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A SYSTEMATIC REVIEW OF ANIMAL MODELS FOR *STAPHYLOCOCCUS AUREUS* OSTEOMYELITIS

W. Reizner, J.G. Hunter, N.T. O'Malley, R.D. Southgate, E.M. Schwarz and S.L. Kates*

Department of Orthopaedics and Rehabilitation, University of Rochester Medical Center, Rochester, NY 14642, USA

Abstract

Staphylococcus aureus (*S. aureus*) osteomyelitis is a significant complication for orthopaedic patients undergoing surgery, particularly with fracture fixation and arthroplasty. Given the difficulty in studying *S. aureus* infections in human subjects, animal models serve an integral role in exploring the pathogenesis of osteomyelitis, and aid in determining the efficacy of prophylactic and therapeutic treatments. Animal models should mimic the clinical scenarios seen in patients as closely as possible to permit the experimental results to be translated to the corresponding clinical care. To help understand existing animal models of *S. aureus*, we conducted a systematic search of PubMed and Ovid MEDLINE to identify *in vivo* animal experiments that have investigated the management of *S. aureus* osteomyelitis in the context of fractures and metallic implants. In this review, experimental studies are categorised by animal species and are further classified by the setting of the infection. Study methods are summarised and the relevant advantages and disadvantages of each species and model are discussed. While no ideal animal model exists, the understanding of a model's strengths and limitations should assist clinicians and researchers to appropriately select an animal model to translate the conclusions to the clinical setting.

Keywords: Osteomyelitis; animal models, methicillin-resistant *Staphylococcus aureus* (MRSA), infection; fracture, implant.

*Address for correspondence:
Stephen L. Kates
601 Elmwood Ave, Box 665
Rochester, NY 14642, USA
Email: Stephen_Kates@urmc.rochester.edu

Introduction

Infection and osteomyelitis represent significant complications of orthopaedic surgery. Infection rates associated with total hip arthroplasty and total knee arthroplasty have been historically reported to be 0.88 % and 0.92 %, respectively, and result in substantial morbidity (Kurtz *et al.*, 2008). As the number of total hip and knee replacements are projected to grow between 174 % and 673 %, during the next 20 years in the United States alone (Kurtz *et al.*, 2007), controlling these rates is of great concern. A recent report by Cram *et al.* has shown the infection rate after total knee replacement to be increasing with time to 3 % (Cram *et al.*, 2012). In contrast to the rates associated with the controlled environment of arthroplasty, the infection rates of open fractures range from several percentage points to a staggering 50 % (Gustilo *et al.*, 1984; Zalavras *et al.*, 2005). Implant associated osteomyelitis inflicts significant morbidity and mortality to the patient and proves a notable challenge for the orthopaedic surgeon.

The increase in antibiotic resistant pathogens has further complicated the management of osteomyelitis (Cardo *et al.*, 2004). *S. aureus* and coagulase-negative *Staphylococci* account for 65-80 % of infections (Schmidt and Swiontkowski, 2000); Darouiche, 2004 and a steep rise in the incidence of methicillin-resistant *S. aureus* and the ongoing emergence of vancomycin-resistant *S. aureus* is well documented (Parvizi *et al.*, 2009; Loomba *et al.*, 2010). Recent studies have shown that patients with a confirmed methicillin-resistant *Staphylococcus aureus* (MRSA) infection experience longer hospital stays, require prolonged antibiotic therapy (usually intravenous) and experience a 2.7 fold increase in mortality compared to non-infected inpatients (Nixon *et al.*, 2006; Lee *et al.*, 2010). One estimate of the mean cost attributable to an MRSA infection is $35,367 (Stone *et al.*, 2002); and, in a 2011 APIC (Association for Professionals in Infection Control and Epidemiology) publication (Rebmann and Aureden, 2011), the annual cost to treat MRSA in hospitalised patients in the United States was quoted between $3.2 billion to $4.2 billion. Accordingly, the successful development of novel therapies to prevent and manage MRSA infections promises to deliver vast benefits in patient care and for the healthcare community.

In the face of such infections, infectious disease and orthopaedic researchers collaborate to refine the management of osteomyelitis. Human clinical trials are inherently difficult to conduct secondary to the low incidence of implant-associated osteomyelitis, the heterogeneous population, various treatment modalities

and the broad range of causative pathogens and associated virulence patterns (Lazzarini *et al.*, 2006). Experimental studies in animal models serve to fill this void. While it is difficult to precisely replicate disease onset and progression characteristics of human infection, animal models facilitate our understanding of osteomyelitis and often inform clinical practice (Norden, 1988). In order to address implant associated osteomyelitis, investigators must ask disease specific questions and build on established animal models capable of answering these questions. For this purpose, we have systematically reviewed experimental studies in animals that explore *S. aureus* osteomyelitis to provide both clinicians and basic scientists with an understanding of current animal models that address the prevention and management of osteomyelitis.

Methods

Search strategy

Two databases, PubMed and Ovid MEDLINE, were used to systematically identify studies exploring osteomyelitis in *S. aureus* animal models. The PubMed database was systematically searched with the following search string and included articles through December 31st, 2012: '(infection) AND (animal model) AND (arthroplasty OR fracture OR internal fixation OR prosthesis).' The search strategy for Ovid MEDLINE (1902 – December 31st, 2012) is described in Table 1. Three reviewers participated in screening and study selection. Two authors (NO'M and WR) individually reviewed all abstracts of identified articles. Studies that met eligibility criteria during this initial screening were retrieved and reviewed in full. A third reviewer (JH) resolved any discrepancies. Additional publications were identified within the reference section of acquired studies, and additional publications of interest were included to supplement those identified by means of the systematic search.

Inclusion/exclusion criteria

Inclusion criteria included: (1) *in vivo* experimental study, (2) investigation of the management of *S. aureus* osteomyelitis, and (3) an experimental model based on an animal species. Exclusion criteria included (1) vertebral osteomyelitis, (2) craniofacial osteomyelitis, and (3) infections not clearly defined as osteomyelitis. Furthermore, letters, abstracts, cases and reviews were excluded. Non-English reports that were unavailable in an English transcript were excluded.

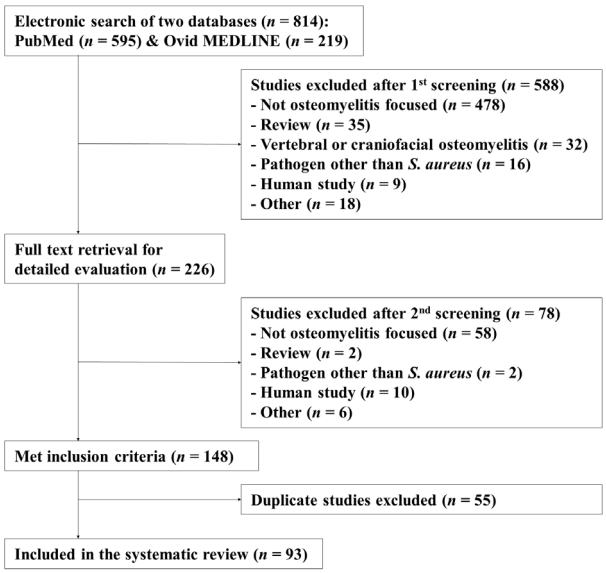

Fig. 1. Systematic Literature Search Flow Diagram

Summary of the literature search

Fig. 1 depicts the results of our systematic search. A total of 814 articles were identified. 588 articles were excluded during the initial screening of titles and abstracts and seventy-eight articles were excluded during the second screening. Of the 148 articles that met eligibility criteria, fifty-five were duplicates. Ultimately, ninety-three experimental studies of *S. aureus* were included to be discussed in this systematic review.

Within the initial screening, 478 of the 588 excluded studies did not explore osteomyelitis. The majority of these articles investigated non-orthopaedic infections (i.e. cardiovascular infections, gastrointestinal infections, etc.). Additionally, any orthopaedic study that did not include an infection-related endpoint did not meet eligibility criteria. Thirty-five articles were reviews, thirty-two studies investigated vertebral or craniofacial osteomyelitis, sixteen studies explored non-*S. aureus* osteomyelitis (pathogens included *S. epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter baumannii*), and nine articles were human studies. The remaining eighteen excluded studies consist of seventeen non-English articles that could not be reviewed and one case study.

Once the remaining 226 articles were retrieved in full, the reviewers excluded fifty-eight studies which did not directly address osteomyelitis. Ten experimental studies were primarily *in vivo* human studies with a minor animal species component, two manuscripts were classified as

Table 1. Ovid MEDLINE Advanced Search Strategy

#	Search Words
1	exp Infection/
2	exp Models, Animal/
3	exp Arthroplasty
4	exp Fracture Fixation, Internal/ or exp Fracture Fixation/ or exp Fracture Healing/ or exp Fracture Fixation, Intramedullary/
5	exp Bone Screws/ or exp Femoral Fractures/ or exp Fractures, Bone/ or exp Fracture Fixation, Internal/ or exp Bone Plates
6	exp "Prostheses and Implants"/
7	1 and 2 and (3 or 4 or 5 or 6)
8	Limit 7 to yr = "1902-2012"

reviews, and two studies did not focus on *S. aureus*. Of the six additional excluded articles, three were non-English studies, two articles were letters, and one was a detailed study proposal that did not possess outcomes. Of note, the number of excluded articles is overestimated as the screening of the PubMed and Ovid MEDLINE searches were done in parallel. Duplicates were calculated only for included studies following the second screening.

Model design characteristics

The experimental studies obtained using the systematic search utilise various animal species, study varying strains of *Staphylococcus aureus*, and explore distinct aspects of osteomyelitis including diagnostic, prophylactic, and therapeutic measures. Table 2 highlights the major elements of each of the ninety-three experimental studies that met our inclusion criteria. Tables 2-5 provide specific details: (1) the animal species utilised, (2) the pathogenic strain studied, (3) the amount of inoculum and the method of inoculation, (4) the study question/purpose, and (5) the principal findings for each reference. Experimental studies are organised according to the use of an internal fixation, open fracture, periprosthetic, external fixation, or haematogenous model. Of note, this systematic review did not identify any rabbit or murine studies employing an external fixation model.

Animal species

Thirty-six references utilised the rabbit as the animal model (Tables 2a, and 2b), thirty-three of which employed the New Zealand white rabbit (NZW). Twenty-nine references utilised a rat model (Tables 3a and 3b), the majority of which employed either the Wistar rat or the Sprague Dawley rat. Seven references utilised a mouse model (Table 4): either the BALB/c or C57BL/6 strain. Seven references utilised an ovine model, six employed a canine model, four a goat model, two a porcine model, one a guinea pig model, and one a hamster model (Table 5).

***Staphylococcus aureus* strain**

Researchers utilised a wide range of *Staphylococcus aureus* strains to investigate osteomyelitis. Of note, twenty-eight references possessed a clinical isolate or unspecified strain. The sensitivities of clinical isolates are often detailed in the accompanying reference. Common strains include ATCC 25923, ATCC 29213, ATCC 49230 as well as bioluminescent strains (i.e. ATCC12600). Thirteen experiments explored osteomyelitis secondary to methicillin-resistant *Staphylococcus aureus*. Nine of these utilised a clinical isolate. MRSA strains included ATCC 33591, ST-021 (a clinical isolate from University of Maryland, Shock Trauma) (Craig *et al.*, 2005), an MLST-80 clone (Poultsides *et al.*, 2008), and the bioluminescent USA300 LAC::*lux* strain (Niska *et al.*, 2012).

Methods of evaluation

Animal experiments exploring osteomyelitis aim to either investigate the pathogenesis of osteomyelitis, examine diagnostic tools, study the efficacy of prophylactic modalities and consider varying therapeutic options. These studies typically confirmed the presence of osteomyelitis, and those studies exploring the natural course of osteomyelitis or examining the efficacy of prophylactic and therapeutic protocols monitored the progression of the infection in a reproducible and an informative manner.

Studies commonly defined the primary endpoint as rate of clinical infection. This was commonly documented by one or more of the following criteria: gross infection (i.e., purulent drainage from the infection site), histologic analysis, X-ray, serum markers of inflammation or capture of photons using bioluminescent imaging. Nonetheless, the manner in which a study establishes the presence of infection varies. Beyond noting local (erythema, swelling or abscess formation) or systemic (fever or lethargy) clinical signs of infection, studies often conducted advanced imaging (i.e., X-ray, computed tomography (CT) scan or magnetic resonance imaging (MRI)), as well as microbiologic and histologic evaluations. Additionally, researchers cultured intracardial blood samples, bone samples, or swabs from pins sites or hardware. Cultures were grown over nutrient agar or in tryptic soy broth. Growth was often considered a binary result (i.e., with growth or without growth). Pin site swabs were assessed in a similar manner. When quantifying the magnitude of infection in bone, researchers pulverised bone samples, suspended the samples in sterile phosphate buffered saline solution and determined colony forming units per gram of bone. To expand on the colony forming units per gram of bone measurement, select studies performed polymerase chain reaction (PCR) assays to assess the presence of bacterial DNA and potentially quantify the bacterial load (Nijhof *et al.*, 2001).

