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Innovations in osteomyelitis research: A review of animal models

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Abstract

Infection of bone tissue, or osteomyelitis, has become a growing concern in modern healthcare due in no small part to a rise in antibiotic resistance among bacteria, notably *Staphylococcus aureus*. The current standard of care involves aggressive, prolonged antibiotic therapy combined with surgical debridement of infected tissues. While this treatment may be sufficient for resolving a portion of cases, recurrences of the infection and associated risks including toxicity with long-term antibiotic usage have been reported. Therefore, there exists a need to produce safer, more efficacious options of treatment for osteomyelitis. In order to test treatment regimens, animal models that closely mimic the clinical condition and allow for accurate evaluation of therapeutics are necessary. Establishing a model that replicates features of osteomyelitis in humans continues to be a challenge to scientists, as there are many variables involved, including choosing an appropriate species and method to establish infection. This review addresses the refinement of animal models of osteomyelitis to reflect the clinical disease and test prospective therapeutics. The aim of this review is to explore studies regarding the use of animals for osteomyelitis therapeutics research and encourage further development of such animal models for the translation of results from the animal experiment to human medicine.

KEYWORDS
animal model, bone infection, orthopedic implant, osteomyelitis, *Staphylococcus aureus*

1 | INTRODUCTION

Osteomyelitis is a disease of bone characterized by the presence of an infectious organism that causes inflammation and the destruction of osseous tissue.[1,2] While there are many methods of classifying osteomyelitis, this review focuses on the broader classifications of acute and chronic osteomyelitis. Although no evidence supports an exact time interval dividing them, acute osteomyelitis is generally accepted as a recent infection of several days or weeks that involves purulent debris and local inflammation. With acute osteomyelitis, aggressive and prompt antibiotic therapy can often resolve the infection before it progresses to a chronic state.[2,3] Figure 1 illustrates a clinical example of acute osteomyelitis secondary to *Staphylococcus intermedius* in a dog, following surgical

Kylie M. Roux and Leah H. Cobb have equal contribution.
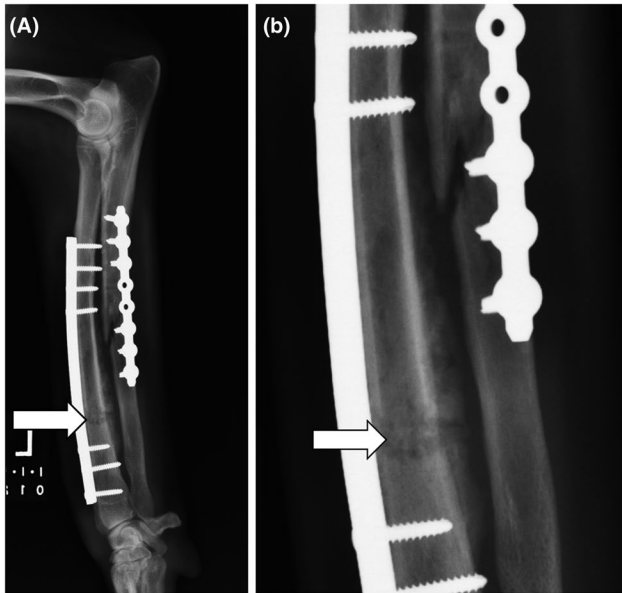
FIGURE 1 Lateral radiograph (A) and inset (B) of a canine radius and ulna with implant-associated osteomyelitis 1 month following repair of an open traumatic fracture. Osteolysis of the radial diaphysis (white arrow) is seen as a large, irregularly marginated, rectangular, lucent region with heterogeneous bony sclerosis that obscures the fracture margins. This abnormal region of bone is bordered caudally (between the radius and ulna) by moderate and irregularly marginated periosteal new bone formation. Similar but fainter osteolytic and osteoproliferative changes are seen surrounding the long oblique fracture within the ulna. There is also regional soft tissue swelling characterized by increased soft tissue opacity and undulating cutaneous margins. The infection resolved with prolonged antibiotic therapy based on culture and sensitivity testing and eventual removal of the plates and screws following healing of the fractures

repair of an open traumatic fracture of the left radius and ulna 1 month prior. In contrast, chronic osteomyelitis is a long-standing, more complex infection characterized by the death of bone tissue.[2,3] Treatment for chronic osteomyelitis is typically surgical debridement coupled with irrigation and drainage followed by prolonged antibiotic therapy. Particularly for chronic osteomyelitis, there remain many challenges in devising effective treatment plans, including the decision of whether to remove any colonized orthopedic hardware, which antimicrobial(s) to use, and delivery method for the antimicrobial(s).[1,3] Figure 2 provides a clinical example of chronic osteomyelitis in a Quarter Horse stallion with a sequestrum (i.e., a necrotic piece of bone) and its associated draining tract. Although horses are not used for research models of osteomyelitis, this clinical manifestation of post-traumatic, chronic osteomyelitis exhibits features that many of the models discussed herein attempt to achieve.

