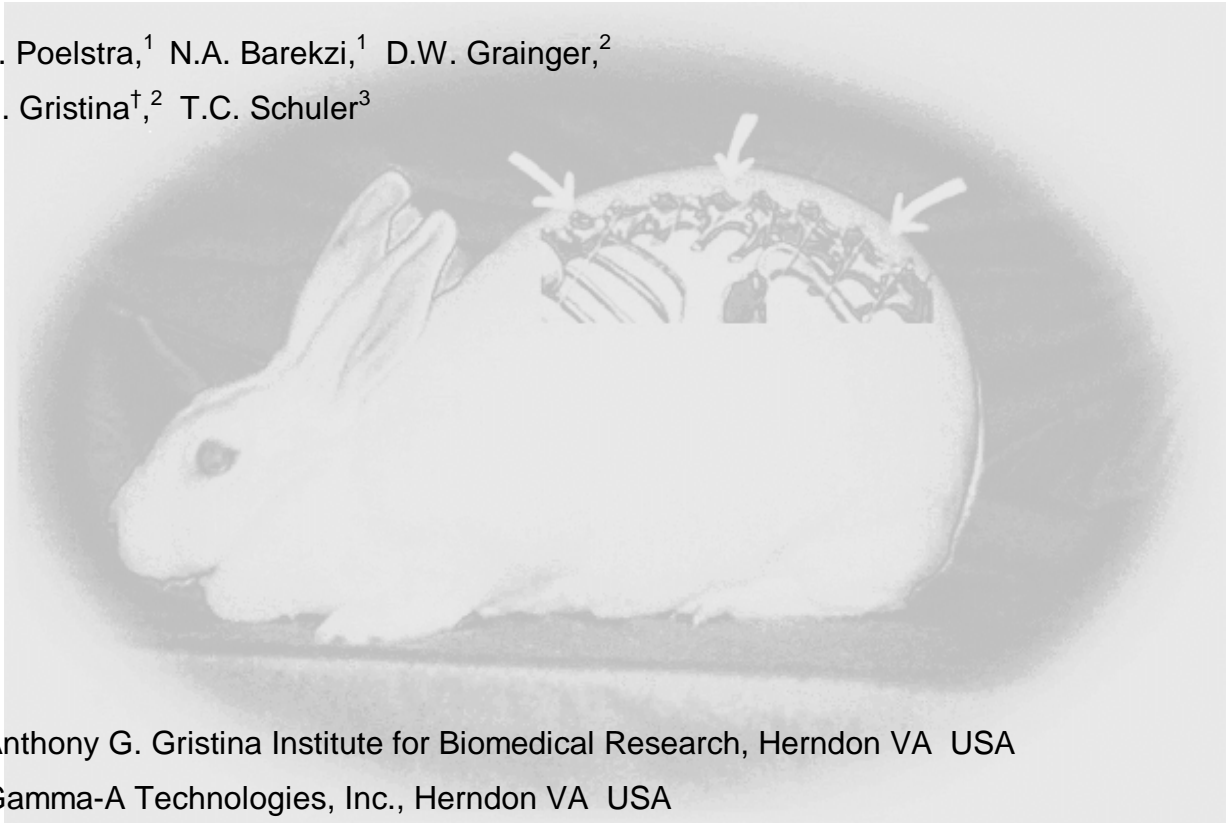# A novel spinal implant infection model in rabbits

K.A. Poelstra,[1] N.A. Barekzi,[1] D.W. Grainger,[2]
A.G. Gristina[†],[2] T.C. Schuler[3]

[1.] Anthony G. Gristina Institute for Biomedical Research, Herndon VA USA

[2.] Gamma-A Technologies, Inc., Herndon VA USA

[3.] Northern Virginia Spine Institute, Reston VA USA

[†] deceased, April 30, 1998

Abstract

A new spinal implant model was designed to study device-centered infection with methicillin resistant Staphylococcus aureus (MRSA) in multiple non-contiguous surgical sites in the lumbar spine region of a rabbit. Large numbers of recent studies show that postoperative wound infection following spinal implant surgery, and the rise in antibiotic resistant bacteria, are a concern. Anti-infective strategies must be tested in relevant animal models that will lead to appropriate clinical studies. Eight anesthetized New Zealand white rabbits underwent completely isolated partial laminectomies and subsequent stainless steel K-wire implantations directly into the transverse processes of vertebrae Th13, L3 and L6. The middle sites (L3) were used as sterile control sites while the outer sites (Th13, L6) were challenged with different amounts of MRSA. Rabbits were sacrificed after seven days and biopsied to provide evidence for device-centered infection. Bacterial growth on the implant surfaces and in surrounding tissues and bone was assayed. Overall device-centered infection was established after seven days in 100% of the sites challenged with 10^3 CFU MRSA or higher. No infection was seen in any of the control sites located between infected vertebrae. Multiple blood and liver samples showed that the separate localized infections did not systemize after seven days. This new animal model demonstrates that multiple biomaterial implants can be evaluated in the same animal and provides a technique for investigating postoperative device-centered infection of the spine. Infection was demonstrated in non-contiguous lumbar sites of the spine, while adjacent control sites remained sterile. Since there was no cross-contamination or systemic spread of the infection, multiple anti-infective strategies or implant materials can now be tested for efficacy in a single animal to combat dramatic and costly postoperative implant infections.