Infections were also qualitatively assessed by radiologic, macroscopic and histologic evaluation. Radiographic evaluation of infected bone is commonly performed according to a score by An *et al.* (An and Friedman, 1998; Lucke *et al.*, 2003a), grading the involved bone on periosteal reaction, osteolysis, soft-tissue swelling, deformity, sequestrum formation, spontaneous fracture and general impression. One macroscopic approach or lesion scoring employs the Rissing scale (Rissing *et al.*, 1985b; Shandley *et al.*, 2012), converting the appearance of infected bone into a quantitative scale: 0 = no visible evidence of infection; 1 = minimal erythema without bone destruction, without abscess; 2 = erythema with bone formation and minimal bone destruction; 3 = abscess with new bone formation, bone destruction and with purulent exudates; 4 = severe bone resorption, abscess, and total bone involvement. Studies performing a histologic examination of bone samples, such as Huneault *et al.*, analysed periosteal proliferation, cortex remodelling, endosteal proliferation, periosteal neutrophilic inflammation, periosteal lymphoplasmocytic inflammation, marrow lymphoplasmocytic inflammation, sequestrum and bacteria (Petty *et al.*, 1985; Huneault *et al.*, 2004).

In recent years, several research groups have adopted the use of bioluminescent imaging (Kadurugamuwa *et al.*, 2003; Li *et al.*, 2008). Through this technique, bacteria are genetically modified to emit photons when metabolically active following the alteration of a *lux* operon. Bioluminescent imaging offers *in vivo* monitoring

Table 2a. Experimental rabbit studies of *Staphylococcus aureus* osteomyelitis

	Species	Strain	CFU	Inoculation Method	Study Purpose	Principal Findings	Reference
Internal Fixation	NZW	Giorgio (Phage 80-81)	2 x 10^8	Injected *via* drill hole into medullary canal (MC).	Establish a model of chronic OM secondary to an internal fixation device.	Chronic staphylococcal OM developed in 88 % of rabbits receiving 2 x 10^8 CFU after tibial fracture & rod insertion.	Andriole *et al*., 1973 & 1974
	NZW	ATCC 8096	1 x 10^8	Injected at femoral defect.	Explore the effect of internal fixation on infection rates.	Inserting a screw significantly increased the infection rate (61.2 % *versus* 35.8 %).	Grewe *et al*., 1987
	NZW	Clinical isolate	3 x 10^5	Injected in MC *via* burr hole after rod placed.	Establish a model of post-traumatic OM: femoral IM rod with midshaft bony defect	Chronic OM developed in all rabbits with rod & inoculum without need for infection-promoting chemical agents.	Eerenberg *et al*., 1994
	NZW	V 8189-94	4 x 10^3 to 4 x 10^6	Injected alongside tibial plate.	Susceptibility of stainless steel (SLS) *versus* titanium (Ti) plates to infection.	Infection rates associated with SLS was statistically significantly (SS) higher than for Ti plates (75 % *vs*. 35 %).	Arens *et al*., 1996
	NZW	V 8189-94	2 x 10^3 to 2 x 10^7	Direct injection in vicinity of implants.	Infection susceptibility between open surgical approach & minimally invasive plate osteosynthesis (MIPO).	Tendency (not SS) toward lower infection rates both superficially & at bone-implant interface with MIPO.	Arens *et al*., 1999
	NZW	NC012973: ATCC 29213	(1) *in vitro*: wires in 10^8/mL (2) *in vivo*: 4 x 10^5 to 4 x 10^6	(1) Inoculation of wires prior to implantation (2) Direct injection in MC before wire implantation	- Establish a biofilm model using a femoral IM-implanted device. - Effect of silver coating on biofilm adhesion to Ti & SLS implants.	*In vitro* inoculation did not produce adequate levels of bacterial adhesion; whereas, direct inoculation resulted in reproducible biofilm formation. Silver coating demonstrated no appreciable effect on biofilm formation in either metal.	Sheehan *et al*., 2004
	NZW	ATCC 25923	5 x 10^6	Percutaneously 48 h after surgery with sodium morrhuate.	Efficacy of adenoviral transfer of the BMP-2 gene (Ad-BMP-2) in enhancing healing of an infected defect fracture model	Addition of Ad-BMP-2 led to earlier initial- & bridging-callus formation & higher overall callus grade *versus* Ad-luciferase suggesting Ad-BMP-2 enhances early healing.	Southwood *et al., 2004*
	NZW	P1	5 x 10^2	*Via* drill hole at inter-condylar notch before pin.	Prophylactic efficacy of coating Ti implant with minocycline & rifampin.	Antibiotic(abx)-coated pins had a SS lower colonisation rate & a SS lower rate of device-related OM.	Darouiche *et al*., 2007
	NZW	MRSA (Clinical isolate)	1 x 10^6	Injected in femoral MC after suctioning & saline flush.	Efficacy of gentamicin - vancomycin-impregnated (2:1) PMMA coating nail to treat bone & intramedullary infections.	Abx-impregnated PMMA nails led to a SS better radiologic & histologic assessment compared to rabbits with (a) non-impregnated nail, (b) no therapy, or (c) systemic teicoplanin.	Giavaresi *et al*., 2008
	NZW	ATCC 6538	2.45 x 10^7	Injected into femoral MC 2 weeks before treatment.	Treatment efficacy of ciprofloxacin implants.	Rabbits with abx-implants demonstrated reduced bacterial counts and fewer clinical & radiologic signs of infection.	Alvarez *et al*., 2008
	NZW	V 8189-94	2 x 10^4 to 1 x 10^8	Innoculated at implant after implantation.	Effect of polishing an internal fixation device on infection rate.	No significant difference in infection rates between polished & unpolished groups with the same material.	Moriarty *et al*., 2009
	NZW	JAR 06.01.31	4.3 x 10^1 to 4.3 x 10^4	Injected directly to implant site.	Susceptibility of non-polished Ti-aluminium-niobium (TAN), polished TAN, & electropolished SLS IM nails.	No SS difference in infection rates among non-polished TAN, polished TAN, & electropolished SLS IM nails.	Moriarty *et al*., 2010
	JW	ATCC 25923	1 x 10^5/mL	*In vitro* inoculation of screws.	Antibacterial efficacy of copper-containing 317L stainless steel.	317L-Cu screws reduced inflammation & infection compared to 317L screws. No difference in bone formation.	Chai *et al*., 2011

as to the degree and extent of infection and offers valuable insight to the metabolic status of the bacteria. These quantitative measurements are made over time in the same animal, in contrast to gross and histologic evaluations that require sacrifice of the test subject. Consequently, one is able to observe the temporal response of infection to an intervention in real-time.

The following section summarises many of the articles that met inclusion criteria and references additional experiments that inform and supplement those found by our systematic approach.

Literature Review of Animal Models

Rabbit models of osteomyelitis

Internal fixation models: acute and chronic osteomyelitis

The original tibial rabbit model was described by Schemen and then later modified by Norden (Norden, 1970), and involved inoculation of a sclerosing agent (5 % sodium morrhuate) *via* an 18 gauge needle followed with *S. aureus* to produce chronic osteomyelitis of the tibia. Insertion of a foreign body (i.e. metallic implants) was introduced by Andriole *et al.* (Andriole *et al.*, 1973). They fractured the tibia using a simple three-pronged clamp and then used a stainless steel pin for fixation, and were able to monitor infection for up to 18 months. Other authors have used the same technique without fracture (Moriarty *et al.*, 2010). Arens *et al.* created a model using plate fixation for the tibiae using 6-hole dynamic compression plating (DCP), and in one study, performed it using minimally invasive plate osteosynthesis (MIPO) (Arens *et al.*, 1999). However, no fracture was created and only unicortical screws were inserted, limiting its applications.

With respect to the femur, investigators historically accessed the femoral canal through the greater trochanter (Eerenberg *et al.*, 1994); however, recently published models are accessing the canal through the intercondylar notch using a parapatellar incision (Castro *et al.*, 2003; Sheehan *et al.*, 2004; Darouiche *et al.*, 2007; Giavaresi *et al.*, 2008). Authors report ease of exposure and access to the canal, though this technique compromises the knee joint and thus infection may not be limited to the femoral shaft. Once access is obtained after reaming, the investigator can perform the bacterial inoculation, place the cement (Castro *et al.*, 2003) or screw (Darouiche *et al.*, 2007), and finally, seal the intercondylar notch with bone wax. As such, these models provide multiple options for treatment and testing techniques. These models could be improved upon by creating a closed fracture to more closely mimic the clinical scenario. Using a simple lateral approach to the femur, Southwood *et al.* dissected through the soft tissue

Table 2b. Experimental rabbit studies of *Staphylococcus aureus* osteomyelitis (...continued)

	Species	Strain	CFU	Inoculation Method	Study Purpose	Principal Findings	Reference
OF	NZW	V 8189-94	4 x 10^3 to 4 x 10^7	Injected adjacent to tibial implant *via* catheter.	Determine the reliability of radiograph findings for the diagnosis of osteitis.	Radiographically verified periosteal reaction is constant & early feature of OM and correlates with degree of infection.	Kraft *et al*., 2001
	NZW	MRSA (ATCC 33591)	1 x 10^6	Injected into tibial MC.	Develop & evaluate a biodegradable implantable gatifloxacin delivery system.	At 2 and 4 weeks, there was a SS decrease in the bacterial count in rabbits treated with the gatifloxacin implant.	El-Kamel & Baddour, 2007
Periprosthetic	NZW	Clinical isolate	3.25 x 10^1 to 3.9 x 10^4	Injected into suprapatellar pouch.	Explore the progression of a knee infection & the role of abx, metallic implant debris, intra-articular blood & steroids.	SS increase in ID_{50} with prophylactic methicillin, cephacetrile, or clindamycin. Presence of metallic debris or autologous blood did not increase susceptibility to infection.	Schurman *et al*., 1975
	ES	Phage type 95	0 to 10^8	Inoculated into MC before cement placed.	Establish a model of OM in hip arthroplasty.	ID_{50} ranged from 50 CFU in rabbits receiving prosthesis & cement to 1 x 10^4 CFU in rabbits without implants.	Southwood *et al*., 1985
	NZW	MRSA (Clinical isolate)	10^5 to 10^8	Injected into knee joint immediately after implantation.	Establish a prosthetic joint infection model due to MRSA. [Silicone elastomer implant]	5 x 10^6 CFU induced infection in 100 % of animals with no mortality. Natural spread of infection without sodium morrhuate. Pathologic & radiologic characteristics defined.	Belmatoug *et al*., 1996
	NZW	Newman	1 x 10^4	Injected into femoral MC before SLS screw placed.	Explore the virulence of a mutant strain that possesses several surface adhesins.	No SS difference in infection rate or histopathologic assessment between strains with & without surface adhesins.	Darouiche *et al*., 1997
	NZW	Wood-46 (ATCC 10832)	10^3, 10^4, 10^5, 10^6 & 10^7	Injected into femoral MC immediately before placement of cement.	Prophylactic efficacy of tobramycin-containing bone cement in arthroplasty.	Premixed tobramycin-containing bone cement prevented implant-related infection. In controls, *SA* had a higher infection rate, & more pronounced periosteal reaction and associated signs of infection compared to *S. epidermidis*.	Nijhof *et al*., 2000a
	NZW	Wood-46	1 x 10^6	Injected into femoral MC before placement of cement.	Prophylactic efficacy of prophylactic tobramycin-containing bone cement & systemic cefazolin.	Tobramycin-containing bone cement & systemic cefazolin each prevented infection as demonstrated by culture & PCR-hybridisation assay.	Nijhof *et al*., 2000b
	NZW	Wood-46	10^5 or 10^6	Bacterial suspension introduced to tibial MC before implant.	Tobramycin-containing bone cement *vs*. systemic cefazolin for treatment of infection in a one-stage revision.	Both tobramycin-containing bone cement and systemic cefazolin reduced the size & rate of infection. Bacterial DNA persisted after antibiotic treatment.	Nijhof *et al*., 2001
	NZW	Clinical isolate	1 x 10^7	Intra-articular injection.	Evaluate ^{99m}Tc-ciprofloxacin as a marker used in scintigraphy for joint infection.	In rabbits with *SA,* increased uptake observed at day 5 in 60 % & at day 12 and 19 in 100 %. In those without *SA*, increased uptake at days 12 & 19 in 83 %. Poor specificity.	Sarda *et al*., 2002
	NZW	MRSA (Clinical isolate)	1 x 10^8	Injected into knee joint after implantation.	Treatment efficacy of teicoplanin cement with & without systemic teicoplanin.	Teicoplanin cement spacers combined with systemic intramuscular teicoplanin was most effective.	Ismael *et al*., 2003
	NZW	MRSA (ST-021)	0, 10^2, 10^3 & 10^4	Injected into knee joint after SLS screw & PMMA cement placed.	Establish a total knee arthroplasty infection model.	1 x 10^3 CFU found to be ID_{70}. 1 x 10^4 CFU did not result in increased infection rates. No animal in any group developed septicaemia. Successfully established paired knee model.	Craig *et al*., 2005
	NZW	EDCC 5055	1 x 10^7	Injected into tibial MC before implant.	Efficacy of two distinct gentamicin-hydroxyapatite (HA) coatings.	Gentamicin-HA-coated & gentamicin-RGD-HA-coated steel K-wires had SS lower infection rates *versus* HA-coating.	Alt *et al*., 2006
	NZW	ATCC 17848	1 x 10^7	Injected in knee near prosthesis.	Efficacy of a levofloxacin & rifampin combination.	Combination significantly reduced bacterial titers as compared to rifampin alone & control.	Muller-Serieys *et al*., 2009
	NZW	ATCC 49230C	1 x 10^8	Injected at implant site.	Prophylactic efficacy of pexiganan acetate for pin tract infections.	75 % reduction of pin tract infections in rabbits receiving Ti-Pexiganan compared to Ti-control with no antimicrobial.	Chou *et al*., 2010
Hematogenous	NZW	ATCC 25953	7 x 10^7 to 1.1 x 10^8/mL	0.2 mL/kg injected in ear vein.	Histologic study of haematogenous OM following physeal fracture.	Combination of a closed physeal injury & bacteraemia led to a reproducible metaphyseal infection.	Whalen *et al*., 1988
	NZW	ATCC 25953	4.65 x 10^8/mL	0.01 mL/kg or 0.02 mL/kg *via* ear vein.	Establish a model of acute haematogenous osteomyelitis.	Animals with fracture & bacteraemia developed OM in nearly all cases; those with only bacteraemia had occasional OM foci	Morrissy & Haynes, 1989
	NZW	Wood-46	1 x 10^8 to 2 x 10^9	Injected into auricular vein several days after implant.	Susceptibility of SLS *versus* Ti plates (traditional or PC-FIX design used).	SLS was SS more susceptible to infection than Ti plates. Ti-PC-FIX plate had infection risk equivalent to 0 CFU.	Johansson *et al*., 1999
	NZW	MRSA (MLST-80 clone)	5 x 10^8 or 3 x 10^8	*Via* femoral artery four weeks after tibial IM implants were placed.	Establish a haematogenous OM model due to a community-acquired MRSA strain.	10/10 animals receiving 5 x 10^8 CFU died within 72 h of septic shock. 8/10 receiving 3 x 10^8 CFU developed periprosthetic infection, OM & septic arthritis.	Poultsides *et al*., 2008

