situ* hybridization for staphylococcal 16S-rRNA can give additional information whether the detection of bacterial DNA represents the presence of the causative organism at the site of an implant or that it is caused by contamination.[31]

RLB confirmed the presence of *S. aureus* DNA in only six of the nine rabbits in which *S. aureus* was cultured 14 days after revision. Because an internal spike was added in the PCR samples, inhibition of amplification in the three control rabbits could be excluded with a negative reverse line blot hybridization result and a positive culture. These rabbits had a relatively low bacteria load as compared to the six rabbits with a positive reverse line blot hybridization result and positive cultures (respectively 3.8 ± 0.8 and 6.3 ± 0.6, mean ^{10}log CFU/g). The PCR samples of the three rabbits with negative reverse line blot hybridization results may have originated from a part of the tibial cortex where infection was minimal, causing sampling error. Furthermore, the differences between the weights of the bone samples taken for culture or PCR, and the magnitude of dilution of the original volumes during the two techniques, should be considered. The amount of a bone used for a PCR sample was approximately 500 times less than that for culture.

It is concluded from the current study that both tobramycin-containing bone cement and systemic cefazolin used in a one-stage revision for an infected implant can reduce size and rate of infection. However, in cases of virulent infections, a combination of systemic and local antibiotics may be necessary in a one-stage revision procedure. Further studies on the feasibility of PCR-reverse line blot hybridization are advised.

REFERENCES

1. Brandt CM, Sistrunk WW, Duffy MC, Hanssen AD, Steckelberg JM, Ilstrup DM, Osmon DR. Staphylococcus aureus prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis 1997;24**:**914–919.
2. Buchholz HW, Elson RA, Engelbrecht E, Lodenkamper H, Rottger J, Siegel A. Management of deep infection of total hip replacement. J Bone Joint Surg [Br] 1981;63**:**342–353.