Staphylococcus aureus is a gram-positive, coccus bacterium typically arranged in clusters. This infectious agent is the leading cause of osteomyelitis in humans, causing 80% of cases.[4] The bacterium expresses adhesins specific to bone matrix and displays a propensity to bind to plasma proteins and host tissues, including fibrinogen

that is known to coat orthopedic hardware after implantation.[5,6] Furthermore, *S aureus* has developed several antimicrobial evasion methods that complicate treatment and allow the infection to persist to a chronic state. Among these are biofilms, a community of bacteria with altered phenotypes capable of evading antibiotics and the host immune system,[7] which secrete enzymes and toxins that damage host cells to allow for nutrient acquisition and the spread of infection.[8] A rise in resistance has been seen in *S aureus* following extensive antimicrobial use, leading to development of phenotypes such as methicillin-resistance *S aureus* (MRSA).[9]

Animal models may provide the most promising outlet for advancing our understanding of the pathogenesis of *S aureus* osteomyelitis and efficacy of treatments to mitigate infection. While in vitro models are useful for initial testing of therapeutics against different phenotypic states of *S aureus* (e.g., biofilms), therapeutics may demonstrate high efficacy in vitro but in an infected animal model provide little remedy for infection.[10,11] Compared to clinical cases, animal models offer a more reproducible, controlled environment that can be manipulated to reflect different disease presentations.[12-14] Small animal models are often preferable due to lower costs associated with housing and providing adequate care, ease of handling, and ability to evaluate larger sample sizes in a single study. After success in a small animal model, translation of promising therapeutics necessitates demonstration of efficacy in large animal models, as large animals more closely resemble humans in many aspects including bone density, weight, and immune system functions. Large animal models are also particularly useful for the study of orthopedic implants, as minimal if any sizing adjustments are needed to evaluate human-scale hardware.[15] In this review, we will focus on three main methods used to induce infection in animal models: post-traumatic, implant, and hematogenous, as illustrated in Figure 3. Several foundational models mentioned briefly in this review are covered more thoroughly in a 2009 review by Patel et al.[16]

2 | SMALL ANIMAL MODELS

2.1 | Mouse (murine)

Murine models of osteomyelitis are advantageous in their ability to reflect the clinical manifestation of the disease in humans.[17,18] Non-invasive monitoring via bioluminescent community-associated methicillin-resistant *S aureus* strains and whole-animal bioluminescent imaging has demonstrated utility.[19] One unique aspect of murine models is the ability to evaluate the effect of type I and type II diabetes on osteomyelitis pathophysiology through manipulation of the mouse genome. Examples include the NOD/ShiLtJ mouse, which exhibits symptoms similar to type I diabetes.[20] Diabetic patients are a population at particular risk for osteomyelitis infection, oftentimes arising from foot ulcers, which makes these models particularly appealing. Limitations of murine models include lack of bone integrity and general hardiness, making hardware adjustments or delaying treatment for longer observation intervals challenging.[18,21]
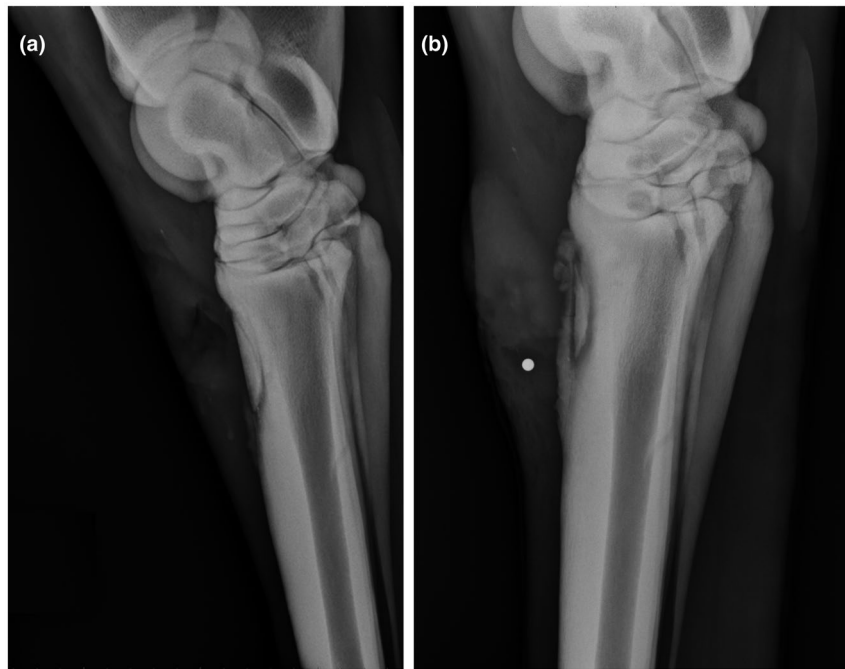
FIGURE 2 Lateral radiographs of a Quarter Horse stallion depicting an open traumatic chip fracture of the left third metatarsal bone both 2 wk (A) and 5 wk (B) following the initial traumatic injury. After 2 weeks, only soft tissue changes associated with the original open wound and presumptive acute osteomyelitis are seen overlying the fracture dorsally. By 5 wk (B), osseous changes of chronic osteomyelitis with a sequestrum are seen, characterized by irregularly marginated periosteal new bone formation and a sharply demarcated zone of lucency and sclerosis (i.e., involucrum) surrounding the fracture fragment. A round metal opaque radiography marker was placed in a cutaneous draining tract (i.e., cloaca) overlying the sequestrum. The patient fully recovered with surgical removal of the necrotic fracture fragment, debridement, and antibiotic therapy based on culture and sensitivity testing