KEY: NZW – New Zealand White; JW – Japanese White; ES – English Shorthair; SA – *S. aureus*; SS – significantly, statistically significant; OM – osteomyelitis; MC – medullary canal; SLS – stainless steel; Ti – titanium

and periosteum of the femoral diaphysis where a 10 mm defect could be made with a burr (Southwood *et al.*, 2004). Fixation followed with stacked bone plates, 2.0 mm cortical screws and cerclage wire. While these authors used sodium morrhuate to aid in creating osteomyelitis, this is somewhat controversial and may not be needed. Many investigators referenced in this article have shown reproducible models without the aid of a sclerosing agent, and it may also skew outcomes if host response is being studied, as the infection cannot all be attributed to the bacteria.

Open fracture models

Open fracture rabbit models primarily utilise the tibia. Ashhurst *et al.* first described a fracture model; fractures were created by a saw and were stabilised with plates (Ashhurst *et al.*, 1982). Worlock *et al.* modified this scheme to generate a model with fixation of an intramedullary rod (Worlock *et al.*, 1988); this method has in turn been employed by others (Kraft *et al.*, 2001). An essential feature of this model is creating a wound over the fracture site where the contamination can take place. Worlock *et al.* report 10^7 colony forming units (CFU) as the minimal dose to produce a consistent infection (> 80 %). Subsequently, fixation can take place at the discretion of the investigator to appropriately address the experimental question. The intramedullary (IM) nail described by Worlock lacked rotational stability, differing from human cases where interlocking bolts are used to provide stability.

Table 3a. Experimental rat studies of *Staphylococcus aureus* osteomyelitis

	Species	Strain	CFU	Inoculation Method	Study Purpose	Principal Findings	Reference
Internal Fixation	Sprague Dawley	ATCC 49230	3.95 x 10^6	Injected into tibial MC seven weeks before beads.	Efficacy of gentamicin-loaded hydroxy-apatite cement (HAC) & PMMA beads.	Animals with gentamicin-loaded HAC or PMMA beads had SS less bacterial counts than those with debridement only.	Solberg *et al.*, 1999
	Sprague Dawley	Unspeci-fied	1 x 10^4	Injected in tibial MC after K-wire inserted in MC.	Efficacy of granulocyte-macrophage colony-stimulating factor (GM-CSF) in acute OM.	Addition of GM-CSF to ampicillin-sulbactam regimen led to greater resolution of infection compared to abx only group.	Subasi *et al.*, 2001
	Wistar	ATCC 9213 SP	-Biofilm implant with 10^6/mL -10^4, 10^5 or 10^6	*In vitro* inoculation of implant or injected near implantation.	Explore four models of OM with varying combinations of implant biofilms & suspensions.	4 Groups: (1) biofilm implant, (2) biofilm implant + 10^4 CFU, (3) sterile implant + 10^5, (4) sterile implant + 10^6 Group 1 produced tibial & implant infection most reliably.	Monzon *et al.*, 2002
	Sprague Dawley	ATCC 49230	10^2, 10^3 or 10^6	Injected into tibial MC at time of K-wire placement.	Establish a model of implant-associated osteomyelitis.	Induction of acute OM was possible with 10^2 CFU. Radiographic & histologic appearance of rats receiving 10^6 were SS worse compared to those receiving 10^2 or 10^3 CFU.	Lucke *et al.*, 2003b
	Sprague Dawley	ATCC 49230	1 x 10^3	Injected in tibial MC after K-wire placed.	Efficacy of gentamicin-loaded poly(D,L-lactide) (PDLLA) coating.	PDLLA + 10 % gentamicin coating SS reduced bacterial growth and radiologic & histologic signs of infection.	Lucke *et al.*, 2003a
	Sprague Dawley	MRSA (Clinical isolate)	5 x 10^5	Injected in tibial MC before placement of rod.	Efficacy of the combination of fusidic acid impregnated cement & systemic teicoplanin.	Combination treatment had an infection elimination rate of 81.8 %, while teicoplanin alone was 55.6 % – not SS.	Ersoz *et al.*, 2004
	Sprague Dawley	ATCC 49230	1 x 10^2	Injected into tibial MC before K-wire placed.	Prophylactic efficacy of local (coated Ti implant) *versus* systemic gentamicin.	Systemic abx reduced infection rate by 15 %, while local abx reduced infection rate by 90 %. No additive benefit.	Lucke *et al.*, 2005
	Sprague Dawley	Clinical isolate	10^3, 10^4, 10^5 or 10^6	Femoral defect was packed by collagen with inoculum.	Establish a model of chronic OM – segmental defect sta-bilised with polyacetyl plate & K-wire.	10^4 CFU was smallest inoculum that led to reproducible infection with detectable bony lysis & loss of fixation stability.	Chen *et al.*, 2005
	Sprague Dawley	Xen29	K-wire incu-bated until biofilm visible.	Biofilm-coated K-wire placed in tibial MC.	Treatment efficacy of 5-aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT).	ALA-mediated PDT inhibited biofilm implants in bone as observed by bioluminescent imaging.	Bisland *et al.*, 2006
	Sprague Dawley	MRSA (Clinical isolate)	5 x 10^6	Injected in lateral femoral condyle before rod placement.	Prophylactic efficacy of teicoplanin- & $CaSO_4$-loaded PMMA bone cement.	Addition of $CaSO_4$ to teicoplanin-loaded PMMA cement led to reduction in bacterial counts, in periosteal reaction & in osteolysis.	Tuzuner *et al.*, 2006
	Sprague Dawley	Unspeci-fied	1 x 10^4	*Via* bovine collagen packed into defect.	Effect of recombinant human osteogenic protein-1 (rhOP-1) on bone formation.	High dose (200 µg) rhOP-1 with abx led to SS more mineralised callus than control, low dose (20 µg), or no abx.	Chen *et al.*, 2006
	Sprague Dawley	Unspeci-fied	1 x 10^4	*Via* bovine collagen packed into defect.	Effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on bone formation in OM.	Significantly more mineralised callus was induced by high dose rhBMP-2 than with low dose and with systemic abx therapy than without.	Chen *et al.*, 2007
	Wistar	ATCC 9213	1.8 x 10^8	Injected into tibial MC after placement of SLS needle.	Study effect of age on cytokine response in acute osteomyelitis. (3 *versus* 22 month old rats)	Implants of old rats had a SS higher number of bacteria than in young rats. Surgery plus inoculum induced SS increases of IL-2 & IL-10 in young rats & IL-6 in old rats.	Garcia-Alvarez *et al.*, 2009
	Wistar	MRSA (Clinical isolate)	2 x 10^6	Injected into tibial defect after K-wire implantation.	Treatment efficacy of micro-spherical implants containing teicoplanin.	Teicoplanin-containing implants reduced the bacterial burden greater than intramuscular injection in chronic OM.	Orhan *et al.*, 2010
	Wistar	ATCC 29213	1 x 10^7	Injected in femoral MC at intercondylar notch before K-wire placed.	Treatment efficacy of moxifloxacin versus teicoplanin in chronic methicillin-sensitive *S. aureus* (MSSA) OM.	Bacterial counts in animals receiving abx were SS reduced as com-pared to controls. After implant removal, moxifloxacin significantly reduced counts more than teicoplanin.	Ozturan *et al.*, 2010

In a rabbit osteomyelitis model that involved inoculation of bacteria into devascularised bone, Smeltzer *et al.* demonstrated the importance of virulence factors in establishing osteomyelitis (Smeltzer *et al.*, 1997). Interestingly, the strain Smith diffuse, a strain that had demonstrated enhanced virulence in a murine peritonitis model, exhibited relatively little evidence of osteomyelitis compared to that of the strain UAMS-1. UAMS-1 achieved an infection rate of 75 % with an inoculum of 2 x 10^3 CFU.

Periprosthetic models

Rabbits are the smallest animals in which true models of prosthesis related osteomyelitis are described in the literature. Knee arthroplasty components exist with modification of existing joint replacements for humans. Belmatoug *et al.* used a human first metatarsophalangeal (MTP) silicone-elastomer implant for the tibial tray of a knee arthroplasty (Belmatoug *et al.*, 1996). Inoculants of 5 x 10^6 resulted in consistent infections. Of note, a silicone implant allows for MRI and micro-computed tomography (µCT) without artefact distortion, a significant advantage over most implants. Craig *et al.* produced a novel arthroplasty model in which a metal screw and ultra-high molecular weight polyethylene (UHMWPE) washer was secured to the non-articulating surface of the lateral femoral condyle (Craig *et al.*, 2005). All the various materials seen in a typical arthroplasty are present; however, the same stresses and strains placed on a weight bearing total joint arthroplasty (TJA) are not recreated with this model, which may bias results of therapeutic studies. A one-stage revision model has been described by inserting a standardised cement plug in to the tibial metaphysis, followed by contamination of 10^5 or 10^6 *S. aureus* directly anterior to the insertion of the anterior cruciate ligament of the knee (Nijhof *et al.*, 2001). This plug has a hook, thereby making a single exchange reproducible, even after several weeks of incorporation in the host.

Table 3b. Experimental rat studies of *Staphylococcus aureus* osteomyelitis (…continued)

