# *In Vitro* Inhibition of Coagulase-Negative Staphylococci by Vancomycin/Aminoglycoside-Loaded Cement Spacers

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## Abstract

**Background:** Successful treatment of allograft infections by the temporary implantation of an antibiotic-loaded polymethylmethacrylate cement spacer depends on the diffusion of antibiotics out of the cement and inhibition of bacterial growth in the surrounding tissue. We investigated with an *in vitro* model how long antibiotics are released by the cement and if gentamicin-resistant coagulase-negative staphylococci (CNS) are inhibited by vancomycin mixed with the gentamicin-loaded cement.

**Materials and Methods:** Four formulations of antibiotic-loaded cement disks, i.e. gentamicin, tobramycin, vancomycin and tobramycin combined with vancomycin, respectively, were used to test the inhibition of eight isolates of *Staphylococcus epidermidis* and two reference strains of *Staphylococcus aureus* by an agar diffusion test on Mueller-Hinton (MH) agar similar to the routine laboratory disk diffusion method. Moreover, cement spacer cylinders loaded with gentamicin alone or combined with vancomycin were submerged in MH agar for weeks and the capacity to inhibit five different isolates of *S. epidermidis* was measured.

**Results:** The size of the inhibition zones around the antibiotic-loaded cement disks correlated with the minimal inhibitory concentration (MIC) of the antibiotics against the tested strains. All five strains of *S. epidermidis* were inhibited by vancomycin-loaded cement spacers for at least 30 days. However, two gentamicin-resistant *S. epidermidis* strains with MICs of 4 mg/l and 16 mg/l could not be inhibited longer than 3 days by the gentamicin-loaded cement spacer.

**Conclusion:** The *in vitro* data suggest that antibiotic-loaded cement spacers inhibit susceptible bacteria for 4–6 weeks. The addition of vancomycin to commercial aminoglycoside-loaded cements might be helpful in allograft infections in tumor patients to inhibit a broad range of bacteria including gentamicin-resistant CNS very commonly found in such infections.

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## Introduction

*Buchholz* and *Engelbrecht* [1] introduced the concept of antibiotic-loaded polymethylmetacrylate (PMMA) bone cement over 30 years ago. After successful prophylactic use of gentamicin-loaded cement with a reduced rate of infected endoprosthesis, they treated deep infections of total hip replacements by revision arthroplasty that comprised, in one stage, excision of soft tissue, removal of implant and cement and replacement with an appropriate implant using antibiotic-loaded cement [2]. Staged revision of infected prosthesis by using temporary antibiotic-loaded cement spacers may be preferred [3]. Systemic antibiotic treatment of infected bone reconstructions in tumor patients may be compromised by devascularization. Bacteria colonize the surface of inert biomaterial and adjacent, damaged tissue cells [4]. Therefore, antibiotic-impregnated cement spacers should inhibit bacteria locally before they form a biofilm. However, it is not well defined how long an antibiotic-loaded cement spacer should be left in place before the definitive reconstruction can take place.

Aminoglycosides are widely used because of their broad-spectrum activity and thermal stability [5, 6]. The high prevalence of highly resistant coagulase-negative staphylococci (CNS) in infected reconstructions may justify the use of vancomycin in bone cement. The present study investigates the *in vitro* inhibition of clinical isolates of *Staphylococcus epidermidis* by bone cement loaded with single antibiotics, i.e. gentamicin, tobramycin and vancomycin, and loaded with vancomycin combined with tobramycin, respectively. In addition, we developed a simple method to measure the time of the inhibition of bacteria which combines the standardized method of disk diffusion and some of the *in vivo* conditions of cement spacers. Our *in vitro* model should demonstrate in an easier way that the antibiotic-loaded bone cement can elute antibiotic over weeks.

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## Materials and Methods

Eight isolates of *S. epidermidis*, isolated from patients with orthopedic infections and two reference strains of *Staphylococcus aureus* (ATCC 29213, susceptible to methicillin; ATCC 43300, resistant to methicillin) were included in our *in vitro* inhibition assay by antibiotic-loaded cement disks. Five of those isolates were used to investigate their inhibition over weeks by cylindrical antibiotic-loaded cement spacers.