2.1.1 | Post-traumatic

Despite the limited size of the murine tibia, a post-traumatic model was created by drilling a 1 mm diameter unicortical hole into the proximal medial tibia, which was then infected with 2×10^3 S aureus bacteria via injection in the medullary cavity. Debridement was performed 2 weeks later using a 20 gauge needle and unsurprisingly, debridement significantly reduced bacterial counts compared to nondebrided tissues.[22] In another study, a fixation plate served to stabilize an 8 mm osteotomy, into which 2×10^3 CFU of S aureus was injected. At days 7 and 14 post-surgery, debridement, and lavage were performed. There was no evidence of fracture healing by sacrifice on day 28.[23]

2.1.2 | Implant

A model by Xiao et al used Vicryl suture as the agent of bacterial contamination. The suture, after soaking in 1×10^7 CFU/mL of S aureus for 30 minutes, was inserted into a hole in the proximal tibia. Bioluminescence and radiography were used to confirm localized, stable infection.[24] In another compelling study, a Kirschner wire (K-wire) was inserted through the femoral canal so 1 mm protruded into the knee joint space. 5×10^2, 5×10^3, or 5×10^4 CFU of S aureus were injected adjacent to the implant where it extended into the surrounding soft tissues.[25] For translational work aimed at improving

therapeutics for diabetic patients, NOD/ShiLtJ mice were used for intramedullary nail implantation and injection of 1×10^3 CFU of S aureus into the femoral canal, determining that prostaglandin E_1 administered with cephalosporin improved recovery outcomes.[26] In another approach, the implant served as a delivery vehicle for antimicrobials, specifically phosphatidylcholine-coated K-wire for release of loaded amikacin, cis-2-decenoic acid, or both.[27]

For osteotomy implant models in which a fixation plate is needed, the femur is commonly utilized. In one study, a commercially pure titanium fixation plate was contaminated via submerging in a bacterial suspension of 4×10^8 CFU of S aureus, resulting in 9×10^5 CFU delivered per implant, and placed onto the femur, and a 0.44 mm osteotomy was created. In uninfected animals, bridging of the osteotomy gap was observed by 35 days. In contrast, the infected mice exhibited dramatic bone damage, and defects were not bridged at 35 days. Increased expression of transforming growth factor-β and platelet-derived growth factor genes, indicative of bone healing, were noted in all uninfected groups compared to all infected groups.[28]

2.1.3 | Hematogenous

Hematogenous murine models of osteomyelitis are also feasible, typically via S aureus injected into the lateral tail vein. Over the course of one experiment, using 2×10^5, 5×10^5, or 1×10^6 CFU
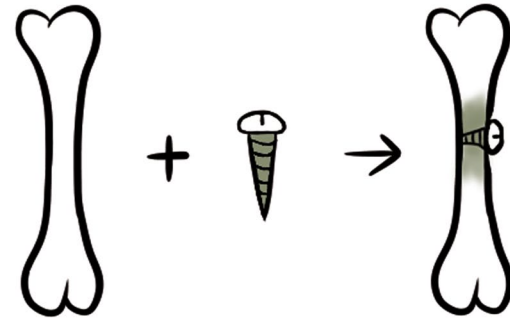
FIGURE 3 Common methods used to induce osteomyelitis in animal models include post-traumatic, implant, and hematogenous

Post-Traumatic
A defect (fracture, hole, etc.) is created in the bone followed by inoculation with S. aureus. Some models may stabilize the defects with plates, but these do not serve as a source of contamination or focus of the study.
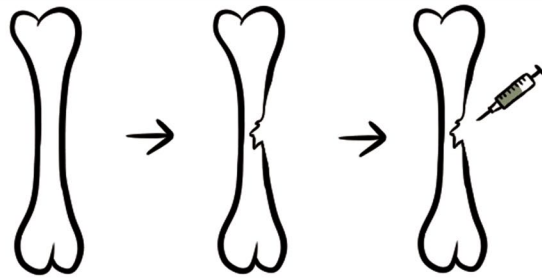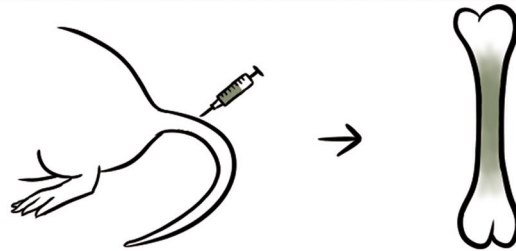
Implant
A contaminated implant (screw, nail, etc.) is placed into the bone to induce infection. Implants may also serve as antimicrobial delivery methods and a surface for bacterial colonization.