	Species	Strain	CFU	Inoculation Method	Study Purpose	Principal Findings	Reference
Internal Fixation	Sprague Dawley	Clinical isolate	1 x 10^4	Injected at tibial osteotomy after IM device placement.	Establish a model of implant-related infected non-unions with detection by fluorescent *in situ* hybridisation.	All rats with 0 CFU had normal bone healing without infection at 6 weeks. 10/11 rats with 10^4 CFU developed infected non-union & all 11 IM implants had biofilm growth.	Alt *et al*., 2011
	Sprague Dawley	Clinical isolate	1 x 10^4	Injected in femoral MC before IM pin placed & fracture induced.	Establish a model of induced implant-associated OM following a fracture. [Control; *SA*; *SA* + ceftriaxone]	SS difference in CFU/femur & CFU/pin among three groups – highest in *SA* only. OM present radiographically & histopathologically in both *SA* groups – not in controls.	Robinson *et al*., 2011
Open Fracture	Sprague Dawley	Clinical isolates	1 x 10^8	Inoculated in wound 15 minutes before irrigation.	Efficacy of surfactant wound irrigation to prevent infection.	Sequential surfactant irrigation protocol led to a SS lower rate of infection than a single 3 L normal saline irrigation.	Marberry *et al*., 2002
	Sprague Dawley	SMH ATCC 700260	3 x 10^2 to 6.8 x 10^4	Introduced into tibial MC *via* dorsal defect.	Establish a traumatised (Gustilo Type III) tibiae model for acute osteomyelitis.	ID_{50} & ID_{95} values were 1.8 x 10^3 CFU & 9.2 x 10^3 CFU. Lavage reduced bacterial load but did not prevent infection.	Buxton *et al*., 2005
	Sprague Dawley	Cowan 1 ATCC 12598	Dose-dependent study. 5.5 x 10^2 & 1.7 x 10^3 used.	Introduced into tibial MC *via* dorsal defect.	Establish an acute OM model secondary to trauma with a foreign body & thermal injury.	ID_{50} & ID_{95} values were 72 & 977 CFU, respectively. At 1.7 x 10^3 CFU, all tibiae were infected when exposed to thermal injury, while 4 of 10 of non-thermal group were sterile. Sand increased bacterial load.	McPherson *et al*., 2008
	Sprague Dawley	Clinical isolate	1 x 10^2	Injected directly at fracture site.	Prophylactic efficacy of interleukin-12 (IL-12) coatings for implant infection.	Local IL-12 significantly reduced the infection rate in a dose-dependent fashion. Systemic IL-12 did not reduce rates.	Li *et al*., 2009
	Sprague Dawley	Clinical isolate	1 x 10^2	Injected directly at fracture site.	Efficacy of monocyte chemo-attractant protein-1 (MCP-1) & IL-12 p70.	Local MCP-1 & IL-12 p70 significantly reduced infection, independently. No synergistic effect when combined.	Li *et al*., 2010a
	Sprague Dawley	Clinical isolate	10^1 or 10^2	Injected at both ends of fracture 1 h before K-wire.	Establish an open fracture OM model. [Blunt trauma, IM K-wire fixation & *SA*]	10^2 had infection rate of 90-100 %. Rate with 0 CFU improved from 20 % to 10 % as surgical technique improved.	Lindsey *et al*., 2010b
	Sprague Dawley	Clinical isolate	1 x 10^2	Injected at both ends of fracture 1 h before K-wire.	Evaluate effect of interleukin-12 systemic therapy in open fracture osteomyelitis.	Infection rate unchanged by IL-12 therapy; but at day 10, bacterial qualitative growth scores were SS lower.	Lindsey *et al*., 2010a
	Brown Norway	ATCC 49230	1 x 10^4	Injected at fracture site into femoral MC.	Effect of gentamicin- & bone morphogenetic protein-loaded scaffold on osteomyelitis.	Gentamicin-treated rats exhibited SS increased fracture healing & bridging callous formation. No SS difference in bacterial bone culture between abx & non-abx groups.	Stewart *et al*., 2010
	Sprague Dawley	Xenogen 38 ATCC 49525	1 x 10^5	Collagen soaked in 1 x 10^5 CFU placed in mid-femoral defect.	Effect of early cefazolin & debridement on infection in an open fracture. [2, 6 & 24 h timepoints]	In rats receiving abx at 2 h, delaying surgery from 2 to 6 h SS increased the infection rate. Regardless of the time of surgery, delaying abx to 6 or 24 h SS increased the rate.	Penn-Barwell *et al*., 2012
Periprosthetic	Wistar	ATCC 25923	1 x 10^3	Injected into femoral MC before Ti rod placement.	Prophylactic efficacy of covalently bound vancomycin Ti rods.	Vancomycin-bound Ti rods led to reduced clinical signs of infection, reduced bacterial load & prevented osteolysis.	Antoci *et al*., 2007
	Wistar	ATCC 25923	1 x 10^3	Injected into femoral MC.	Treatment efficacy of vancomycin-loaded thin sol-gel coating.	Coating resulted in SS decrease in bacterial count & bacterial adhesion compared to control.	Adams et al., 2009
	Wistar	ATCC 25923	1 x 10^3	Injected into tibial MC after K-wire fixation.	Efficacy of debridement *vs*. abx-loaded cement *vs*. abx-loaded autogenous bone.	Teicoplanin-loaded cement led to SS lower bacterial counts, but this led to extensive infection in 3/8 rats.	Sener *et al*., 2010

Table 4. Experimental mouse studies of *Staphylococcus aureus* osteomyelitis

	Species	Strain	CFU	Inoculation Method	Study Purpose	Principal Findings	Reference
Internal Fixation	Balb/c	FDA209P	5 x 10^3	Injected at fracture site before K-wire placement.	Treatment efficacy of hyaluronic acid gel as a carrier of gentamicin.	Gentamicin-containing hyaluronic gel suppressed bacterial growth without interfering with bone growth.	Matsuno *et al*., 2006
	Balb/c	Clinical isolate	1 x 10^6	Injected into the osseous cavity of the tibiae.	Study the expression of human β-defensin-2 during osteomyelitis.	The murine homologue, murine β-defensin-3, was upregulated following tibial contamination.	Varoga *et al*., 2008
	C57BL/6	ATCC 49230 Xen29	9.5 x 10^5 ATCC 49230 & 4.2 x 10^5 Xen29 per pin	*In vitro* inoculation of SLS pin before implantation.	Establish a quantitative model of implant-associated OM utilising bioluminescent imaging (BLI).	*In vivo* BLI of Xen29 combined with *nuc* gene real-time quantitative PCR demonstrated the initial exponential growth phase of bacteria that peaks on day 4, followed by the biofilm growth phase at a lower metabolic rate.	Li *et al*., 2008
	C57BL/6	MRSA (Clinical isolate)	2.7 x 10^4	*In vitro* inoculation of SLS pin.	Efficacy of hyperbaric oxygen (HBO) therapy in the prophylaxis & treatment of osteomyelitis.	HBO therapy did not decrease bacterial burden. Of note, a positive correlation was found between the receptor activator of NF-κB ligand concentration and lesion score.	Shandley *et al*., 2012
Periprosthetic	C57BL/6	ALC2906	5 x 10^2, 5 x 10^3, & 5 x 10^4	Injected into joint space after K-wire implantation.	Explore a treatment modality for a post-arthroplasty infection of bioluminescent *S. aureus*.	*Ex vivo* bacterial counts highly correlated with *in vivo* bioluminescent signals. Minocycline/rifampin-loaded implants reduced infection, inflammation & biofilm formation.	Bernthal *et al*., 2010
	C57BL/6	MSSA (Xen29) MRSA (USA300 LAC:lux)	1 x 10^4	Injected into knee joint after device implantation.	Prophylactic efficacy of low-*versus* high-dose daptomycin, tigecycline, & vancomycin.	Low- & high-dose daptomycin & tigecycline and high-dose vancomycin resulted in significantly fewer bacterial counts as compared to control.	Niska *et al*., 2012
	C57BL/6	ALC2906, Xen29, Xen40 & Xen36	10^2, 10^3 or 10^4	Injected into joint space after K-wire implantation.	Establish a chronic post-arthroplasty infection with a bioluminescent *S. aureus* strain.	Xen29, Xen40 & Xen36 had increased bioluminescent signal through day 42; ALC2906 became undetectable at day 10. Xen36 induced the least inflammation.	Pribaz *et al*., 2012

Table 5. Other experimental animal studies of *Staphylococcus aureus* osteomyelitis

	Animal	Strain	CFU	Inoculation Method	Study Purpose	Principal Findings	Reference
Internal Fixation	Canine	Unspecified	10^3, 10^8 or 10^9	Injected into tibial MC *via* cortical window.	Establish a model of subacute OM & explore the role of gentamicin using this model.	Tibial OM was induced with 10^9 CFU. Gentamicin-loaded cement prevented sepsis in dogs with 10^3 to 10^9 CFU; but it was ineffective in treatment of established infection.	Fitzgerald, 1983
	Canine	ATCC 29213	6 x 10^5 to 8 x 10^5	Directly dropped on 2.0 mm screw, then inserted in femoral diaphysis.	Efficacy of ciprofloxacin-loaded crosslinked high amylose starch implants as prophylaxis & treatment.	Preventative group had absence of radiographic, macroscopic & histologic signs of infection. In established OM, systemic & local ciprofloxacin had equivalent curative efficacy.	Huneault *et al.*, 2004
	Guinea Pig	879R4S & 879R4S/1536	4 x 10^6/mL	Explanted miniplates & mini-screws bathed for 1 h.	Explore role of host proteins absorbed on implant in adhesion & colonisation.	SS reduction in adhesion of the fibronectin adhesin-defective mutant of *SA versus* wild-type strain on explanted implants.	Fischer *et al.*, 1996
	Ovine	ATCC 25923	2.5 x 10^6	Inoculated at osteotomy site after surgical closure.	Effect of vancomycin-modified implant on biofilm formation & bone-healing.	Vancomycin-derivatised plate surface inhibited colonisation & supported bone-healing (homogenous remodelling).	Stewart *et al.*, 2012
	Ovine	ATCC 25923	2.5 x 10^6	Injected *via* catheter into osteotomy site.	Effect of hydrophobic polycationic implant coatings on biofilm formation & bone-healing.	Both Ti & SLS implants coated by N,N-dodecyl,methyl-PEI exhibited SS less biofilm formation & greater bone healing compared to uncoated implants.	Schaer *et al.*, 2012
External Fixation	Goat	ATCC 25923	7.6 x 10^5	Inoculated on pin after insertion.	Prophylactic efficacy of tobramycin-loaded PMMA pin sleeve.	Abx-loaded sleeves had no gross evidence of infection. At 48 h, all untreated pins were colonised; treated had no growth.	Voos *et al.*, 1999
	Goat	ATCC 29213	3 x 10^4	Inoculated on pin before insertion.	Efficacy of hydroxyapatite-chlorhexadine coating on SLS & Ti pins.	Coating SS decreased pin tract infections from 100 % in uncoated pins to 83.3 % with no growth in coated pins.	Dejong *et al.*, 2001
	Ovine	ATCC 29213	5 x 10^7	Inoculated in wound around pin.	Explore pathogenesis of pin infections: spread of bacteria & fluid accumulation.	When a fluid reservoir was maintained around the pin, the infection rate was significantly greater (7 of 9 *versus* 0 of 9).	Clasper *et al.*, 2001a
	Ovine	ATCC 29213	2.4 x 10^7	Inoculated in wound at pin two weeks before IM nailing.	Explore the outcome of secondary IM nailing following pin track infection.	IM nailing in the setting of no abx led to sepsis in all sheep. Local debridement, MC lavage, and systemic and local abx controlled spread of infection; did not prevent chronic OM.	Clasper *et al.*, 2001b
Hematogenous	Canine	Phage types 52, 80, & 81	1 x 10^5	Injected into tibial nutrient artery.	Establish a canine model of acute haematogenous OM.	At 48 h, 10 % died of septicaemia. Surviving dogs developed medullary destruction, spontaneous fractures, & notable periosteal bone formation.	Deysine *et al.*, 1976
	Canine	Phage type 80/81	5 x 10^5	Injected into tibial nutrient artery.	Explore progression of acute haematogenous OM into chronic infection.	At 2 years, dogs exhibited clinical, histologic, radiologic & microbiologic changes associated with chronic OM.	Deysine *et al.*, 1983

Several authors have investigated the role of cement with various mixtures of antibiotics. Nijhof *et al.* describe a method of gaining access to the femoral canal by the trochanter tertius (Nijhof *et al.*, 2000a; Nijhof *et al.*, 2000b). Once the bone is exposed and the canal accessed, a silicone sleeve is inserted into the shaft. This sleeve will house both the inoculum and the cement which serves to seal off the bony defect and prevents subsequent leakage of the inoculum into the surrounding soft tissues.

Haematogenous models

Morrisy *et al.* and Whalen *et al.* first published a haematogenous model involving trauma to the proximal epiphysis of the tibia (Whalen *et al.*, 1988; Morrissy and Haynes, 1989). A three point bending force over the proximal part of the tibia creates a reproducible shearing injury to the physis. This is followed by intravenous (IV) injection of a high bacterial load (10^8 CFU) and provides good analysis of histologic parameters. This model was adapted by Johansson *et al.* (Johansson *et al.*, 1999) who implemented the presence of metallic hardware in the distal part of the tibia, although no fracture was created prior to fixation. Exposure to the distal diaphysis was made through a lateral approach, and a 5 x 35 mm dynamic compression plate (DCP) was applied followed by skin closure. Three to five days later, after the incision had healed, 10^8 *S. aureus* were injected into the auricular vein. This model had a relatively poor infection rate with a high mortality rate for the animals. Again, the addition of an injury or fracture may create an area of weakness to the host immune system, theoretically allowing greater infection rates as first published by Morrissy *et al.* (1989).

Poultsides *et al.* produced a haematogenous infection after insertion of a porous tantalum intramedullary implant press-fit into the proximal part of the tibia, which was capped by a silicone cup to permit load transmission within the knee joint (Poultsides *et al.*, 2008). Four weeks later, the femoral artery was cannulated and a catheter was advanced to 20 mm proximal to the knee joint where 1 mL of bacterial inoculum was injected to maximise the implant exposure to bacteria. While a higher inoculum (5 x 10^8) resulted in 100 % mortality due to septic shock, lower inoculates did not consistently produce infection reliably. This reinforces the difficulty of injecting the smallest load to ensure infection without overwhelming the animal immune system.

Advantages and disadvantages

Rabbits are employed in a large portion of the reviewed experiments. As an intermediate-sized animal, they possess distinct versatility and are relatively easy to handle, manipulate and maintain. Consequently, rabbits are relatively inexpensive. While their size lends itself to easy maintenance, their bones remain large enough to perform plate and screw fixation. The medullary canal of both the tibia and femur can easily accommodate a modified nail and are sizeable enough to house implants, in which an investigator can remove and replace in a reproducible

multi-staged fashion. Based on the included rabbit studies, the typical inoculation dose ranges from 10^3-10^8. Higher doses are typically required when manually coating implants with bacteria in contrast to direct inoculation in order to successfully initiate an infection.