The minimal inhibitory concentrations (MIC) of gentamicin, tobramycin and vancomycin for those bacteria were determined by Etest strips containing the corresponding antibiotic calibrated with MIC values covering a scale of 15 twofold dilutions (AB Biodisk, Solna, Sweden). In brief, staphylococcal colonies of an overnight culture on sheep blood agar were suspended in saline and adjusted to match a 0.5 McFarland turbidity standard, i.e. containing approximately 1 to $2 \times 10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and Mueller-Hinton (MH) agar plates were inoculated by streaking the swab over the entire agar surface before one Etest strip per plate was placed onto each agar. They were incubated for 24 h at 35 °C without $CO_2$ and MICs were read where the growth inhibition ellipse intersected the scale. Synergy testing was also performed by Etest as above according to the instructions of the manufacturer. However, a first Etest strip with gentamicin was placed onto the inoculated MH agar for only 1 h and removed again before a second Etest strip with vancomycin was put in exactly the same place as the first; this experiment was also performed in parallel for each strain by placing first the Etest strip with vancomycin for 1 h followed by the Etest strip with gentamicin overnight. Plates were incubated as above but the read MIC is the MIC of the combination of both, gentamicin and vancomycin.

For the *in vitro* inhibition assay by antibiotic-loaded cement disks, four antibiotic-loaded cement formulations were used: (1) Palacos commercially prepared with 500 mg gentamicin per 40.8 g cement powder (Biomet-Merck, Warsaw, Indiana, USA) and (2) Simplex commercially prepared with 1 g tobramycin (Stryker Howmedica Osteonics, Limerick, Ireland); two other formulations were prepared by hand-mixing 3.0 g of vancomycin hydrochloride (Vancocin, Lilly, Indianapolis, Indiana, USA) with a tongue depressor into standard 40 g doses (3) of the standard Simplex P without antibiotic (Stryker Howmedica Osteonics, Limerick, Ireland) and (4) of the Simplex bone cement with tobramycin (Stryker Howmedica Osteonics, Limerick, Ireland). Single doses of each bone cement formulation were vacuum mixed according to manufacturer's instructions using a commercial cement mixer and delivery system (Howmedica Inc., Limerick, Ireland). Immediately after mixing, the cement was delivered into aluminum molds with cavities to produce uniform disk-shaped specimens with a diameter of 14 mm and thickness of 2 mm. Cured samples were inspected and any disks possessing gross voids or surface defects were discarded. The antibiotic-impregnated cement disks were used on the very same day of their production.

Suspensions of the eight isolates of CNS as well as of the two reference strains of *S. aureus* were prepared and streaked onto MH agar plates as above. After a few minutes the disks of antibiotic-loaded cement were placed into the middle of the inoculated MH agar plates and pressed down to ensure complete contact with the agar surface. Each strain was tested in duplicate with each cement disk formulation. Plates were incubated for 24 h at 35 °C without $CO_2$. After 24 h the inhibition zones were measured.

Five of the eight isolates of *S. epidermidis* were included in the inhibition experiment with cylindrical antibiotic-loaded cement spacers. Two antibiotic-loaded cement formulations were used to prepare five of each cylindrical cement spacers: (1) Palacos R-40 (40.8 g powder) commercially prepared with 500 mg gentamicin (Biomet-Merck) and (2) a formulation prepared by hand-mixing 3 g of vancomycin hydrochloride into the standard 40 g doses of Palacos bone cement with 500 mg gentamicin (Biomet-Merck). Briefly, the Palacos R-40 with gentamicin was mixed with the entire contents of two ampoules of 18.8 g liquid component twice as much as normal in a bowl for 50 s with a sterile plastic spatula until a homogeneous fluid cement mixture was formed. The mixture was filled into a perfusor syringe OPS® 50 ml (Braun, Melsungen, Germany) and packed by the gum stamp of the syringe. The syringes were placed with the outflow part upside until the hardened spacers could be taken out of the syringes after 10 min; they were stored at room temperature for 24 h. Further five cylindrical cement spacers were produced in the same way but 3 g vancomycin was added to the solution into the mixing bowl. The cylindrical spacers had approximately the diameter of the spacers used in the tumor patients with allograft infections.