Hematogenous
Bacteria is inoculated into the bloodstream and time is allotted for infection, followed by isolation of the tissue of interest (typically bone for osteomyelitis).

injected into the lateral tail vein, *S aureus* initially invaded and proliferated not only bone but also other organs, most notably the kidneys. After 60 days, however, these organs were progressively cleared, and *S aureus* was present only in the tibia. This was attributed to the tropism of the strain (ATCC 6850) for bone, demonstrating the utility of this strain in hematogenous models, and emphasizing the significance of bacterial strain in infection studies.[17]

2.2 | Rat

First popularized by the publication of Zak et al in 1982,[29,30] rat models offer advantages in ease of care, easier surgical manipulation than mice (due to their increased size), as well as greater general hardiness than mice. Furthermore, rats may be appealing to researchers for their lower regulatory burden, relative to larger species for which the USDA has more intensive requirements. Furthermore, rats tolerate long-term antibiotic therapy, even at high doses.[31,32] Similar to diabetic mouse strains, the immune systems of rats have also been manipulated to reflect human conditions, which enables the study of risk factors for osteomyelitis and their impact on pathophysiology of the disease.[30,31] Thus, rats serve as effective models for screening of therapeutics, before larger animal models that tend to cost more in regards to housing and care requirements.

2.2.1 | Post-traumatic

The first rat osteomyelitis models largely relied on sclerosing agents to induce infection in post-traumatic tibial models, likely due to the ability of sclerosing agents to decrease (re)vascularization, thus facilitating bone necrosis and infection development.[31] These agents played a central role in models by Zak et al[29] and Rissing et al,[33] but were later proven unnecessary for infection development in a study by Spagnolo et al[16,31] Femoral models with no additives have also been explored. In one study, 100 μL of 10^4 CFU/mL *S aureus* was injected directly into a defect created by a needle in the distal femur. After 2 weeks, a bone cement in the femoral cavity was evaluated as an antimicrobial therapeutic.[34]

A study by Bonnarens and Einhorn used a drop-tower apparatus to drop a weight onto the femur, creating a transverse fracture.[35] Prior to fracture, a Steinmann pin was placed into the intramedullary canal of the femur, exiting through the greater trochanter and bent into a 3 mm "handle" buried into the muscle. Although this model did not induce infection, it served as the groundwork for future models involving spontaneous fractures.[35] In one modification, the femur was accessed distally (rather than proximally) for reaming. 10^4 CFU bacterial suspension was inoculated into the medullary canal, then a pin was inserted and the drop apparatus used. All infected femurs failed to bridge the fracture, whereas a bridging fracture callus was noted in all uninfected controls.[36]
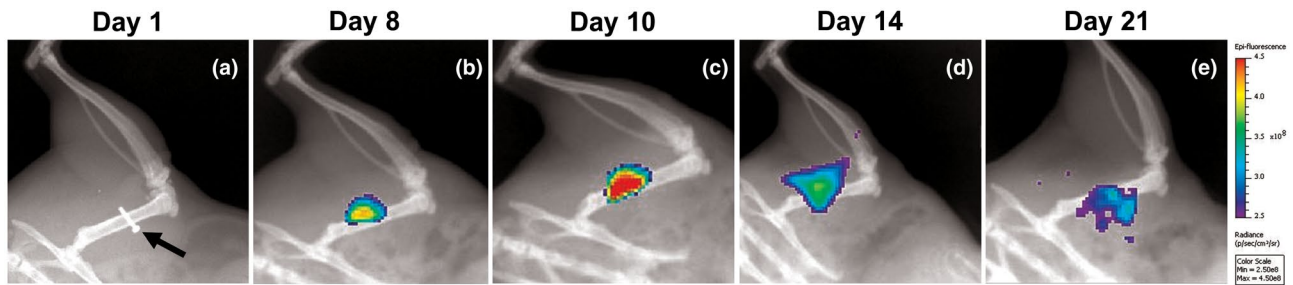
FIGURE 4 In vivo imaging of an untreated rat in the femoral osteomyelitis model induced by a screw (A, arrow) contaminated with fluorescent *Staphylococcus aureus* (ATCC 6538-GFP). A, Radiograph without fluorescence on day 1 post-infection showing location of the contaminated screw, which is removed on day 7. B-E, Longitudinal radiographic and fluorescent imaging 8 d (B), 10 d (C), 14 d (D), and 21 d (E) post-infection

Other fracture models utilize fixation plates and induce fracture surgically, rather than via a drop apparatus, and often involve an osteotomy. Typically, a defect 6mm in length is used, as this is accepted as critically sized in rat femora.[37-43] A popular method of introduction of *S aureus* into the defect is via contaminated type I bovine collagen.[37-43] One of the first publications describing a rat model of fixation plate, osteotomy, and osteomyelitis was Chen et al, in which a polyacetyl plate and Kirshner wires served as fixators.[40] A 10^5 CFU dose of *S aureus* resulted in osteolysis and loss of fixation plate stability over 2 weeks. This model has been adapted in many research projects in recent years, such as the evaluation of a novel osteogenic bone graft.[38] Other investigations include: biofilm formation in diabetic rats,[30] osteogenic protein-1 (OP-1) for inducing bone formation in the presence of *S aureus*,[40] and debridement optimization.[37,43]