Rat models of osteomyelitis: Internal fixation models: acute and chronic osteomyelitis

There are few studies designed to model acute osteomyelitis. The rat is known to possess a strong immune system that at times can complicate infection models in this species. Nonetheless, Subasi *et al.* explored acute osteomyelitis after pre-drilling the medial cortex of the proximal part of the tibia, directly inoculating bacteria and sealing the hole with bone cement in the absence of a sclerosing agent (Subasi *et al.*, 2001). A similar method was used by a group that used photodynamic therapy to eradicate acute *S. aureus* tibial osteomyelitis (Burch *et al.*, 2005).

Chronic tibial osteomyelitis was initially described by Zak *et al.* (Zak *et al*., 1982) and has been modified by several investigators since that time. Zak *et al.* used sodium morrhuate as a sclerosing agent to help establish an infection; arachidonic acid has also been described (Rissing *et al.*, 1985a; Mendel *et al.*, 1999). More recent models, however, do not rely upon a sclerosing agent, which may cause variability in the inflammatory pathology of the infection. They describe a craniomedial approach to the proximal tibia. This is ideal, as there is minimal soft tissue for dissection and for placement of desired instrumentation. Groups have used either a short segment of wire or needle tip as the metallic implant into the medullary canal (Hamblen, 1968; Korkusuz *et al.*, 1993; Solberg *et al.*, 1999; Ersoz *et al.*, 2004). While these are adequate models of developing osteomyelitis, they are not directly comparable to a human subject. A model designed by Lucke *et al.* (Lucke *et al.*, 2003b), though technically more challenging, mimics the human condition more accurately. It requires placement of a Kirschner wire (K-wire) or needle down the length of the medullary canal of the tibia with contamination prior to implanting the metal implant, which has been reproduced by other investigators (Lucke *et al.*, 2005; Bisland *et al.*, 2006; Garcia-Alvarez *et al.*, 2009). Others have modified this technique to include internal fixation of the tibia after performing an osteotomy of the tibial diaphyseal area (Alt *et al.*, 2011).

Several rat models designed to recreate femoral implant associated chronic osteomyelitis. Skott *et al.* (Skott *et al.*, 2006) originally described a medial parapatellar approach, which allowed reaming of the distal femur generating easy access to the canal for intramedullary fracture fixation. This has been modified to implant a bacterial inoculum and steel pin with the hole subsequently sealed by bone wax or cement to develop intramedullary osteomyelitis (Tuzuner *et al.*, 2006; Ozturan *et al.*, 2010; Robinson *et al.*, 2011). A novel model of extramedullary fixation was first described by Chen *et al*. They used a 6-hole polyacetyl plate with threaded K-wires to secure the femur after creation of a 6 mm defect (Chen *et al.*, 2005). A disadvantage to this model is the need to expose the entire femur laterally for application of the hardware.

Open fracture osteomyelitis

The majority of open rat models involved the femur, in which a comminuted midshaft femur fracture was created by dropping a weighted blade onto the hind leg. Lateral exposure of the fracture ensued and the fixation took place by intramedullary K-wire placement. The fracture site is then contaminated and left open to air for 1 hour to mimic environmental exposure of the trauma patient prior to treatment (Li *et al.*, 2009; Li *et al.*, 2010a; Lindsey *et al.*, 2010a). Stewart *et al.* used this same technique to generate the femur fracture, though they removed small pieces of bone to create a 5.0 mm gap and implemented a polypropylene fumarate scaffold, to serve as a carrier for their treatment (Stewart *et al.*, 2010). Similarly, an open femur fracture model with polymethylene plate and threaded K-wires has been performed (Penn-Barwell *et al.*, 2012). A 6.0 mm defect is created after fixation and the bone is contaminated and closed prior to treatment.

Two studies identified described an open tibial fracture model. Both studies used a 1.0 mm burr to create a 10 mm longitudinal trough in the cranial tibial cortex to expose the medullary canal. Using cautery, the endosteal blood supply was subsequently disrupted and bacterial contamination performed (Buxton *et al.*, 2005). No instrumentation was used. This technique was later modified with the addition of sand as the foreign body and *Escherichia coli* and *S. aureus* as bacterial contaminants (McPherson *et al.*, 2008).

Periprosthetic osteomyelitis

Given the small size of the rat, it is difficult to generate a true model for total joint arthroplasty. Antoci *et al.* provide a limited model of arthroplasty-related osteomyelitis (Antoci *et al.*, 2007), which is similar to the chronic femoral model by Skott *et al*. The notable difference is that after bacterial inoculation, a titanium rod is press-fitted into the femoral notch, effectively sealing the canal. Consequently, the titanium implant still has direct exposure to the joint, in contrast to being sealed off by bone wax, effectively simulating the pathogenesis of arthroplasty-related osteomyelitis. It should be stated that models in larger animals, where joint replacement is possible, is preferred as it clinically replicates the infection.

External-fixation osteomyelitis-related model

There are few rat studies specifically addressing the question of external fixation induced osteomyelitis despite being a recognised risk factor for infection. One citation identified independently of the systematic search described a model that inserted three 2.0 mm diameter pins into the third, fourth and fifth tail vertebrae (Holt *et al.*, 2011); however, no direct inoculation took place at the time of implantation. No additional models of external fixation of the rat were identified.

Haematogenous models

Hienz *et al.* established a haematogenous rat model to allow study of the initiating events of osteomyelitis (Hienz *et al.*, 1995). They injected a sclerosing agent (i.e. sodium morrhuate) directly into the mandible and tibia followed by intravenous injection of *S. aureus* into the femoral vein

for haematogenous spread, effectively generating acute osteomyelitis.

Advantages and disadvantages

Rats possess a distinct set of pros and cons. Rat bones are of sufficient size to reproduce fracture patterns, and to perform drilling and fixation, as well as intramedullary nailing. The medullary canal is large enough to implant foreign objects and despite their small size, screw and plate fixation has been well documented (Histing *et al.*, 2011). As an alternative to larger animals, such as the rabbit or sheep, rats are inexpensive, abundant in supply, and are easily housed and maintained for prolonged periods of time during an experiment. Rats require inoculation doses ranging from 10^3-10^6 CFU, with several open fracture models reporting inoculation doses as low as 10^2 CFU.

Mouse models of osteomyelitis

Acute and chronic osteomyelitis

The work by Funao *et al.* (Funao *et al.*, 2012) utilised a femoral implant and monitored the infection with bioluminescence imaging. A midline lower limb arthrotomy was performed to allow introduction of an intramedullary inoculate of *S. aureus* through the distal part of the femur, without contaminating the actual joint space. Bone wax was used to seal the access burr hole and sequential study of the infectious process and establishment of osteomyelitis was observed. Both Sottnik *et al.* (Sottnik *et al.*, 2010) and Yoshii *et al.* (Yoshii *et al.*, 2002a; Yoshii *et al.*, 2002b) utilise a transcortical hole in the proximal part of the tibia with implanted *S. aureus* seeded suture material as the source of the osteomyelitis. Varoga *et al.* (Varoga *et al.*, 2008; Varoga *et al.*, 2009) modified a rat model initially described by Lucke *et al.* (Lucke *et al.*, 2003b), in which an access hole in the proximal part of the tibia allowed access to the medullary canal for the introduction of an inoculum. Mariott *et al.* (Marriott *et al.*, 2004; Marriott *et al.*, 2005) accessed the femur with a burr for inoculation with *S. aureus*, based on original work by Spagnolo *et al.* (Spagnolo *et al.*, 1993). In 2006, Matsuno and colleagues (Matsuno *et al.*, 2006) described a mouse model in which the femur was surgically exposed and fractured with surgical scissors, followed by placement of *S. aureus* and an intramedullary Kirschner wire. They do not specify whether the fracture site is closed at the superficial level or left open, both methods would be suitable depending on the desired clinical scenario being modelled.

Li *et al.* described a reproducible murine model with the use of bioluminescent *S. aureus* (Xen29) for an implant-associated osteomyelitis in which a stainless steel pin is coated with *S. aureus* and implanted transcortically, medial to lateral, through the tibial metaphysis (Li *et al.*, 2008; Li *et al.*, 2010b). This has the advantage of not involving the knee joint. This led to a highly reproducible localised abscess in greater than 90 % of the mice, without any detectable haematogenous spread, sepsis or mortality. The method was subsequently reproduced by Shandley *et al.* (Shandley *et al.*, 2012). Johansson *et al.* (Johansson *et al.*, 2001) similarly inserted 0.4 mm cerclage wire through a 0.6 mm drill hole in the medial tibial metaphysis, before an inoculation suspension was injected close to the metaphyseal drill hole.

Periprosthetic models

Bernthal *et al.* (Bernthal *et al.*, 2010) used a model of periprosthetic infection established through a medial parapatellar approach and then introduced a Kirschner wire into the murine femur with 1.0 mm left protruding into the knee joint before inoculation with *S. aureus*. This model is also similar to an arthroplasty infection and could be used to assess synovial response, but fails to adequately recreate the environment of a total joint arthroplasty. Other authors have published long term (42 days) evaluation with this model (Pribaz *et al.*, 2012).

Advantages and disadvantages

In recent years, mice have served as the animal of choice to establish models utilising bioluminescent imaging. Given the superior knowledge into the function and regulation of their immune system, the choice of utilising a mouse model is not surprising (Patel *et al.*, 2009). Supplement this advantage with their small size, ease of handling, and overall lower cost, and it becomes evident why mouse models are quickly becoming a frequently used model for the study of osteomyelitis. While the size of mice offers many advantages, it is noteworthy that the smaller size makes two-stage revisions and multiple procedures in a single mouse more challenging.

Nonetheless, with the advent of bioluminescent imaging, the advantages of mouse models abound. As imaging photon emissions through thicker tissues skews the ability of researchers to interpret the metabolic activity of bacteria, small animals, such as mice and rats, prove valuable. As bioluminescent imaging permits real-time assessment of the degree of infection in a single animal longitudinally without sacrificing the animal, the sheer number of animals needed in an experiment is reduced; and more importantly, researchers are able to examine the response of bacteria to an intervention in a single animal over time. Researchers need not compare the degree of infection in mouse 'a' at time 't' to the degree of infection in mouse 'b' at time 't + 1.' Consequently, animals can serve as their own control and experimental variability is greatly reduced. No models of external fixation for the mouse were identified in this review.

Other animal models of osteomyelitis

Internal fixation models: acute and chronic osteomyelitis

In 1983, Fitzgerald developed a dog model of chronic osteomyelitis (Fitzgerald, 1983). The authors removed a 1.0 cm^2 cortical window, injected *S. aureus* and filled the defect with intramedullary polymethylmethacrylate (PMMA) cement. After twelve weeks, each specimen revealed radiographic, clinical, histological and microbiologic evidence of osteomyelitis. Another canine model uses the lateral aspect of the femur, in which *S. aureus* is directly dripped onto a 2.0 mm cortical screw, which is subsequently placed through cortex with the end of the screw resting in the intramedullary cavity (Huneault *et al.*, 2004).

The Schaer lab has established an internal fixation model of the proximal part of the tibia of a sheep. They exposed the tibia using a standard medial incision. Next, they pre-drilled screw holes, they then used an oscillating saw to create the fracture. The internal fixation was inserted into the previous drilled holes after being inoculated with *S. aureus* (Stewart *et al.*, 2012).

Experimental models in guinea pigs have been described. One group of authors described an experimental model to reproduce internal fixation devices and study bacterial colonisation in guinea pigs. Pure titanium mini-plates and screws (Synthes) were implanted into the proximal aspects of the iliac wings. Identical plates and screws were implanted subcutaneously as controls (Fischer *et al.*, 1996).

Open fracture models

Williams *et al.* recently published a model simulating type IIIB Gustilo open fracture using the proximal part of the tibia of sheep (Williams *et al.*, 2012b). They used two 20 x 20 mm metal plates separated by 10 mm that could accommodate a membrane that was coated with a MRSA biofilm. Khodaparast *et al.* incised the skin over the proximal part of canine tibiae and created a fracture. The fracture was stabilised with a locked intramedullary nail and *S. aureus* was inoculated into the medullary canal proximal and distal to the fracture (Khodaparast *et al.*, 2003).

Another study of open fractures in sheep utilised a fracture created in the hind limb using a chevron osteotomy. A patch of bovine type I collagen was used to locally deliver a standard inoculum of *S. aureus* to the fracture site. An 8.0 mm IM nail was placed into the tibia (Hill *et al.*, 2002). This model was unique compared to other models used to study IM nailing in open fractures, in that it placed contamination at the osteotomy site prior to IM nailing. Consequently, the pathogen is disseminated with reaming and insertion of the nail distal to the fracture site. This appropriately mimics the clinical complications when placing an IM nail after an open fracture.

In a goat model of open fractures, a unicortical circular defect was created using a coring reamer distal and medial to the tibial tubercle (Wenke *et al.*, 2006). This defect was inoculated with *S. aureus*. After three weeks, animal groups treated with antibiotics demonstrated no evidence of infection, whereas, animals that were not treated developed necrosis and abscess formation at the site of the cortical defect.