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Infection is a known and problematic complication in posterior or posterolateral reconstructive spine surgery. The introduction of spinal instrumentation in the 1960's increased the incidence of postoperative spinal infection[14,16,22] and, although modern spinal surgical techniques have decreased the incidence of this complication, postoperative spinal implant infection still occurs at a significant rate. The average incidence under antibiotic prophylaxis can be 0.1 percent but is reported to be 8.2

percent and higher for complicated cases, depending on patient- and procedure-related risk factors.[4,7,9,13,15,17,24,25] Geriatric, immunocompromised, diabetic and obese patients all have greater risks of infection.[1,7,17,25] The most common organism isolated from postoperative spinal wound infections is *Staphylococcus aureus*.[6,7,15,16,17,25]

Foreign bodies, dead space, devitalized or even necrotic tissue all contribute to a decrease in the body's ability to eliminate bacteria that inevitably end up inside the wound after long surgical procedures.[21] Many pathogenic bacteria require adhesion-dependent colonization to establish infection. The formation of a surface-adherent and protective 'biofilm' is characteristic of a mature infection, particularly on implanted devices. Once established, this biofilm is very difficult to eradicate despite the use of antibiotics that are highly active in standard *in vitro* susceptibility tests.[10,11,20] In addition, the unfortunate emergence of methicillin and vancomycin resistant pathogen strains and of a large immunocompromised patient population add significant magnitude to the surgical infection problem.[8,10,23]

Apart from patient discomfort, the cost of treating spinal implant infections ranges from $6,000 to $900,000 per case, and in approximately 50% of these cases, operative intervention is required.[9,15,19] Prevention of postoperative wound infection (prophylaxis) is therefore the first line of defense in the battle against rising healthcare costs[4,7] with antibiotic prophylaxis as current routine.[21]

In order to direct new anti-infective therapies to spinal implant surgery, more appropriate *in vivo* animal models are required. Many animal models have been designed to study artificial joint infection or osteomyelitis in long bone, but none of these models accurately represent the local environment of current postoperative wound infections after spinal implant surgery.[2,3,5,18] The model described herein is based on the separate implantation of three biomaterials in isolated laminectomy defect sites in the lumbar spine of a single rabbit, mimicking aspects of posterior spinal instrumentation used in lumbar fusion surgery. Individual sites were intentionally challenged with different amounts of methicillin resistant *Staphylococcus aureus* to establish a consistent, localized device-centered infection.

Materials and Methods

Animals. After approval of all protocols by the institutional animal care and use committee, eight New Zealand white (NZW) female rabbits were obtained, weighing 2.5 – 3.0 kg each.

Bacterial inoculum. One day prior to surgery, methicillin resistant *Staphylococcus aureus* (MRSA, ATCC 33593) was suspended in 20 ml trypticase soy broth and incubated at 37°C, shaking at 150 RPM. After 18 hours, the culture was two times diluted in sterile saline and centrifuged (8,000 RPM) for 10 minutes. Final concentrations of bacteria were obtained by making different dilutions in sterile saline. The final bacterial concentration (Colony Forming Units (CFU) per milliliter) was estimated using a spectrophotometric assay and determined by plating on Trypticase Soy Agar (TSA).

Surgical procedure. The entire back and major parts of the rabbit gluteal region were thoroughly shaved one day prior to the surgical procedure. The animals were fasted 12 hours before premedication with butorphanol tartrate (0.1 mg/kg) and injected intramuscularly with an anaesthetic cocktail comprising ketamine HCl (44 mg/kg), xylazine (5 mg/kg) and acepromazine maleate (0.75 mg/kg) thirty minutes later. The positions of the desired vertebrae, thoracic 13 (*Th13*), lumbar 3 (*L3*) and lumbar 6 (*L6*) were marked on the back of the animal, and 0.5 - 1.0 ml of antibiotic-free Marcaine® 0.5% was injected subcutaneously at every site as local anaesthesia. After preparing the back with povidone-iodine, sterile drapes were used to cover the entire animal, leaving a circular area open (5 cm diameter) above the desired vertebral surgical site.