Although less common, tibial fracture models also exist. In one unique study, an open tibial fracture model, akin to a Gustilo type III wound, was developed. To induce infection, a trough was first created in the tibial medullary cavity by drilling first into the anterior cortex, in a proximal-to-distal motion. After the trough was created, a cautery device was used to damage the endosteal blood supply. 10 µL of 1×10^7 CFU/mL *S aureus* suspension was then placed into the trough, followed by curettage and lavage. This model successfully established acute osteomyelitis, designated as the time within 15 days from initial infection.[44] Another unique study utilized an acute tibial open fracture model to evaluate factors that play a role in infection development and to determine a minimum dose of inoculum for infection. For creation of infection, a 1 cm trough was drilled into the medullary cavity of the craniomedial tibia and contaminated with inoculum ranging from 5 to 6500 CFU of *S aureus*. After 10 minutes, the troughs lavaged. Twenty-four hours later, the tibias were removed for bacterial counting. It was found that 72 CFU/g of bone was sufficient to cause infection in 50% of the rats, while 977 CFU/g of bone resulted in a 95% infection rate. This model was then used to evaluate the effect of thermal injury, bimicrobial contamination with the addition of *Escherichia coli*, and foreign bodies of soil and sand on infection development. Thermal injury contributed to infectivity when challenged with low doses of inoculum, but neither the addition of *E coli* nor contamination with sand or soil had a significant effect on infectivity.[45]

2.2.2 | Implant

Perhaps, the most common use of rats in osteomyelitis studies has been for implant-based models. A popular non-fractured implant model of osteomyelitis was published by Lucke et al, in which acute osteomyelitis was localized in the tibia with a K-wire.[16,46] In a similar model, the same methodology was used and an extended experimental period was chosen to generate chronic osteomyelitis, characterized by sclerotic bone and a lack of vascularization. Lavage and debridement were performed at days 7 and 14, with sacrifice at day 28. Systemic teicoplanin treatment with or without extracorporeal shockwave therapy was evaluated.[47] Another study placed a K-wire after a fracture occurred, from the proximal fragment to the distal fragment, until it was partially seated into the epiphysis. This antegrade K-wire placement resulted in a consistent infection rate (90%-100%) after 3 weeks with a low dose of 10^1 or 10^2 CFU of *S aureus*.[48]

Similar to K-wires, stainless steel tubing has been used as an implant for infection development. In one study, this tubing was presoaked in 1×10^4 CFU/mL *S aureus* and inserted into the femur, after which a supplementary 50 CFU *S aureus* suspension was injected inside the tubing. At days 6 and 45 post-initial infection, respectively, signs of acute and chronic osteomyelitis were confirmed by biofilm protein isolation and histology.[49]

Although not as common as wires, screws[50] and nails[16,51] have also been used to simulate implant-related osteomyelitis in rats. In one study, a polyether ether ketone (PEEK) screw coated in titanium was used as the agent to introduce bacteria into the tibia after soaking in 3.3×10^7 CFU/mL bacterial solution. The animals were monitored through 28 days, during which micro-computed tomography (µCT) was used to evaluate bone formation and resorption.[50] Bone formation decreased and bone resorption increased in the groups with colonized screw implants.[50] To observe osseointegration and antimicrobial effects of hydroxyapatite- and hydroxyapatite-silver-coated nails, a unicortical hole 8 mm in depth was drilled into the proximal lateral tibial metaphysis, followed by *S aureus* injection of 10^2 or 10^3 CFU and for the experimental groups, nail placement.[51] In a recent study, a contaminated screw was placed in a solution of $\sim 1 \times 10^8$ *S aureus* for 5-10 minutes, resulting in approximately 5×10^4 CFU of fluorescent ATCC 6538-GFP *S aureus* on the screw, which was placed

into the mid-diaphysis of the femur, establishing infection in the bone and surrounding soft tissue. On day 7 post-infection, the screw was removed, and treatments were administered: fosfomycin, bacteriophage, fosfomycin, and bacteriophage, or blank alginate hydrogel. On day 8 post-infection, histology revealed establishment of infection through neutrophilic inflammation as well as fibrosis and the presence of Gram-positive bacteria. Bone bacterial load was reduced only in the fosfomycin group, while soft tissue bacterial load was lower in all three treatment groups compared to controls.[52] More recently, this model has been used to longitudinally evaluate infection using in vivo radiographic and fluorescent imaging, as shown in Figure 4.

2.2.3 | Hematogenous

Although rare, in one hematogenous rat model of osteomyelitis, a medial parapatellar arthrotomy was created, and a cannulated needle was used to clear the medullary canal, into which a K-wire was inserted. After surgery, S aureus was delivered systemically via catheter in the tail vein. A high dose of S aureus (10^7 CFU) was necessary to induce infection of both the femur and the implant. Furthermore, after 14 days, the addition of the K-wire significantly increased the rate of infection. Interestingly, the dose of 10^7 CFU was not sufficient to induce osteomyelitis in rats without the implant.[53]

2.3 | Rabbit

Rabbits models are useful for those who need an animal model larger than rodents but do not have the infrastructure to support large animals. Rabbits are also a great choice for studies on implant devices or coatings, as evaluating these products using rodents is limited due to their smaller size.[54] Some products designed for humans can even be evaluated in rabbits with no modifications, and rabbit immune responses can reflect those of humans.[54,55] Challenges of rabbits include their hindgut fermenting gastrointestinal system, limiting their utility in evaluation of oral antibiotics due to the disruption of their crucial gut flora, and their tendency to undergo respiratory depression when put under anesthesia.[56]

2.3.1 | Post-traumatic

As with rat models, there exist post-traumatic models of osteomyelitis in the rabbit utilizing sclerosing agents.[57] In one tibial model, microbiological, and histological evidence of chronic osteomyelitis were noted at 4 weeks after inoculation with 1×10^8 CFU of S aureus, at which time the rabbits underwent debridement and either placement of novel bioactive glass implants, or daily intravenous injections of teicoplanin for 4 weeks.[57] These post-traumatic models using sodium morrhuate are also ideal for mimicking blast wound trauma seen in the battlefield, and resulting bone infection, because sclerosing agents induce necrosis of bone similar to blast wound trauma.