Periprosthetic models

In their canine total joint arthroplasty model, Petty *et al.* reamed the femoral canals of dogs and simultaneously introduced PMMA cement as well as *S. aureus*, *S. epidermidis*, or *E. coli* resulting in infection by all three organisms at a later time point (Petty *et al.*, 1988). The authors cautioned against the use of their model for the more common setting of chronic infections of joint prostheses, as the femoral medullary canals in their experiment were acutely infected. The transition from an acute to chronic infection is typically based on development of radiographic changes including periosteal reaction, new bone formation, sequestered bone, and histologic changes of acute and chronic inflammation. All models of chronic infection begin as acute infections that progress with time.

External fixation models

Clasper *et al.* examined sheep models of osteomyelitis associated with external fixators in tibiae. Three bicortical pins were placed and *S. aureus* was applied to the wound around the pin (Clasper *et al.*, 2001a). After only 1 hour, one group of sheep that had a stagnant fluid reservoir at the pin site displayed evidence of extension of the infection. Another ovine model addressed secondary intramedullary (IM) nailing after pin tract infections from external fixators (Clasper *et al.*, 2001b). Two external fixator pins were placed into the tibia and contaminated with *S. aureus*. After fourteen days, the pins were removed and an 8.0 mm modified IM nail was placed. Without treatment, animals developed effusions of the stifle joints (joint of hind limb equivalent to the human knee) on which they were reluctant to bear weight; they were euthanised at a mean of 10.5 days with sepsis of the stifle joint, soft tissues, and entire tibia.

In order to study external fixation-related infections in goats, Voos *et al.* placed three 4.0 mm half-pins through one cortex of an iliac crest (Voos *et al.*, 1999). Sixteen days after contamination with *S. aureus*, infection was apparent in 100 % of untreated controls. Another group put two 3.0/4.0 mm external fixator pins into the anteromedial surface of the tibia and inoculated the pins with *S. aureus* (DeJong *et al.*, 2001). After 14 days, untreated controls showed clinical signs of infection in 96 % of pins and were culture positive in 100 %.

Haematogenous models

Emslie and Nade describe a model of acute haematogenous osteomyelitis in chickens caused by intravenous injection of *S. aureus* into the wing veins of one-month-old chickens (Emslie *et al.*, 1983). After 24 h, 96 % of animals had evidence of osteomyelitis. This highly reproducible model is unique and closely mimics human disease of acute haematogenous osteomyelitis seen in paediatric populations and permits researchers to study the natural progression of osteomyelitis seen in children. Unlike the other animals presented in this section, chickens also have the advantage of being economical to maintain and the materials required for this model are inexpensive.

Johansen *et al.* investigate the potential of human and porcine *S. aureus* isolates to induce acute haematogenous osteomyelitis upon inoculation in the femoral artery (Johansen *et al.*, 2012). As supported by computed tomography, microscopic evaluation and peptide nucleic acid fluorescence *in situ* hybridisation, the onset of haematogenous osteomyelitis and biofilm formation was dependent on bacterial strain.

Advantages and disadvantages

A variety of other animals are utilised in the study of osteomyelitis; albeit less frequently than rabbits, rats, and mice. These animals (i.e. dogs, sheep, goats) tend to be larger and are consequently more expensive and difficult to maintain. In general, these animals have larger bones and joints, which enable the use of hardware that better

approximates the prosthetics used in humans. In fact, such animal models may employ commercially available implants, enabling an additional level of reproducibility among research groups. Moreover, these larger animals are able to tolerate multiple procedures and undergo more blood draws. This size results in increased purchase price and maintenance costs, a notable concern when studying chronic conditions such as osteomyelitis. For this reason, there are relatively few osteomyelitis studies performed in these other animals when compared to rabbits, rats and mice. Inoculation doses for these studies is as follows: 10^5-10^8 CFU in canine models, 10^6-10^9 CFU in ovine models, 10^8-10^{10} CFU in porcine models, 10^3-10^5 CFU in goat models, and 10^6 CFU in a guinea pig model.

Non-bone infection models and their impact on clinical practice

One non-bone infection models has had a profound effect on clinical practice in treatment of bony infection. This is the tissue cage model. The tissue cage model is a simple one in which a plastic tissue cage is placed in the subcutaneous tissue of the animal and infected with bacteria. John *et al*. (John *et al.*, 2009) described the efficacy of daptomycin in implant-associated infection due to methicillin-resistant *Staphylococcus aureus*: importance of combination with rifampin. Other important findings have also been made with the tissue cage model but this is beyond the scope of this manuscript.

Conclusion

Osteomyelitis caused by *Staphylococcus aureus* remains a prevalent complication in clinical orthopaedics. A comprehensive review of available animal models to mimic human osteomyelitis has been presented in this paper and their implementation has generated several novel techniques. To trial the effectiveness of novel treatment adjuvants for osteomyelitis, an appropriate understanding of the current models is required to ensure that clinical questions of interest are adequately addressed. Experimental animal studies need to be reproducible and meticulously detailed to enable future groups to build upon established results. Such details include animal species, bacterial strain, amount and method of inoculation, and the methods of evaluation: radiologic, microscopic, macroscopic, and histologic assessment. One need, identified by the authors, is a unified definition and a gold standard for diagnosis of osteomyelitis for animal models. Traditional approaches to quantify bacterial load by means of CFU and PCR assays, which can only be used in cross-sectional studies, have recently been replaced with *in vivo* bioluminescence (BLI) and radiological outcomes that have their own challenges as indirect measures of bacterial load. Thus, establishment of a quantitative standard is considered to be the next major advance in this field.

While there may be no ideal animal model, increasingly effective modes of inoculation and refined methods of assessing infection continue to aid in the understanding and management of osteomyelitis.

The authors recognise the need for some new clinically relevant models to mimic prosthetic joint infection with two stage exchange. A large animal model for two stage exchange of infected internal fixation hardware would be another useful adjunct in the study of *Staphylococcus* osteomyelitis. Another model that would be useful would be an animal model for culture negative infection mediated by biofilm. This is a serious clinical problem and is very challenging to study without a validated animal model. Finally, development of genetically modified animals may permit future research on the host immune response to *Staphylococcus aureus*, which has developed numerous methods of tricking the host immune response. For assessment, the authors anticipate increased utilisation of newer biomarkers including host antibody response, PCR, fluorescent *in situ* hybridisation (FISH) and Ibis Assay (Ibis, Carlsbad, CA, USA).

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References

Adams CS, Antoci V Jr, Harrison G, Patal P, Freeman TA, Shapiro IM., Parvizi J, Hickok NJ, Radin S, Ducheyne P (2009) Controlled release of vancomycin from thin sol-gel films on implant surfaces successfully controls osteomyelitis. J Orthop Res **27**: 701-709.

Alt V, Bitschnau A, Osterling J, Sewing A, Meyer C, Kraus R, Meissner SA, Wenisch S, Domann E, Schnettler R (2006) The effects of combined gentamicin-hydroxyapatite coating for cementless joint prostheses on the reduction of infection rates in a rabbit infection prophylaxis model. Biomaterials **27**: 4627-4634.

Alt V, Lips KS, Henkenbehrens C, Muhrer D, Oliveira Cavalcanti MC, Sommer U, Thormann U, Szalay G, Heiss C, Pavlidis T, Domann E, Schnettler R (2011) A new animal model for implant-related infected non-unions after intramedullary fixation of the tibia in rats with fluorescent *in situ* hybridization of bacteria in bone infection. Bone **48**: 1146-1153.

Alvarez H, Castro C, Moujir L, Perera A, Delgado A, Soriano I, Evora C, Sanchez E (2008) Efficacy of ciprofloxacin implants in treating experimental osteomyelitis. J Biomed Mater Res B Appl Biomater **85**: 93-104.

An YH, Friedman RJ (1998) Animal models of orthopedic implant infection. J Invest Surg **11**: 139-146.

Andriole VT, Nagel DA, Southwick WO (1973) A paradigm for human chronic osteomyelitis. J Bone Joint Surg Am **55**: 1511-1515.

Andriole VT, Nagel DA, Southwick WO (1974) Chronic staphylococcal osteomyelitis: an experimental model. Yale J Biol Med **47**: 33-39.

Antoci V, Jr., Adams CS, Hickok NJ, Shapiro IM, Parvizi J (2007) Vancomycin bound to Ti rods reduces periprosthetic infection: preliminary study. Clin Orthop Relat Res **461**: 88-95.

Arens S, Schlegel U, Printzen G, Ziegler WJ, Perren SM, Hansis M (1996) Influence of materials for fixation implants on local infection. An experimental study of steel *versus* titanium DCP in rabbits. J Bone Joint Surg Br **78**: 647-651.

Arens S, Kraft C, Schlegel U, Printzen G, Perren SM, Hansis M (1999) Susceptibility to local infection in biological internal fixation. Experimental study of open *vs*. minimally invasive plate osteosynthesis in rabbits. Arch Orthop Trauma Surg **119**: 82-85.

Ashhurst DE, Hogg J, Perren SM (1982) A method for making reproducible experimental fractures of the rabbit tibia. Injury **14**: 236-242.

Belmatoug N, Cremieux AC, Bleton R, Volk A, Saleh-Mghir A, Grossin M, Garry L, Carbon C (1996) A new model of experimental prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus*: a microbiologic, histopathologic, and magnetic resonance imaging characterization. J Infect Dis **174**: 414-417.

Bernthal NM, Stavrakis AI, Billi F, Cho JS, Kremen TJ, Simon SI, Cheung AL, Finerman GA, Lieberman JR, Adams JS, Miller LS (2010) A mouse model of post-arthroplasty *Staphylococcus aureus* joint infection to evaluate *in vivo* the efficacy of antimicrobial implant coatings. Plos One **5**: e12580.

Bisland SK, Chien C, Wilson BC, Burch S (2006) Pre-clinical *in vitro* and *in vivo* studies to examine the potential use of photodynamic therapy in the treatment of osteomyelitis. Photochem Photobiol Sci **5**: 31-38.

Burch S, Bisland SK, Bogaards A, Yee AJ, Whyne CM, Finkelstein JA, Wilson BC (2005) Photodynamic therapy for the treatment of vertebral metastases in a rat model of human breast carcinoma. J Orthop Res **23**: 995-1003.

Buxton TB, Travis MT, O'Shea KJ, McPherson JC 3rd, Harvey SB, Plowman KM, Walsh DS (2005) Low-dose infectivity of *Staphylococcus aureus* (SMH strain) in traumatized rat tibiae provides a model for studying early events in contaminated bone injuries. Comp Med **55**: 123-128.

Cardo D, Horan T, Andrus M, Dembinski M, Edwards J, Peavy G, Tolson J, Wagner D, Syst N (2004) National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control **32**: 470-485.

Castro C, Sanchez E, Delgado A, Soriano I, Nunez P, Baro M, Perera A, Evora C (2003) Ciprofloxacin implants for bone infection. *In vitro-in vivo* characterization. J Control Release **93**: 341-354.

Chai H, Guo L, Wang X, Fu Y, Guan J, Tan L, Ren L, Yang K (2011) Antibacterial effect of 317L stainless steel contained copper in prevention of implant-related infection *in vitro* and *in vivo*. J Mater Sci Mater Med **22**: 2525-2535.

Chen X, Tsukayama DT, Kidder LS, Bourgeault CA, Schmidt AH, Lew WD (2005) Characterization of a chronic infection in an internally-stabilized segmental defect in the rat femur. J Orthop Res **23**: 816-823.

Chen X, Schmidt AH, Tsukayama DT, Bourgeault CA, Lew WD (2006) Recombinant human osteogenic protein-1 induces bone formation in a chronically infected, internally stabilized segmental defect in the rat femur. J Bone Joint Surg Am **88**: 1510-1523.

Chen X, Schmidt AH, Mahjouri S, Polly DW Jr, Lew WD (2007) Union of a chronically infected internally stabilized segmental defect in the rat femur after debridement and application of rhBMP-2 and systemic antibiotic. J Orthop Trauma **21**: 693-700.

Chou TG, Petti CA, Szakacs J, Bloebaum RD (2010) Evaluating antimicrobials and implant materials for infection prevention around transcutaneous osseointegrated implants in a rabbit model. J Biomed Mater Res A **92**: 942-952.

Clasper JC, Cannon LB, Stapley SA, Taylor VM, Watkins PE (2001a) Fluid accumulation and the rapid spread of bacteria in the pathogenesis of external fixator pin track infection. Injury **32**: 377-381.

Clasper JC, Stapley SA, Bowley DM, Kenward CE, Taylor V, Watkins PE (2001b) Spread of infection, in an animal model, after intramedullary nailing of an infected external fixator pin track. J Orthop Res **19**: 155-159.

Craig MR, Poelstra KA, Sherrell JC, Kwon MS, Belzile EL, Brown TE (2005) A novel total knee arthroplasty infection model in rabbits. J Orthop Res **23**: 1100-1104.

Cram P, Lu X, Kates SL, Singh JA, Li Y, Wolf BR (2012) Total knee arthroplasty volume, utilization, and outcomes among Medicare beneficiaries, 1991-2010. JAMA **308**: 1227-1236.