A 2.5 cm dorsal skin incision was made longitudinally in the midline, followed by a single incision in the fascia to expose the spinous process (Figure 1a). Using a small rongeur, the entire spinous process, with surrounding musculature and ligaments, was excised from the base (weighing 0.08 – 0.10 g.), creating a hollow self-contained defect, mimicking a partial laminectomy defect (Figure 1b). The ligamentum flavum was not violated, and the dura was not exposed.
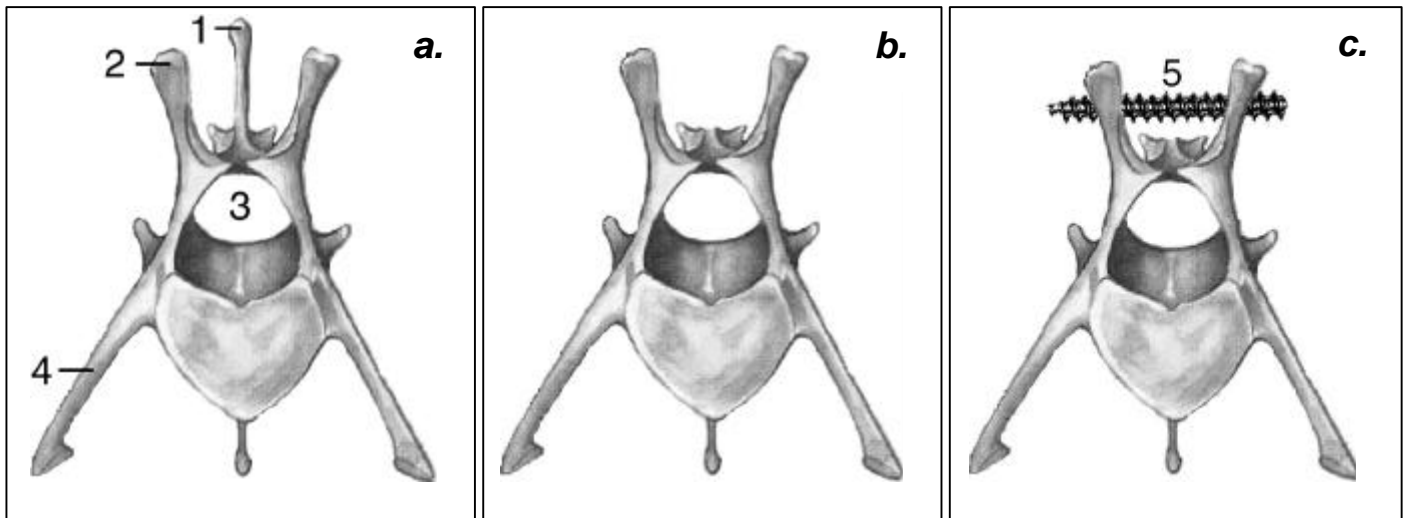
Figure 1:

a. *Cranial view on intact rabbit lumbar vertebra*
    (1. Spinous process; 2. Transverse process; 3. Spinal canal; 4. Costal process),
b. *After creating the laminectomy defect,*
c. *After implantation of the stainless steel K-wire (5).*

From the left side of the animal, a 0.85 mm diameter stainless steel threaded Kirschner wire (generously donated by Smith&Nephew-Richards, ASTM F138) was screwed through both transverse processes, crossing the laminectomy defect, and cut adjacent to the lateral wall of the left transverse process at the appropriate length (Figure 1c). Bacterial inoculum or sterile isotonic solution (both volumes: 100 µl) was squirted from a sterile syringe needle (30G) onto the implant and inside the defect pocket.   The fascia was closed tightly by running sutures with braided, biodegradable Vicryl™ 2/0. The skin was closed with interrupted Ethilon nylon™ 2/0 sutures.

Immediately after uncovering the animal, the back was again prepared with povidone-iodine and covered with new sterile cloth. Using a new set of sterile instruments, the second implantation was performed at the next randomly selected vertebra, with approximately 4 cm separating adjacent incisions. The same procedure was repeated at the third vertebral site. The closed wounds were left uncovered to prevent bandage irritation of the skin.