The cylindrical spacers were placed in the middle of cylindrical glasses (Duran®, Schott, Mainz, Germany) containing 440 ml of liquid (50 °C) sterile MH agar so that 2–3 cm of the spacer were still above the level of the agar surface. After the agar became solid at room temperature, suspensions of staphylococci were prepared as above and streaked with a swab tightly over the entire agar surface around the cylindrical cement spacer. The cylindrical glasses with the spacer in the agar were incubated at 37 °C without $CO_2$ and diameters of inhibition were measured after 24 h and incubation continued for some days (Figure 1). The cylindrical spacers that showed an inhibition of the staphylococci were then transferred to a new cylindrical glass with liquid (50 °C) sterile MH agar but the amount of agar was diminished by

**Figure 1.** Cement spacer in a cylindrical glass with a zone of inhibition of the added staphylococci.

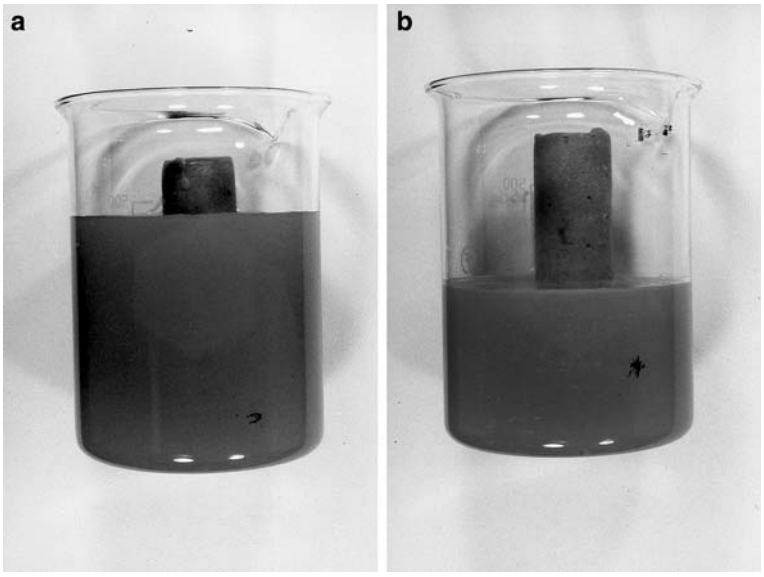


**Figure 2.** a) Cement spacer in the cylindrical glass at the beginning of the experiments; b) Cement spacer in the cylindrical glass 5 weeks later.

Table 1
**Minimal inhibitory concentrations (MIC) of all isolates determined by Etest.**

| Organism | MIC in mg/l | | | | |
|---|---|---|---|---|---|
| | Tobramycin | Vancomycin | Gentamicin plus vancomycin | Vancomycin plus gentamicin | Gentamicin |
| *Staphylococcus aureus* | | | | | |
| ATCC 43300 | > 256 | 1.5 | 0.75 | 0.38 | 32 |
| ATCC 29213 | 1.0 | 1.0 | 1.0 | 1.0 | 0.38 |
| *Staphylococcus epidermidis* | | | | | |
| V07 33499 | 0.38 | 2 | 0.19 | 0.125 | 0.125 |
| V10 33001 | 2 | 2 | 0.25 | 0.125 | 0.125 |
| V10 22259 | 8–12 | 1.0 | 0.38 | 0.19 | 0.25 |
| V01 17734 | > 256 | 1.5 | 2 | 2 | 16 |
| V07 21204 | > 256 | 2 | 1.5 | 1.5 | 4 |
| V01 15748 | > 256 | 2 | 2 | 2 | 4 |
| V10 28533 | > 256 | 2 | 0.25 | 0.19 | 0.19 |
| V09 15128 | > 256 | 2 | 2 | 2 | > 256 |

20 ml (Figure 2a, b) so that the contact of the agar with the spacer cement not yet submerged in the former experiment can be excluded; otherwise, the contact of agar with the cement not submerged in the earlier experiments would allow diffusion of antibiotics of the upper part of the spacer. Suspensions from overnight grown colonies from the same bacteria were streaked onto the agar and the inhibition zone was measured again after 24 h. At the beginning the intervals were short to detect an early loss of release of antibiotics by bone cement; the volume of the agar had to be reduced for each new transfer by 20 ml to be sure that the contact zone of the agar was not at a level of the cement spacer not yet submerged before; therefore, the interval was prolonged so that we could perform the experiment over 2 months. The experiment was stopped earlier for those spacers that did not show any more inhibition zones or which were contaminated by molds.