Curtis MJ, Brown PR, Dick JD, Jinnah RH (1995) Contaminated fractures of the tibia: a comparison of treatment modalities in an animal model. J Orthop Res **13**: 286-295.

Darouiche RO (2004) Treatment of infections associated with surgical implants. N Engl J Med **350**: 1422-1429.

Darouiche RO, Landon GC, Patti JM, Nguyen LL, Fernau RC, McDevitt D, Greene C, Foster T, Klima M (1997) Role of *Staphylococcus aureus* surface adhesins in orthopaedic device infections: are results model-dependent? J Med Microbiol **46**: 75-79.

Darouiche RO, Farmer J, Chaput C, Mansouri M, Saleh G, Landon GC (1998) Anti-infective efficacy of antiseptic-coated intramedullary nails. J Bone Joint Surg Am **80**: 1336-1340.

Darouiche RO, Mansouri MD, Zakarevicz D, Alsharif A, Landon GC (2007) *In vivo* efficacy of antimicrobial-coated devices. J Bone Joint Surg Am **89**: 792-797.

DeJong ES, DeBerardino TM, Brooks DE, Nelson BJ, Campbell AA, Bottoni CR, Pusateri AE, Walton RS, Guymon CH, McManus AT (2001) Antimicrobial efficacy of external fixator pins coated with a lipid stabilized hydroxyapatite/chlorhexidine complex to prevent pin tract infection in a goat model. J Trauma **50**: 1008-1014.

Deysine M, Rosario E, Isenberg HD (1976) Acute hematogenous osteomyelitis: an experimental model. Surgery **79**: 97-99.

Deysine M, Isenberg HD, Steiner G (1983) Chronic haematogenous osteomyelitis; studies on an experimental model. Int Orthop **7**: 69-78.

Eerenberg JP, Patka P, Haarman HJ, Dwars BJ (1994) A new model for posttraumatic osteomyelitis in rabbits. J Invest Surg **7**: 453-465.

El-Kamel AH, Baddour MM (2007) Gatifloxacin biodegradable implant for treatment of experimental osteomyelitis: *in vitro* and *in vivo* evaluation. Drug Deliv **14**: 349-356.

Emslie KR, Ozanne NR, Nade SM (1983) Acute haematogenous osteomyelitis: an experimental model. J Pathol **141**: 157-167.

Ersoz G, Oztuna V, Coskun B, Eskandari MM, Bayarslan C, Kaya A (2004) Addition of fusidic acid impregnated bone cement to systemic teicoplanin therapy in the treatment of rat osteomyelitis. J Chemother **16**: 51-55.

Fischer B, Vaudaux P, Magnin M, el Mestikawy Y, Proctor RA, Lew DP, Vasey H (1996) Novel animal model for studying the molecular mechanisms of bacterial adhesion to bone-implanted metallic devices: role of fibronectin in *Staphylococcus aureus* adhesion. J Orthop Res **14**: 914-920.

Fitzgerald RH Jr (1983) 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 **65**: 371-380.

Funao H, Ishii K, Nagai S, Sasaki A, Hoshikawa T, Aizawa M, Okada Y, Chiba K, Koyasu S, Toyama Y, Matsumoto M (2012) Establishment of a real-time, quantitative, and reproducible mouse model of *Staphylococcus* osteomyelitis using bioluminescence imaging. Infect Immun **80**: 733-741.

Gaines RJ, DeMaio M, Peters D, Hasty J, Blanks J (2012) Management of contaminated open fractures: a comparison of two types of irrigation in a porcine model. J Trauma Acute Care Surg **72**: 733-736.

Garcia-Alvarez F, Navarro-Zorraquino M, Castro A, Grasa JM, Pastor C, Monzon M, Martinez A, Garcia-Alvarez I, Castillo J, Lozano R (2009) Effect of age on cytokine response in an experimental model of osteomyelitis. Biogerontology **10**: 649-658.

Giavaresi G, Borsari V, Fini M, Giardino R, Sambri V, Gaibani P, Soffiatti R (2008) Preliminary investigations on a new gentamicin and vancomycin-coated PMMA nail for the treatment of bone and intramedullary infections: An experimental study in the rabbit. J Orthop Res **26**: 785-792.

Grewe SR, Stephens BO, Perlino C, Riggins RS (1987) Influence of internal fixation on wound infections. J Trauma **27**: 1051-1054.

Gustilo RB, Mendoza RM, Williams DN (1984) Problems in the management of type-III (severe) open fractures – a new classification of type-III open fractures. J Trauma **24**: 742-746.

Hamblen DL (1968) Hyperbaric oxygenation. Its effect on experimental staphylococcal osteomyelitis in rats. J Bone Joint Surg Am **50**: 1129-1141.

Hienz SA, Sakamoto H, Flock JI, Morner AC, Reinholt FP, Heimdahl A, Nord CE (1995) Development and characterization of a new model of hematogenous osteomyelitis in the rat. J Infect Dis **171**: 1230-1236.

Hill PF, Clasper JC, Parker SJ, Watkins PE (2002) Early intramedullary nailing in an animal model of a heavily contaminated fracture of the tibia. J Orthop Res **20**: 648-653.

Holt J, Hertzberg B, Weinhold P, Storm W, Schoenfisch M, Dahners L (2011) Decreasing bacterial colonization of external fixation pins through nitric oxide release coatings. J Orthop Trauma **25**: 432-437.

Huneault LM, Lussier B, Dubreuil P, Chouinard L, Desevaux C (2004) Prevention and treatment of experimental osteomyelitis in dogs with ciprofloxacin-loaded crosslinked high amylose starch implants. J Orthop Res **22**: 1351-1357.

Ismael F, Bleton R, Saleh-Mghir A, Dautrey S, Massias L, Cremieux AC (2003) Teicoplanin-containing cement spacers for treatment of experimental *Staphylococcus aureus* joint prosthesis infection. Antimicrob Agents Chemother **47**: 3365-3367.

Johansen LK, Koch J, Frees D, Aalbaek B, Nielsen OL, Leifsson PS, Iburg TM, Svalastoga E, Buelund LE, Bjarnsholt T, Hoiby N, Jensen HE (2012) Pathology and biofilm formation in a porcine model of staphylococcal osteomyelitis. J Comp Pathol **147**: 343-353.

Johansson A, Lindgren JU, Nord CE, Svensson O (1999) Material and design in haematogenous implant-associated infections in a rabbit model. Injury **30**: 651-657.

Johansson A, Flock JI, Svensson O (2001) Collagen and fibronectin binding in experimental staphylococcal osteomyelitis. Clin Orthop Relat Res: 241-246.

John AK, Baldoni D, Haschke M, Rentsch K, Schaerli P, Zimmerli W, Trampuz A (2009) Efficacy of daptomycin in implant-associated infection due to methicillin-resistant *Staphylococcus aureus*: importance of combination with rifampin. Antimicrob Agents Chemother **53**: 2719-2724.

Kadurugamuwa JL, Sin L, Albert E, Yu J, Francis K, DeBoer M, Rubin M, Bellinger-Kawahara C, Parr TR Jr, , Contag PR (2003) Direct continuous method for monitoring biofilm infection in a mouse model. Infect Immun **71**: 882-890.

Khodaparast O, Coberly DM, Mathey J, Rohrich RJ, Levin LS, Brown SA (2003) Effect of a transpositional muscle flap on VEGF mRNA expression in a canine fracture model. Plast Reconstr Surg **112**: 171-176.

Korkusuz F, Uchida A, Shinto Y, Araki N, Inoue K, Ono K (1993) Experimental implant-related osteomyelitis treated by antibiotic-calcium hydroxyapatite ceramic composites. J Bone Joint Surg Br **75**: 111-114.

Koschmieder R, Ritzerfield W, Homeyer L (1975) [Addition of gentamicine to polymethyl methacrylate for therapy of infectious bone diseases. Experimental *in vivo* tests]. Z Orthop Ihre Grenzgeb **113**: 147-149.

Kraft CN, Schlegel U, Pfluger D, Eijer H, Textor J, Hansis M, Arens S (2001) Radiological signs of osteitis around extramedullary metal implants. A radiographic-microbiological correlative analysis in rabbit tibiae after local inoculation of *Staphylococcus aureus*. Arch Orthop Trauma Surg **121**: 338-342.

Kurtz S, Ong K, Lau E, Mowat F, Halpern M (2007) Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am **89**: 780-785.

Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J (2008) Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty **23**: 984-991.

Lazzarini L, Overgaard KA, Conti E, Shirtliff ME (2006) Experimental osteomyelitis: what have we learned from animal studies about the systemic treatment of osteomyelitis? J Chemother **18**: 451-460.

Lee BY, Wiringa AE, Bailey RR, Lewis GJ, Feura J, Muder RR (2010) *Staphylococcus aureus* vaccine for orthopedic patients: an economic model and analysis. Vaccine **28**: 2465-2471.

Li D, Gromov K, Søballe K, Puzas JE, O'Keefe RJ, Awad H, Drissi H, Schwarz EM (2008) Quantitative mouse model of implant-associated osteomyelitis and the kinetics of microbial growth, osteolysis, and humoral immunity. J Orthop Res **26**: 96-105.

Li B, Jiang B, Boyce BM, Lindsey BA (2009) Multilayer polypeptide nanoscale coatings incorporating IL-12 for the prevention of biomedical device-associated infections. Biomaterials **30**: 2552-2558.

Li B, Jiang B, Dietz MJ, Smith ES, Clovis NB, Rao KM (2010a) Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. J Orthop Res **28**: 48-54.

Li D, Gromov K, Proulx ST, Xie C, Li J, Crane DP, Søballe K, O'Keefe RJ, Awad HA, Xing L, Schwarz EM (2010b) Effects of antiresorptive agents on osteomyelitis: novel insights into the pathogenesis of osteonecrosis of the jaw. Ann N Y Acad Sci **1192**: 84-94.

Lindsey BA, Clovis NB, Smith ES, Salihu S, Hubbard DF (2010a) An animal model for open femur fracture and osteomyelitis – Part II: Immunomodulation with systemic IL-12. J Orthop Res **28**: 43-47.

Lindsey BA, Clovis NB, Smith ES, Salihu S, Hubbard DF (2010b) An animal model for open femur fracture and osteomyelitis: Part I. J Orthop Res **28**: 38-42.

Loomba PS, Taneja J, Mishra B (2010) Methicillin and vancomycin resistant S. aureus in hospitalized patients. J Glob Infect Dis **2**: 275-283.

Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Haas NP, Raschke M (2003a) Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. Bone **32**: 521-531.

Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Haas NP, Raschke M (2003b) A new model of implant-related osteomyelitis in rats. J Biomed Mater Res B Appl Biomater **67**: 593-602.

Lucke M, Wildemann B, Sadoni S, Surke C, Schiller R, Stemberger A, Raschke M, Haas NP, Schmidmaier G (2005) Systemic *versus* local application of gentamicin in prophylaxis of implant-related osteomyelitis in a rat model. Bone **36**: 770-778.

Marberry KM, Kazmier P, Simpson WA, Christensen GD, Phaup JG, Hendricks KJ, Anglen JO, Gainor BJ (2002) Surfactant wound irrigation for the treatment of staphylococcal clinical isolates. Clin Orthop Relat Res **403**: 73-79.

Marriott I, Gray DL, Tranguch SL, Fowler VG, Jr., , Stryjewski M, Scott Levin L, Hudson MC, Bost KL (2004) Osteoblasts express the inflammatory cytokine interleukin-6 in a murine model of *Staphylococcus aureus* osteomyelitis and infected human bone tissue. Am J Pathol **164**: 1399-1406.

Marriott I, Gray DL, Rati DM, Fowler VG, Jr., Stryjewski ME, Levin LS, Hudson MC, Bost KL (2005) Osteoblasts produce monocyte chemoattractant protein-1 in a murine model of *Staphylococcus aureus* osteomyelitis and infected human bone tissue. Bone **37**: 504-512.

Matsuno H, Yudoh K, Hashimoto M, Himeda Y, Miyoshi T, Yoshida K, Kano S (2006) A new antibacterial carrier of hyaluronic acid gel. J Orthop Sci **11**: 497-504.

McPherson JC 3rd, Runner RR, Shapiro B, Walsh DS, Stephens-DeValle J, Buxton TB (2008) An acute osteomyelitis model in traumatized rat tibiae involving sand as a foreign body, thermal injury, and bimicrobial contamination. Comp Med **58**: 369-374.

Mendel V, Reichert B, Simanowski HJ, Scholz HC (1999) Therapy with hyperbaric oxygen and cefazolin for experimental osteomyelitis due to *Staphylococcus aureus* in rats. Undersea Hyperb Med **26**: 169-174.

Merritt K, Dowd JD (1987) Role of internal fixation in infection of open fractures: studies with *Staphylococcus aureus* and *Proteus mirabilis*. J Orthop Res **5**: 23-28.

Monzon M, Garcia-Alvarez F, Lacleriga A, Amorena B (2002) Evaluation of four experimental osteomyelitis infection models by using precolonized implants and bacterial suspensions. Acta Orthop Scand **73**: 11-19.

Moriarty TF, Debefve L, Boure L, Campoccia D, Schlegel U, Richards RG (2009) Influence of material and microtopography on the development of local infection *in vivo*: experimental investigation in rabbits. Int J Artif Organs **32**: 663-670.