To prevent unwanted contamination, the sterile control site was consistently operated on first, and positioned deliberately (L3) between the two inoculated sites (Th13 and L6) to investigate the possibility for any cross-contamination. Variability was minimized and using the same surgeon (KP) to perform all operations standardized surgical trauma. After the procedure, the animals were housed individually in standard cages, and provided with water and standard antibiotic-free rabbit chow. The animals were monitored daily, particularly with regard to their wounds, temperature, signs of sepsis and body weight.

Evaluation. According to the approved protocol, the animals were sacrificed after seven days, using an intravenous injection of pentobarbital (10mg/kg). Prior to euthanasia, blood was drawn from the ear vein of each animal, and cultured to determine systemic sepsis. Under sterile conditions, biopsies of the skin (suture area), the fascia and muscle (suture area), the vertebral lamina, the K-wire implants and both transverse processes were removed from all sites. Also, a piece of the right liver lobe (approximately 5 grams) was removed to monitor systemic infection. Harvested tissue samples were immediately homogenized (Omni-International GLH homogenizer) and implants were sonicated (NEY Ultrasonik 100) for 30 minutes to detach bacteria. Serial dilutions were plated on TSA and incubated for 24 hours at $37°C$. Finally, the CFU burden was determined per gram tissue sample to enumerate bacterial amounts at every site. Biomaterial-centered infection was arbitrarily defined to occur at sites where MRSA was present on the K-wire, and a positive bacterial finding was evident in two or more tissue samples from that same site.

Results

Procedure, dose and observation time. No problems with either the anaesthetic or the surgical procedures were observed during the evaluation of these eight rabbits. The data for all eight rabbits are combined in Table 1. Blood and liver sample cultures were consistently sterile for all rabbits after 7 days, indicating that the separate localized infections did not produce systemic sepsis. Vital functions (weight, temperature, food and water intake) supported the observation of lack of systemic spread of infection.

Infection analysis. When a surgical implantation site was infected, bacterial growth was consistently found in all tissue samples from that particular site. Bacterial growth localized, for example, only on the K-wire implant or the bone, was never observed. In infected sites containing pus in the muscle area and in the defect, a translucent film covered the metal implant surface. Other than MRSA, no other bacterial growth was seen on any of the TSA culture plates post-harvest.

Evaluation. The eight animals were divided in two groups of two, and one group of four animals, respectively. In the two animals from Group 1, bacterial amounts of 10^4 and 10^5 CFU MRSA were randomly applied as local inocula in the spinal 'bacterial-sites' (Th13, L6). Subsequent bacterial growth in these sites created consistently large amounts of debris and pus, indicating high levels of infection. Post-mortem quantification of bacteria in these sites, shown in Table 1, indeed revealed extremely high bacterial burdens. No evidence for infection was, however, observed in the 'sterile control sites' (L3) located in between these contaminated sites (Th13, L6), and no bacteria were detected in liver or blood samples. Hence, all three implant sites (Th13, L3 and L6) were considered to have isolated, localized infection only. Subsequently, the next two rabbits (Group 2) were used to determine optimal inocula doses for this model using randomly applied 10^2 and 10^3 CFU MRSA.

As shown in Table 1, the lower dose (10^2 CFU MRSA) did not cause a consistent infection in these two animals after seven days. The higher dose (10^3 CFU MRSA)

produced a consistent localized infection as evidenced by pus, debris and post-mortem pathogen quantification. No evidence of systemic sepsis was found in liver or blood samples. We chose therefore to continue with a bacterial challenge of 10^3 CFU MRSA in the next four rabbits (Group 3). A sterile control site (L3) located between Th13 and L6 was again included to confirm that three completely isolated test sites could be established in a single animal spine without causing cross-contamination.

The overall infection rate for 'positive control sites', as calculated from the data in Table 1, was 89% (16:2) including the ineffective inoculum of 10^2 CFU MRSA. When only 10^3 CFU challenges in Group 3 are considered, a 100% infection rate after seven days was achieved. All control-sites (L3), lacking inoculum, remained sterile and post-mortem analysis of blood and liver samples showed no signs of systemic sepsis after seven days in all animals. Data in Table 1 for Group 3 animals show that biopsied tissue samples from the different infected sites post-mortem contained on average ~ 1.8 x 10^6 CFU MRSA/gram tissue. No differences (t-test) were found between averaged CFU values post-mortem between the different spine sites (Th13, L6).

Discussion

Antibiotic resistant, biofilm-forming infection is a dramatic and costly complication of surgical procedures and limits the use of implanted biomaterial devices.[4,17] Host defenses and administered antibiotics are often only partially effective against biomaterial-centered spinal infections, with complications sometimes mandating complete removal of the device. Rates for spinal infection increase up to three times when metallic spinal implants are used.[12,25]

Many animal models have been designed to date to study surgical infection, but none link a spinal implant infection with the ability to test specific preventative treatments. Soft tissue damage and dead space areas characteristics of posterior spinal implant surgery are now combined as factors in a single model.