### Statistics

The MICs of the combination of gentamicin plus vancomycin or vancomycin plus gentamicin (Table 1) were compared with the MICs of the single compound with the lower MIC by the Wilcoxon signed-rank test to detect synergy, i.e. lower MICs of the combination than the MICs of the single compounds. The mean values of the duplicate zones of inhibition of the cement disks with vancomycin combined with tobramycin (Table 2) were compared with the mean values of the single compound with the bigger zone by the Wilcoxon signed-rank test to detect synergy, i.e. greater inhibition zones of the disks with combined antibiotics.

### Results

The MICs of the eight strains of *S. epidermidis* and of the two reference strains of *S. aureus* are shown in table 1. The MICs of the synergy testing by Etest were not different from the MICs of the single component which was most active against the corresponding strain ($p = 0.38$ for the gentamicin plus vancomycin combination, $p = 0.69$ for the vancomycin plus gentamicin combination). Inhibition zones of the antibiotic-loaded cement disks were measured after an overnight incubation (Table 2). The duplicate values did not differ more than 2 mm with the following exception: the inhibition zones of the two cement disks with vancomycin combined with tobramycin were 30 mm and 37 mm for strain V10 21204. The diameters of the zones around the cement disks loaded with vancomycin and tobramycin were not different from those of the most active single antibiotic ($p = 0.96$). Furthermore, the zones were greater for strains with low MICs than for strains with higher MICs and strains with MICs greater than 12 mg/l did not show any inhibition zone with the corresponding cement disks.

The zones of inhibition of the ten cement spacers are shown in table 3. Only one of the spacers loaded with gentamicin alone (V10 22259) showed a zone of inhibition until the 8th week. The two spacers incubated with strains with elevated MICs against gentamicin (V01 17734 and V10 21204, see Table 2) stopped showing a zone of inhibition after 2 and 3 days. The corresponding spacers loaded with gentamicin and vancomycin inhibited the same strains of *S. epidermidis* over 7 weeks. The zones of inhibition of

Table 2
**Zones of inhibition of the antibiotic-loaded cement disks with the agar diffusion method**

| Organism | Zone of inhibition of duplicate cement disks (first, second disk) in mm | | | |
|---|---|---|---|---|
| | Tobramycin | Vancomycin | Vancomycin plus tobramycin | Gentamicin |
| *Staphylococcus aureus* | | | | |
| ATCC 43300 (MRSA) | No zone | 27, 28 | 26, 27 | No zone |
| ATCC 29213 | 30, 30 | 28, 28 | 32, 32 | 31, 31 |
| *Staphylococcus epidermidis* | | | | |
| V07 33499 | 35, 36 | 28, 28 | 38, 39 | 38, 37 |
| V12 33001 | 26, 26 | 30, 29 | 29, 28 | 36, 37 |
| V10 22259 | 23, 24 | 28, 30 | 29, 28 | 34, 33 |
| V01 17734 | No zone | 31, 31 | 30, 28 | No zone |
| V10 21204 | No zone | 30, 31 | 30, 37 | 24, 23 |
| V01 15748 | No zone | 34, 33 | 34, 32 | 26, 26 |
| V07 28533 | No zone | 31, 32 | 31, 32 | 40, 40 |
| V09 15128 | No zone | 28, 28 | 26, 28 | No zone |

the other two gentamicin-loaded spacers were only measurable for 3 and 8 days, respectively, because they were contaminated by environmental strains.

Three of five spacers loaded with gentamicin and vancomycin (V07 33499, V10 22259, V10 21204) still showed a zone of inhibition at the end of the study after more than 15 diffusion experiments; spacer V10 22259 showed a transient contamination by fungus. Two spacers (V01 17734, V07 28533) produced after 7 and 4 weeks, respectively, irregular zones and were not transferred for further experiments.