One study used this method to evaluate S aureus, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Acinetobacter baumannii* in monocultures or delivered in combination. All monoculture inoculations (0.15 mL of 10^7 CFU/mL) as well as the combinations with MRSA (0.15 mL of 10^5 CFU/mL) were able to reliably induce infection.[58] In a femoral model, a novel 3D printed scaffold composed of poly D,L-lactic acid powder laced with levofloxacin and tobramycin in concentric, alternating layers was evaluated.[59]

Another form of post-traumatic model by Kishor et al involved the production of a unicortical defect 5 mm in diameter to reflect a more severe situation. To induce infection, 10 μL of 5×10^6 CFU/mL S aureus was injected at the femoral defect site.[60] In another study, a bicortical defect was made in the femoral condyle. Here, a polymethylmethacrylate (PMMA) rod, contaminated by immersion in a S aureus inoculum (concentration not reported), was placed for 7 days, at which point various calcium phosphate cements loaded with doxycycline were implanted for an additional 21 days and evaluated for bacterial clearance.[61] Other femoral models avoid the creation of cortical defects. In one investigation, femoral trepanation was performed, and a suspension of 10^9 CFU of S aureus was inoculated into the knee cavity through a parapatellar injection. Animals were euthanized at 1, 2, 3, 9, and 14 days for evaluation of bacterial load in bone marrow over time. Histological signs and bacterial counts indicated acute osteomyelitis at all time points, though no significant differences in acute inflammation, intraosseous chronic inflammation, or bone necrosis scores were observed between days 3, 9, or 14.[62]

Models involving segmental defects, often involving the radius, are also reported. One major advantage of radial models is that a fixation device is usually not required, as the ulna serves this role. One study, aiming to induce chronic osteomyelitis, employed the removal of a 1 cm segment, where inoculum as small as 2×10^3 CFU of S aureus was injected directly into the medullary canal, and the excised segment was replaced.[63] In another study utilizing a similar methodology, 1 cm of the radius was removed, a K-wire was placed through the medullary cavity, and intramedullary inoculation of 7.5×10^6 CFU of S aureus was performed on the removed segment, which was then replaced into the defect space. Three weeks later, the site was debrided, the K-wire was replaced, and a novel biodegradable poly(N-isopropylacrylamide-co-dimethyl-γ-butyrolactone acrylate-co-Jeffamine® M-1000 acrylamide) hydrogel was utilized to deliver gentamicin.[64] The segmental defect model has since been adapted for the femur.[65] Notable research involving the humerus utilized custom-designed intramedullary nails or a seven-hole locking compression plate to stabilize a 0.45 mm osteotomy, into which S aureus was injected (6×10^3 to 6×10^6 CFU for the plate groups and 6×10^2 to 6×10^6 CFU for the nail groups).[54]

2.3.2 | Implant

Due to their larger physiology, rabbits enable evaluation of a broader range of implants than mouse and rat models. Implants reported

TABLE 1 Species utilized in osteomyelitis models, and characteristics of each that mimic human osteomyelitis

Species	Similarities to human osteomyelitis
Mouse	• Diabetic NOD/ShiLtJ mouse line mimics human type 1 diabetes (major risk factor for osteomyelitis)[20]
Rat	• Sclerosing agents used for production of a model with a histopathology similar to human chronic osteomyelitis[32] • Spagnolo's model reported radiographic changes and new bone formation that closely resembles what is seen in human osteomyelitis[31]
Rabbit	• The rabbit opsonic antibody response to chronic osteomyelitis closely resembles the human antibody response to the infection[55]
Pig	• Porcine bone fracture stress values closely align with human bone[75] • Bone composition is very similar to humans (second only to dogs)[75] • Similar bone regenerative capabilities[84] • Bacterial localization in the bones of juvenile pigs reflects what is seen with hematogenous osteomyelitis in human children[74]
Dog	• Very similar bone composition and density to when compared to human bone[75]
Goat/sheep	• Size allows for the use of human implants rather than adapted hardware[84] • Represents a more accurate depiction of human body weight[89] • Similar bone healing capacity[84] • Vascular supply to the tibia (useful for hematogenous studies) is like that of humans.[89]

in literature include but are not limited to: nails,[56,66,67] screws,[68] wires,[64,69] rods,[70,71] and plates.[54,70,72,73]

In one work, a biofilm-coated stainless steel fixation plate, attached via four screws on the midshaft of the femur, was used to induce osteomyelitis. The plate was contaminated by incubation in 5 mL of 10^6 CFU/mL of *S aureus*. A 1 mm defect was created after placement of the plate, and after 21 days, symptoms included: implant failure, callus formation away from the defect site, swelling, pus, and tissue damage.[72]