Table 1 Spinal Implant Infection Model Experimental Summary

Implantation site, CFU challenge, Operating time per site and CFU post-mortem per gram tissue, after 7 days implantation.

Spinal Implantation Site

Animal	Th13			L3			L6		
	Inoculation[A] CFU MRSA	time[B] (min)	post-mortem[C] CFU MRSA	Inoculation[A] CFU MRSA	time[B] (min)	post-mortem[C] CFU MRSA	inoculation[A] CFU MRSA	time[B] (min)	post-mortem[C] CFU MRSA
Group 1									
1	10^4	12	5.0×10^7	None	11	Sterile	10^5	11	1.6×10^7
2	10^5	11	6.3×10^6	None	13	Sterile	10^4	12	5.0×10^7
Group 2									
3	10^2	11	sterile	10^3	9	7.9×10^5	10^2	7	sterile
4	10^2	13	1.6×10^6	10^3	9	3.2×10^5	10^3	9	1.0×10^6
Group 3									
5	10^3	11	1.2×10^6	None	9	Sterile	10^3	11	6.3×10^6
6	10^3	12	1.0×10^6	None	9	Sterile	10^3	10	1.6×10^6
7	10^3	9	3.1×10^6	None	11	Sterile	10^3	14	5.0×10^5
8	10^3	10	2.0×10^5	None	10	Sterile	10^3	9	2.5×10^5
Mean (Group 3)[D]		10.30min	1.4×10^6		9.45min	Sterile		11.00min	2.2×10^6

A. Total CFU MRSA applied to each site at the time of surgery (in100 µl aqueous carrier).

B. Surgical time per site (min).

C. Average CFU determined from biopsied tissue samples and the harvested implant (CFU/g sample).

D. Average CFU values and operating time showed no significant differences between the infected spinal implant sites.

Dead space areas such as laminectomy defects tend to fill up with blood, providing an ideal medium for pathogens to multiply. In the presence of a foreign body, the risk of long-lasting antibiotic resistant infections in spinal surgery is high. Results from this three-site biomaterial-centered infection model in New Zealand white rabbits successfully demonstrate the utility of this novel animal infection model for use in spinal surgery research.

Multiple surgical sites remain isolated, despite substantial bacterial inoculation, indicating that cross-contamination is prevented. Lack of detectable systemic sepsis also provides evidence that sterile site contamination cannot occur via this route. The sterile sites in Groups 1 and 3 were operated on first by design to prevent inadvertent spread of pathogens from surfaces of inoculated sites to the sterile wounds. No correlation was found between the surgical site sequence and the post-mortem bacterial burden between Th13 and L6. Additionally, no correlation between the time the animal was under anesthesia and the CFU values found post-mortem in randomly operated infected sites could be detected. It is arguable whether host response to infection is different in this multi-site model over one in which only one site is challenged. However, the intrinsic advantage of comparing different sites within the same animal with the observed lack of site communication or systemic complications is highly significant because the localized inflammatory reactions observed at each site in one animal are then directly comparable. This design decreases the statistical variance and therewith the number of animals required in a given study, compared to one-site models.

Methicillin resistant *Staphylococcus aureus* (ATCC 33593) demonstrated a consistent capability to establish a device-centered infection after seven days in this model when inocula exceeded 10^2 CFU/site. Infection efficiency was targeted by design at 100% in inoculated sites in order to study cross-contamination and systemic spread of the infection. Consistent infection might well be feasible using lower doses of MRSA, but infection using 10^2 CFU/site was unreliable. Greater clinical relevance would be achieved using low doses of inoculum and monitoring progression of infection over longer time frames.

Future research using this multi-site biomaterial-centered model will focus on testing different pathogens and varying bacterial burdens in single animals with and without the use of a biomaterial implants. Different implanted biomaterials (metals, composites, polymers and tissue-engineered scaffolds) will also be analyzed for susceptibility to spinal infection, in tandem with application of systemic or local antibiotics and lavages to prevent and clear device-centered infections.

Further research is required to prevent the devastating and costly complications resulting from postoperative wound infections. This model provides an experimental vehicle to move forward with this testing. In the presence of implanted biomaterials and antibiotic resistant bacteria, routine use of antibiotics no longer protects against infection. In addition to improved surgical techniques and antibiotic prophylaxis, new methods to reduce device-centered infection must be studied in animal models relevant to clinical practice. Future work will consider these exact experimental scenarios using this model.

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