## Discussion

It is known that many commonly used antibiotics are released from bone cement in such a way that the local antibiotic levels vastly exceed the MICs needed for treating most susceptible pathogens, and that the levels are much higher than those achieved with parenteral therapy [7]. When used in PMMA, the antibiotic agent must be heat stable and effective against the common pathogens and exhibit good elution characteristics [8]. Previous studies measured *in vitro* the released antibiotics in fluids surrounding antibiotic-loaded cement [9–11] or *in vivo* in wound drainage, urine and serum [12–14].

The time period of antibiotic release out of the bone cement reached days to several months in earlier studies and the diffusion is influenced by the cement-, antibiotic- and environment-dependent factors [15]. The elution characteristics of different bone cements depend on handling, physical states and physical characteristics, i.e. speed of creep and relaxation [16]. Some authors reported that Palacos releases more gentamicin and vancomycin [12, 14, 17–20] and has more rapid elution characteristics than Simplex-P, which is probably due to its greater porosity than other cements [7, 21]. In contrast, Simplex-P bone cement has superior handling characteristics [7]. Other studies claimed that the elution capacity of CMV (DePuy International, Blackpool, UK) may be greater than that of Palacos R [20, 22].

Our freshly prepared antibiotic-loaded cement disks released tobramycin, gentamicin and vancomycin into the agar, as shown in previous studies [8, 19, 23]. The concentration of antibiotics is highest near the disk and the bacterium is inhibited if the MIC is lower than this concentration. The staphylococci were only inhibited by the aminoglycoside-loaded cement disks if the corresponding MIC in the Etest was lower than 16. The concentration of the antibiotic at the edge of the inhibition zone corresponds to the MIC of the bacterium and the inhibition zones correlated – with one exception – with the MIC of the corresponding staphylococci, i.e. the lower the MIC the bigger was the inhibition zone. The addition of vancomycin to tobramycin resulted in an inhibition of all staphylococci including those six strains against which MICs of tobramycin were > 256. However, the inhibition zones of tobramycin combined with vancomycin were not significantly greater for the other staphylococci than those of the more potent antibiotic alone. This was also observed by Etest where the combination of gentamicin with vancomycin revealed MIC similar to the MIC of the most active antibiotic. We could not observe a synergistic inhibition of tobramycin or gentamicin with vancomycin *in vitro*. However, we did not measure the bactericidal effect of the combination of vancomycin and gentamicin. A bactericidal synergy of two substances that interact with different targets of the bacteria might be helpful to eliminate the bacteria from the bone similar to synergistic action of vancomycin or penicillin with gentamicin in enterococcal endocarditis [24]. Furthermore, aminoglycosides alone might promote the formation of small colony variants of staphylococci [25, 26]. Small colony variants of staphylococci have been recovered from patients with unusually persistent infections which are chronically exposed to aminoglycosides. The addition of vancomycin might prevent the formation of small colony variants but this has to be studied further. The reason for the addition of vancomycin is, however, to inhibit those staphylococci that are not inhibited by the eluted amounts of aminoglycosides out of the cement.

Our experiments with cement spacers show that enough antibiotic was eluted into the agar to inhibit bacteria. However, the two strains of *S. epidermidis* with MIC 4 and 16 against gentamicin were not inhibited after 3 days. The spacers with vancomycin released antibiotics until the end of the experiment over 8 weeks. This is in contrast with previous reports that vancomycin is not well eluted from antibiotic-impregnated bone cement in total hip arthroplasty [13].

Table 3
**Zone of inhibition of the antibiotic-loaded cement spacer from day 1 to 12 weeks in mm.**