By drilling a defect 4 mm in diameter in the tibia, injecting 3.8×10^5 CFU of *S aureus*, and placing a titanium nail, one study established an acute osteomyelitis model characterized by early post-operative infection according to hematological analysis. Interestingly, different calcium-binding fluorophores were administered at 14, 28, and 41 days to enable longitudinal evaluation of bone formation.[56] Another unique nail-based study generated a bifocal osteotomy in the tibia to evaluate internal versus external fixation in osteomyelitis. This is of particular interest, as most animal models utilize only a single osteotomy. After this osteotomy was created, the tibial fragment was submerged in a 10^8 CFU/mL MRSA culture and replaced into the defect, where an intramedullary nail was used for stabilization. Four days later, surgical debridement was performed, nail was removed, and rabbits received either sterile internal nail fixation or bilateral external fixation with a custom device. This study revealed that removal of the internal osteosynthesis device improved recovery outcomes, based on bacterial counts recovered from purulent discharge.[66]

In one chronic model, sodium morrhuate was used to induce infection in the tibia over a period of 4 weeks. At this time, debridement was performed and either two Mg-Cu alloy nails or two pure titanium intramedullary nails were placed in the intramedullary canal.[67] Similarly to nail models, screw models have also been explored. For bacterial contamination, screws of 317L-copper, 317L-stainless steel, and titanium alloy (Ti-6Al-4V) were submerged in 10^5 CFU/mL of both *S aureus* and *E coli* for 6 minutes prior to implantation into a 2.5 mm cylindrical hole in the femur. The ability of the copper screw to mitigate implant colonization was compared to stainless steel and titanium screw controls, and copper was deemed superior based on ex vivo bacterial loads.[68]

Although uncommon, PMMA cylinders have also been used as implants. An 8.5 mm long femoral transcondylar defect was drilled and irrigated. Then, a contaminated (CFU/mL not reported) PMMA cylinder was pressed into the defect space. Four days later, the cylinder was removed, and infected soft tissues surrounding the bony defect were debrided. Rabbits then received either uncoated titanium or polyelectrolyte-film-coated titanium implants, for four or seven additional days.[71]

3 | LARGE ANIMAL MODELS

3.1 | Pig (porcine)

One benefit of using a pig model is that pigs are omnivores, and therefore have gut biomes that respond to antibiotics similarly as those of humans, making pigs a good candidate for oral and/or systemic antibiotic treatment evaluation.[74] Furthermore, although the composition of canine bones most closely resembles humans, pigs are the next closest match. Notably, fracture stress is higher in dogs (6.12 MPa) when compared to pigs (2.40 MPa) and humans (1.21 MPa) (Table 1). Studies on fracture and related stress from weight bearing may, thus, be more easily translated for human applications using the pig.[75] One disadvantage of pigs is their rate of bone growth, which is considerably faster than that of humans.[74] Additionally, the porcine tibia and fibula are shorter than those of a human, limiting their utility in the evaluation of implants.[74]

3.1.1 | Post-traumatic

For simulation of gunshot wounds, one porcine post-traumatic osteomyelitis model fired a 200 mg steel fragment into the right

tibial metaphysis. After this procedure, approximately 10^7 CFU of *S aureus* was inoculated into the defect site on a strip of bovine collagen. Experimentally, pigs received benzylpenicillin and flucloxacillin through intramuscular injection every 6 hours for 7 days. After 14 days, radiographs were taken and bone and soft tissue were collected and processed for histology. All animals in the control group had developed acute osteomyelitis, while the treatment group showed no signs of infection.[76]

3.1.2 | Implant

An implant-associated tibial osteomyelitis model has been established in pigs, utilizing a stainless steel implant. Briefly, fluoroscopic guidance was utilized to clear the medullary cavity of the tibia using a K-wire (4 mm), a low *S aureus* inoculum of 10^2, 10^3, or 10^4 CFU was delivered into the cavity, and a small steel implant was placed into the medullary cavity. Although animals were observed for only 5 days, signs of localized, acute osteomyelitis were noted to varying degrees on CT scans and implant cavity cultures in all groups.[74]

3.1.3 | Hematogenous

A benefit of porcine models is their pulmonary intravascular macrophages, which inhibit bacteremia and allow for prolonged survival after hematogenous inoculation of bacteria, in contrast to other species that may have to be euthanized due to septicemia.[77] In one study, a catheter was inserted into the left ear vein of juvenile pigs. Pigs then received either an inoculation of 10^8 CFU *S aureus* at the time of surgery, or the initial inoculation followed by another at 12 hours post-surgery.[78] In groups euthanized at 12, 24, and 48 hours, infection was successfully induced in the long bones and lungs without affecting the vertebrae, with no signs in those euthanized at 6 hours. However, after 48 hours the pulmonary bacterial load decreased, and bacteremia tests were negative, attributed primarily to the pulmonary intravascular macrophages of the pig that can effectively phagocytose *S aureus*.[77,78] This model is promising for evaluating therapeutics for juvenile osteomyelitis, which is often characterized by long bone infection that initiates deep within the metaphysis and spreads to the capillary loops near the growth plate, with the absence of vertebral lesions.[78,79] In one revision of this model, the use of the brachial artery rather than the ear vein resulted in 62.5% (5/8) of subjects euthanized for lameness.[80] The use of the right femoral artery was also explored, and found to be superior to other routes of bacterial inoculation in porcine hematogenous models.[81,82] Thus, in a subsequent study, Nielsen et al. used the right femoral artery for a diagnostic study. However, three subjects (38% of the experimental group) had to be prematurely euthanized due to complications including elevated C-reactive protein (CRP), neutrophilia, sepsis, and lameness. Interestingly, one animal from the experimental group was excluded due to lack of infection, leaving only four animals for diagnostic testing.[83] Overall, the current hematogenous porcine models

fail to be reliable due to high complication rates, but remain a promising area for future animal model development.