3. Raut VV, Siney PD, Wroblewski BM. One-stage revision of total hip arthroplasty for deep infection. Long-term followup. Clin Orthop 1995;321:202–207.
4. Garvin KL, Hanssen AD. Infection after total hip arthroplasty. Past, present, and future. J Bone Joint Surg [Am] 1995;77: 1576–1588.
5. Pagnano MW, Trousdale RT, Hanssen AD. Outcome after reinfection following reimplantation hip arthroplasty. Clin Orthop 1997;338:192–204.
6. Nijhof MW, Dhert WJ, Fleer A, Vogely HC, Verbout AJ. Prophylaxis of implant-related staphylococcal infections using tobramycin-containing bone cement. J Biomed Mater Res 2000; 52:754–761.
7. Nijhof MW, Stallmann HP, Vogely HC, Fleer A, Schouls LM, Dhert WJ, Verbout AJ. Prevention of infection with tobramycin-containing bone cement or systemic cefazolin in an animal model. J Biomed Mater Res 2000;52:709–715.
8. Vogely HC, Oosterbos CJ, Puts EW, Nijhof MW, Nikkels PG, Fleer A, Tonino AJ, Dhert WJ, Verbout AJ. Effects of hydroxyapatite coating on Ti-6Al-4V implant-site infection in a rabbit tibial model. J Orthop Res 2000;18:485–493.
9. Wilbrink B, van der Heijden IM, Schouls LM, van Embden JD, Hazes JM, Breedveld FC, Tak PP. Detection of bacterial DNA in joint samples from patients with undifferentiated arthritis and reactive arthritis, using polymerase chain reaction with universal 16S ribosomal RNA primers. Arthritis Rheum 1998;41:535–543.
10. Kaufhold A, Podbielski A, Baumgarten G, Blokpoel M, Top J, Schouls L. Rapid typing of group A streptococci by the use of DNA amplification and non-radioactive allele-specific oligonucleotide probes. FEMS Microbiol Lett 1994;119:19–25.
11. Fitzgerald RH, Jr. Experimental osteomyelitis: Description of a canine model and the role of depot administration of antibiotics in the prevention and treatment of sepsis. J Bone Joint Surg [Am] 1983;65:371–380.
12. Gerhart TN, Roux RD, Hanff PA, Horowitz GL, Renshaw AA, Hayes WC. Antibiotic-loaded biodegradable bone cement for prophylaxis and treatment of experimental osteomyelitis in rats. J Orthop Res 1993;11:250–255.
13. Saleh Mghir A et al. Efficacy of teicoplanin and autoradiographic diffusion pattern of [14C]teicoplanin in experimental Staphylococcus aureus infection of joint prostheses. Antimicrob Agents Chemother 1998;42:2830–2835.
14. Nijhof MW, Dhert WJA, Tilman PBJ, Fleer A, Verbout AJ. Release of tobramycin from tobramycin-containing bone cement in bone and serum of rabbits. J Mater Sci Mater Med 1997;8:799–802.
15. Loty B, Postel M, Evrard J, Matron P, Courpied JP, Kerboull M, Tomeno B. One stage revision of infected total hip replacements with replacement of bone loss by allografts. Study of 90 cases of which 46 used bone allografts. Int Orthop 1992;16:330–338.
16. Miley GB, Scheller AD, Jr., Turner RH. Medical and surgical treatment of the septic hip with one-stage revision arthroplasty. Clin Orthop 1982;170:76–82.
17. Mulcahy DM, O'Byrne JM, Fenelon GE. One stage surgical management of deep infection of total hip arthroplasty. Ir J Med Sci 1996;165:17–19.
18. Salvati EA, Chekofsky KM, Brause BD, Wilson PD, Jr. Reimplantation in infection: A 12-year experience. Clin Orthop 1982; 170:62–75.
19. Ure KJ, Amstutz HC, Nasser S, Schmalzried TP. Direct-exchange arthroplasty for the treatment of infection after total hip replacement. An average ten-year follow-up. J Bone Joint Surg [Am] 1998;80:961–968.
20. von Foerster G, Kluber D, Kabler U. Mid- to long-term results after treatment of 118 cases of periprosthetic infections after knee joint replacement using one-stage exchange surgery. Orthopäde 1991;20:244–252.
21. Wroblewski BM. One-stage revision of infected cemented total hip arthroplasty. Clin Orthop 1986;211:103–107.
22. Hoeffel DP, Hinrichs SH, Garvin KL. Molecular diagnostics for the detection of musculoskeletal infection. Clin Orthop 1999; 360:37–46.
23. Mariani BD, Martin DS, Levine MJ, Booth RE, Jr., Tuan RS. The Coventry Award. Polymerase chain reaction detection of bacterial infection in total knee arthroplasty. Clin Orthop 1996; 331:11–22.
24. Mariani BD, Tuan RS. Advances in the diagnosis of infection in prosthetic joint implants. Mol Med Today 1998;4:207–213.
25. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, Gorman SP, Davis RI, Anderson N. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 1999;37:3281–3290.
26. van der Heijden IM, Wilbrink B, Vije AE, Schouls LM, Breedveld FC, Tak PP. Detection of bacterial DNA in serial synovial samples obtained during antibiotic treatment from patients with septic arthritis. Arthritis Rheum 1999;42:2198–2203.
27. Rantakokko-Jalava K et al. Direct amplification of rRNA genes in diagnosis of bacterial infections. J Clin Microbiol 2000;38: 32–39.
28. van der Vliet GM, Schepers P, Schukkink RA, van Gemen B, Klatser PR. Assessment of mycobacterial viability by RNA amplification. Antimicrob Agents Chemother 1994;38:1959–1965.
29. Sheridan GE, Masters CI, Shallcross JA, MacKey BM. Detection of mRNA by reverse transcription-PCR as an indicator of viability in Escherichia coli cells. Appl Environ Microbiol 1998;64:1313–1318.
30. Hellyer TJ, DesJardin LE, Hehman GL, Cave MD, Eisenach KD. Quantitative analysis of mRNA as a marker for viability of Mycobacterium tuberculosis. J Clin Microbiol 1999;37:290–295.
31. Krimmer V, Merkert H, von Eiff C, Frosch M, Eulert J, Lohr JF, Hacker J, Ziebuhr W. Detection of Staphylococcus aureus and Staphylococcus epidermidis in clinical samples by 16S rRNA-directed in situ hybridization. J Clin Microbiol 1999;37:2667–2673.