| Day week | Amount of Mueller-Hinton agar in the cylindrical glass (ml) | V07 33499 Gentamicin | V07 33499 Gentamicin and Vancomycin | V10 22259 Gentamicin | V10 22259 Gentamicin and Vancomycin | V01 17734 Gentamicin | V01 17734 Gentamicin and Vancomycin | V10 21204 Gentamicin | V10 21204 Gentamicin and Vancomycin | V07 28533 Gentamicin | V07 28533 Gentamicin and Vancomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Day 1 | 440 | 72 | 76 | 66 | 61 | 35 | [b] | 44 | 53 | 74 | > 85 |
| Day 2 | 420 | 43 | 61 | 46 | 54 | 30 | 50 | 32 | 50 | 55 | 68 |
| Day 3 | 400 | 44 | 61 | 46 | 54 | No zone | 46 | No zone | 45 | 52 | 65 |
| Day 4 | 380 | [a] | 61 | 42 | 57 | No zone | 46 | No zone | 47 | 47 | 62 |
| Day 8 | 360 | [a] | 56 | 43 | 52 | No zone | 45 | No zone | 46 | 50 | 60 |
| Day 9 | 340 | | 54 | 44 | [a] | No zone | 44 | No zone | 41 | [a] | 57 |
| Day 13 | 320 | | 70 | 48 | [a] | No zone | 43 | No zone | 42 | [a] | 57 |
| Day 16 | 300 | | 51 | 43 | [a] | No zone | 42 | No zone | 40 | | 51 |
| Day 20 | 280 | | a | 40 | [a] | No zone | 41 | No zone | 42 | | 48 |
| Day 24 | 260 | | 39 | 39 | [a] | No zone | 35 | No zone | 40 | | 46 |
| Day 30 | 220 | | 42 | 37 | 39 | No zone | 35 | No zone | 40 | | 46 |
| Week 6 | 200 | | 46 | 37 | 43 | | 35 | | 34 | | [a] |
| Week 7 | 180 | | 44 | [b] | 45 | | 35 | | 32 | | |
| Week 8 | 150 | | 42 | 36 | 44 | | [b] | | 38 | | |
| Week 10 | 130 | | 45 | | 40 | | [b] | | 35 | | |
| Week 12 | 100 | | 45 | | 44 | | | | [b] | | |

[a] Not readable inhibition zones because of contamination with an environmental fungal strain; [b] not readable inhibition zones because of irregular shape around the spacer

However, the addition of vancomycin might be a microbial ecological risk [27]. Gradual development of vancomycin resistance could occur as a result of wide use of vancomycin with prolonged release in subtherapeutic doses or the employment of large amounts. Therefore, vancomycin-loaded cement is suggested only for revisions of infected arthroplastic and for high-risk procedures. Failure of infection control in massive tumor reconstruction may result in the loss of the extremity. Since most CNS of orthopedic infections are resistant to gentamicin, vancomycin might inhibit those bacteria. Aminoglycosides are particularly effective against gram-negative bacteria, and cannot be replaced totally by vancomycin.

We conclude that these *in vitro* experiments support reasonable use of vancomycin loaded spacers in tumour patients with infected reconstructions by CNS. Although we observed antibiotic elution over 8 weeks we suggest that the spacer should be changed every 4–6 weeks since the declining antibiotic concentration may no longer inhibit the CNS, the most prevalent infectious pathogens in infected allografts. These data have to be confirmed by *in vivo* experiments; we recently started to investigate explanted cement spacer from patients to detect their inhibition of CNS.

## References


1. Buchholz HW, Engelbrecht H: Über die Depotwirkung einiger Antibiotica (sic!) bei Vermischung mit dem Kunstharz Palacos. Chirurg 1970; 40: 511–515.
2. Buchholz HW, Elson RA, Engelbrecht E, Lodenkämpfer H, Röttger J, Siegel A: Management of deep infection of total hip replacement. J Bone Joint Surg Br 1981; 63: 342–353.
3. Donati D, Biscaglia R: The use of antibiotic-impregnated cement in infected reconstructions after resection for bone tumours. J Bone Joint Surg Br 1998; 80: 1045–1050.
4. Gristina AG: Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 1987; 237: 1588–1595.
5. Bunetel L, Segui A, Cormier M, Langlais F: Comparative study of gentamicin release from normal and low viscosity acrylic bone cement. Clin Pharmacokinet 1990; 19: 333–340.
6. Seyral P, Zannier A, Argenson JN, Raoult D: The release *in vitro* of vancomycin and tobramycin from acrylic bone cement. J Antimicrob Chemother 1994; 33: 337–339.