Moriarty TF, Campoccia D, Nees SK, Boure LP, Richards RG (2010) *In vivo* evaluation of the effect of intramedullary nail microtopography on the development of local infection in rabbits. Int J Artif Organs **33**: 667-675.

Morrissy RT, Haynes DW (1989) Acute hematogenous osteomyelitis: a model with trauma as an etiology. J Pediatr Orthop **9**: 447-456.

Muller-Serieys C, Saleh Mghir A, Massias L, Fantin B (2009) Bactericidal activity of the combination of levofloxacin with rifampin in experimental prosthetic knee infection in rabbits due to methicillin-susceptible *Staphylococcus aureus*. Antimicrob Agents Chemother **53**: 2145-2148.

Nijhof MW, Dhert WJ, Fleer A, Vogely HC, Verbout AJ (2000a) Prophylaxis of implant-related staphylococcal infections using tobramycin-containing bone cement. J Biomed Mater Res **52**: 754-761.

Nijhof MW, Stallmann HP, Vogely HC, Fleer A, Schouls LM, Dhert WJ, Verbout AJ (2000b) Prevention of infection with tobramycin-containing bone cement or systemic cefazolin in an animal model. J Biomed Mater Res **52**: 709-715.

Nijhof MW, Fleer A, Hardus K, Vogely HC, Schouls LM, Verbout AJ, Dhert WJ (2001) Tobramycin-containing bone cement and systemic cefazolin in a one-stage revision. Treatment of infection in a rabbit model. J Biomed Mater Res **58**: 747-753.

Niska JA, Shahbazian JH, Ramos RI, Pribaz JR, Billi F, Francis KP, Miller LS (2012) Daptomycin and tigecycline have broader effective dose ranges than vancomycin as

prophylaxis against a *Staphylococcus aureus* surgical implant infection in mice. Antimicrob Agents Chemother **56**: 2590-2597.

Nixon M, Jackson B, Varghese P, Jenkins D, Taylor G (2006) Methicillin-resistant *Staphylococcus aureus* on orthopaedic wards: incidence, spread, mortality, cost and control. J Bone Joint Surg Br **88**: 812-817.

Norden CW (1970) Experimental osteomyelitis. I. A description of the model. J Infect Dis **122**: 410-418.

Norden CW (1988) Lessons learned from animal models of osteomyelitis. Rev Infect Dis **10**: 103-110.

Orhan Z, Cevher E, Yildiz A, Ahiskali R, Sensoy D, Mulazimoglu L (2010) Biodegradable microspherical implants containing teicoplanin for the treatment of methicillin-resistant *Staphylococcus aureus* osteomyelitis. Arch Orthop Trauma Surg **130**: 135-142.

Ozturan KE, Yucel I, Kocoglu E, Cakici H, Guven M (2010) Efficacy of moxifloxacin compared to teicoplanin in the treatment of implant-related chronic osteomyelitis in rats. J Orthop Res **28**: 1368-1372.

Parvizi J, Azzam K, Ghanem E, Austin MS, Rothman RH (2009) Periprosthetic infection due to resistant staphylococci: serious problems on the horizon. Clin Orthop Relat Res **467**: 1732-1739.

Patel M, Rojavin Y, Jamali AA, Wasielewski SJ, Salgado CJ (2009) Animal models for the study of osteomyelitis. Semin Plast Surg **23**: 148-154.

Penn-Barwell JG, Murray CK, Wenke JC (2012) Early antibiotics and debridement independently reduce infection in an open fracture model. J Bone Joint Surg Br **94**: 107-112.

Petty W, Spanier S, Shuster JJ, Silverthorne C (1985) The influence of skeletal implants on incidence of infection. Experiments in a canine model. J Bone Joint Surg Am **67**: 1236-1244.

Petty W, Spanier S, Shuster JJ (1988) Prevention of infection after total joint replacement. Experiments with a canine model. J Bone Joint Surg Am **70**: 536-539.

Poultsides LA, Papatheodorou LK, Karachalios TS, Khaldi L, Maniatis A, Petinaki E, Malizos KN (2008) Novel model for studying hematogenous infection in an experimental setting of implant-related infection by a community-acquired methicillin-resistant *S. aureus* strain. J Orthop Res **26**: 1355-1362.

Pribaz JR, Bernthal NM, Billi F, Cho JS, Ramos RI, Guo Y, Cheung AL, Francis KP, Miller LS (2012) Mouse model of chronic post-arthroplasty infection: noninvasive *in vivo* bioluminescence imaging to monitor bacterial burden for long-term study. J Orthop Res **30**: 335-340.

Rebmann T, Aureden K (2011) Preventing methicillin-resistant *Staphylococcus aureus* transmission in hospitals: an Executive Summary of the Association for Professionals in Infection Control and Epidemiology, Inc, Elimination Guide. Am J Infect Control **39**: 595-598.

Rissing JP, Buxton TB, Fisher J, Harris R, Shockley RK (1985a) Arachidonic acid facilitates experimental chronic osteomyelitis in rats. Infect Immun **49**: 141-144.

Rissing JP, Buxton TB, Weinstein RS, Shockley RK (1985b) Model of experimental chronic osteomyelitis in rats. Infect Immun **47**: 581-586.

Robinson DA, Bechtold JE, Carlson CS, Evans RB, Conzemius MG (2011) Development of a fracture osteomyelitis model in the rat femur. J Orthop Res **29**: 131-137.

Sarda L, Saleh-Mghir A, Peker C, Meulemans A, Cremieux AC, Le Guludec D (2002) Evaluation of (99m)Tc-ciprofloxacin scintigraphy in a rabbit model of *Staphylococcus aureus* prosthetic joint infection. J Nucl Med **43**: 239-245.

Schaer TP, Stewart S, Hsu BB, Klibanov AM (2012) Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection. Biomaterials **33**: 1245-1254.

Schmidt AH, Swiontkowski MF (2000) Pathophysiology of infections after internal fixation of fractures. J Am Acad Orthop Surg **8**: 285-291.

Schurman DJ, Johnson BL Jr, Amstutz HC (1975) Knee joint infections with *Staphylococcus aureus* and *Micrococcus* species. J Bone Joint Surg Am **57**: 40-49.

Sener M, Kazimoglu C, Karapinar H, Gunal I, Afsar I, Karatas Sener AG (2010) Comparison of various surgical methods in the treatment of implant-related infection. Int Orthop **34**: 419-423.

Shandley S, Matthews KP, Cox J, Romano D, Abplanalp A, Kalns J (2012) Hyperbaric oxygen therapy in a mouse model of implant-associated osteomyelitis. J Orthop Res **30**: 203-208.

Sheehan E, McKenna J, Mulhall KJ, Marks P, McCormack D (2004) Adhesion of *Staphylococcus* to orthopaedic metals, an *in vivo* study. J Orthop Res **22**: 39-43.

Skott M, Andreassen TT, Ulrich-Vinther M, Chen X, Keyler DE, LeSage MG, Pentel PR, Bechtold JE, Soballe K (2006) Tobacco extract but not nicotine impairs the mechanical strength of fracture healing in rats. J Orthop Res **24**: 1472-1479.

Smeltzer MS, Thomas JR, Hickmon SG, Skinner RA, Nelson CL, Griffith D, Parr TR Jr, Evans RP (1997) Characterization of a rabbit model of staphylococcal osteomyelitis. J Orthop Res **15**: 414-421.

Solberg BD, Gutow AP, Baumgaertner MR (1999) Efficacy of gentamycin-impregnated resorbable hydroxyapatite cement in treating osteomyelitis in a rat model. J Orthop Trauma **13**: 102-106.

Sottnik JL, U'Ren LW, Thamm DH, Withrow SJ, Dow SW (2010) Chronic bacterial osteomyelitis suppression of tumor growth requires innate immune responses. Cancer Immunol Immunother **59**: 367-378.

Southwood LL, Frisbie DD, Kawcak CE, Ghivizzani SC, Evans CH, McIlwraith CW (2004) Evaluation of Ad-BMP-2 for enhancing fracture healing in an infected defect fracture rabbit model. J Orthop Res **22**: 66-72.

Southwood RT, Rice JL, McDonald PJ, Hakendorf PH, Rozenbilds MA (1985) Infection in experimental hip arthroplasties. J Bone Joint Surg Br **67**: 229-231.

Spagnolo N, Greco F, Rossi A, Ciolli L, Teti A, Posteraro P (1993) Chronic staphylococcal osteomyelitis: a new experimental rat model. Infect Immun **61**: 5225-5230.

Stewart RL, Cox JT, Volgas D, Stannard J, Duffy L, Waites KB, Chu TM (2010) The use of a biodegradable,

load-bearing scaffold as a carrier for antibiotics in an infected open fracture model. J Orthop Trauma **24**: 587-591.

Stewart S, Barr S, Engiles J, Hickok NJ, Shapiro IM, Richardson DW, Parvizi J, Schaer TP (2012) Vancomycin-modified implant surface inhibits biofilm formation and supports bone-healing in an infected osteotomy model in sheep: a proof-of-concept study. J Bone Joint Surg Am **94**: 1406-1415.

Stone PW, Larson E, Kawar LN (2002) A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990-2000. Am J Infect Control **30**: 145-152.

Subasi M, Kapukaya A, Kesemenli C, Kaya H, Sari I (2001) Effect of granulocyte-macrophage colony-stimulating factor on treatment of acute osteomyelitis. An experimental investigation in rats. Arch Orthop Trauma Surg **121**: 170-173.

Tuzuner T, Sencan I, Ozdemir D, Alper M, Duman S, Yavuz T, Yildirim M (2006) *In vivo* evaluation of teicoplanin- and calcium sulfate-loaded PMMA bone cement in preventing implant-related osteomyelitis in rats. J Chemother **18**: 628-633.

Varoga D, Tohidnezhad M, Paulsen F, Wruck CJ, Brandenburg L, Mentlein R, Lippross S, Hassenpflug J, Besch L, Muller M, Jurgens C, Seekamp A, „ Schmitt L, Pufe T (2008) The role of human beta-defensin-2 in bone. J Anat **213**: 749-757.

Varoga D, Wruck CJ, Tohidnezhad M, Brandenburg L, Paulsen F, Mentlein R, Seekamp A, Besch L, Pufe, T. (2009) Osteoblasts participate in the innate immunity of the bone by producing human beta defensin-3. Histochem Cell Biol **131**: 207-218.

Voos K, Rosenberg B, Fagrhi M, Seligson D (1999) Use of a tobramycin-impregnated polymethylmethacrylate pin sleeve for the prevention of pin-tract infection in goats. J Orthop Trauma **13**: 98-101.

Wenke JC, Owens BD, Svoboda SJ, Brooks DE (2006) Effectiveness of commercially-available antibiotic-impregnated implants. J Bone Joint Surg Br **88**: 1102-1104.

Whalen JL, Fitzgerald RH, Jr., Morrissy RT (1988) A histological study of acute hematogenous osteomyelitis following physeal injuries in rabbits. J Bone Joint Surg Am **70**: 1383-1392.

Williams DL, Haymond BS, Beck JP, Savage PB, Chaudhary V, Epperson RT, Kawaguchi B, Bloebaum RD (2012a) *In vivo* efficacy of a siliconecationic steroid antimicrobial coating to prevent implant-related infection. Biomaterials **33**: 8641-8656.

Williams DL, Haymond BS, Woodbury KL, Beck JP, Moore DE, Epperson RT, Bloebaum RD (2012b) Experimental model of biofilm implant-related osteomyelitis to test combination biomaterials using biofilms as initial inocula. J Biomed Mater Res A **100**: 1888-1900.

Worlock P, Slack R, Harvey L, Mawhinney R (1988) An experimental model of post-traumatic osteomyelitis in rabbits. Br J Exp Pathol **69**: 235-244.

Worlock P, Slack R, Harvey L, Mawhinney R (1994) The prevention of infection in open fractures: an experimental study of the effect of fracture stability. Injury **25**: 31-38.

Yoshii T, Magara S, Miyai D, Kuroki E, Nishimura H, Furudoi S, Komori T (2002a) Inhibitory effect of roxithromycin on the local levels of bone-resorbing cytokines in an experimental model of murine osteomyelitis. J Antimicrob Chemother **50**: 289-292.

Yoshii T, Magara S, Miyai D, Nishimura H, Kuroki E, Furudoi S, Komori T, Ohbayashi C (2002b) Local levels of interleukin-1beta, -4, -6 and tumor necrosis factor alpha in an experimental model of murine osteomyelitis due to *Staphylococcus aureus*. Cytokine **19**: 59-65.

Zak O, Zak F, Rich R, Tosch W, Kradolfer F, Scheld WM (1982) Experimental staphylococcal osteomyelitis in rats: Therapy with rifampin and claxacillin, alone or in combination. In: Current Chemotherapy and Immunotherapy (Periti P, Gialdroni Grassi G, eds) American Society for Microbiology, pp 973-974.

Zalavras CG, Patzakis MJ, Holtom PD, Sherman R (2005) Management of open fractures. Infect Dis Clin North Am **19**: 915-929.

Editor's Note: All questions/comments by the reviewers were answered by text changes. There is hence no Discussion with Reviewers.