7. Duncan CP, Masri BA: The role of antibiotic-loaded cement in the treatment of an infection after a hip replacement. J Bone Joint Surg Am 1994; 76: 1742–1751.
8. Scott CP, Higham PA, Dumbelton JH: Effectiveness of bone cement containing tobramycin. An *in vitro* susceptibility study of 99 organisms found in infected joint arthroplasty. J Bone Joint Surg Br 1999; 81: 440–443.
9. Perry AC, Rouse MS, Khaliq Y, Piper KE, Hanssen AD, Osmon DR, Steckelberg JM, Patel R: Antimicrobial release kinetics from polymethylmethacrylate in novel continuous flow chamber. Clin Orthop 2002; 403: 49–53.
10. Lawson KJ, Marks KE, Brems J, Rehm S: Vancomycin vs tobramycin elution from polymethylmethacrylate: an *in vitro* study. Orthopedics 1990; 13: 521–524.
11. Nelson CL, Griffin FM, Harrison BH, Cooper RE: *In vitro* elution characteristics of commercially and noncommercially prepared antibiotic PMMA beads. Clin Orthop 1992; 284: 303–309.
12. Wahlig H, Dingeldein E: Antibiotics and bone cements. Experimental and clinical long-term observations. Acta Orthop Scand 1980; 51: 49–56.
13. Brien WW, Salvati EA, Klein R, Brause B, Stern S: Antibiotic impregnated bone cement in total hip arthroplasty. Clin Orthop 1993; 296: 242–248.
14. Chapman MW, Hadley WK: The effect of polymethylmethacrylate and antibiotic combination on bacterial viability. An *in vitro* and preliminary *in vivo* study. J Bone Joint Surg Am 1976; 58: 76–81.
15. Bertazzoni Minelli E, Caveiari C, Benini A: Release of antibiotics from polymethylmethacrylate cement. J Chemother 2002; 14: 492–500.
16. Holm NJ: The relaxation of some acrylic bone cements. Acta Orthop Scand 1980; 51: 727–731.
17. Elson RA, Jephcott AE, McGechie DB, Verettas D: Antibiotic-loaded acrylic cement. J Bone Joint Surg Br 1977; 59: 200–205.
18. Greene N, Holtom PD, Warren CA, Ressler RL, Shepherd L, McPherson EJ, Patzakis MJ: *In vitro* elution of tobramycin and vancomycin polymethylmethacrylate beads and spacers from Simplex and Palacos. Am J Orthop 1998; 27: 201–205.
19. Kuechle DK, Landon GC, Musher DM, Noble PC: Elution of vancomycin, daptomycin and amikacin from acrylic bone cement. Clin Orthop 1991; 264: 302–308.
20. Cerretani D, Giorgi G, Fornara P, Bocchi L, Neri L, Ceffa R, Ghisellini F, Ritter MA: The *in vitro* elution characteristics of vancomycin combined with imipenem-cilastatin in acrylic bone-cements. J Arthroplasty 2002; 17: 619–626.
21. Taggart T, Kerry RM, Norman P, Stockley I: The use of vancomycin-impregnated cement beads in the management of infection of prosthetic joints. J Bone Joint Surg Br 2002; 84: 70–72.
22. Bayston R, Milner RD: The sustained release of antimicrobial drugs from bone cement. An appraisal of laboratory investigations and their significance. J Bone Joint Surg Br 1982; 64: 460–464.
23. Penner MJ, Masri BA, Duncan CP: Elution characteristics of vancomycin and tobramycin combined in acrylic bone-cement. J Arthroplasty 1996; 11: 939–944.
24. Pankey GA, Sabath LD: Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Inf Dis 2004; 38: 864–870.
25. Chuard C, Vaudaux PE, Proctor RA, Lew DP: Decreased susceptibility to antibiotic killing of a stable small colony variant of *Staphylococcus aureus* in fluid phase and on fibronectin-coated surfaces. J Antimicrob Chemother 1997; 39: 603–608.
26. Proctor RA, Peters G: Small colony variants in staphylococcal infections: diagnostic and therapeutic implications. Clin Inf Dis 1998; 27: 419–423.
27. Chohfi M, Langlais F, Fourastier J, Minet J, Thomazeau H, Cormier M: Pharmacokinetics, uses, and limitations of vancomycin-loaded bone cement. Int Orthop 1998; 22: 171–177.