3.2 | Dog (canine)

Canine models are some of the more well-established models for orthopedic research. In a recent study, of all non-human species tested, canine bones most closely resembled human bones with regards to composition and density.[75] Despite these desirable attributes, few canine models exist, most likely due to the ethical concerns associated with the use of animals commonly adopted as household pets.[84]

The first published canine model was in 1976, by Deysine et al. Unique to this study was the use of the nutrient artery of the tibia as the inoculation site of radiopaque barium sulfate (used for radiograph enhancement) and 0.1 mL of a 10^6 CFU/mL culture of *S aureus*, somewhat mimicking hematogenous osteomyelitis.[16,85] Later, Fitzgerald et al established one of the first canine post-traumatic tibial models, which was later modified for the femur by Petty et al.[16,86,87]

More recent work deviating from these models, and simulating open fracture, also exist. In one such project, a captive bolt device delivered 6800 N of force to fracture the proximal tibia. Then, intramedullary nails were used to fix the site of fracture. 10^6 CFU of *S aureus* was injected into the medullary cavity and allowed to flow freely into the surrounding soft tissue. A transpositional muscle flap from the gastrocnemius muscle was then surgically created on some of the subjects, which displayed increased vascular endothelial growth factor (VEGF) mRNA expression versus the fracture only group at 2 hours post-surgery, indicating that the type of closure used in surgery should be carefully selected.[88]

3.3 | Goat (caprine)

Caprine, or goat, osteomyelitis models have not been widely utilized in research, likely due to preference for more well-established sheep models. Nonetheless, their larger anatomy more closely mimics human long bones.[89] This provides an easier translation of research findings for human applications, avoids the increased economic burden associated with custom-made, novel devices designed specifically for animals.[90]

The only caprine osteomyelitis models that fell within the constraints of this review were tibial models. Salgado et al. developed a popular goat osteomyelitis model through a post-traumatic tibial study.[16,91] Concurrently, a similar defect model was published, utilizing a 12 mm unicortical defect in the metaphysis of the tibia, followed by a 3.14×10^6 CFU bacterial inoculation. With the larger defect size, sclerosing agents were omitted and osteomyelitis was still induced.[92]

To compare the infection rate of fractures with external fixation versus intramedullary locking nails, with or without reaming, one

study developed two separate surgical protocols. For simulation of external fixation, a chevron osteotomy was created along the tibia, followed by generation of 4 mm drill holes. For intramedullary nail placement, a medial parapatellar incision was performed, followed by use of a 6 mm drill bit for access into the medullary canal. After fixation, 10^3 CFU of S aureus was introduced to the fracture site on an absorbable gelatin sponge. At 14 days, bacterial growth in the group with reaming and intramedullary nailing was significantly greater than the groups with an external fixation device or no reaming and intramedullary nailing.[90] Intramedullary nails were further analyzed in a using a more recent model, in which a tibial mid-diaphysial osteotomy was performed with intramedullary nail fixation. Micro-CT images, histology, and bacterial counts on explanted hardware indicated the successful development of chronic osteomyelitis, but infected soft tissue interference with the antimicrobial silver hybrid coatings of the intramedullary nails make the results inconclusive.[93]

3.4 | Sheep (ovine)

Sheep are a desirable model of long bone osteomyelitis, as their bones are similar in size to those of humans. Additionally, sheep and humans share a similar rate of osteogenesis. Torsional stiffness of sheep femoral bone has also been shown to closely mimic the torsional stiffness of human bone.[94] However, sheep bone is denser and has fewer Haversian canals than human bone.[94] Sheep models, like all large animal models, come with the burden of increased research costs for appropriate upkeep and housing. It should be noted that goat and sheep bone anatomy have very similar characteristics; largely, these two species could be interchanged depending on availability to research groups.

Kaarsemaker et al pioneered the use of sheep for osteomyelitis models in 1997.[16,95] In a similar femoral model, sclerosing agents were not used. A hole was drilled into the medial femoral condyle, and in the infection groups, 4×10^5 CFU of S aureus was inoculated, before the PLGA-polyethylene glycol scaffold materials were packed in. In both control groups with the scaffold only, and in two treated groups with an antibiotic-impregnated scaffold, no bacteria were isolated from blood samples, while the group with bacteria and no treatment had bacteria isolated from the bony defect, indicating that localized infection was produced. This provides evidence that sclerosing agents are not necessary in post-traumatic ovine models.[96]

Other efforts have been directed toward implants models. From a 2002 ovine tibial chronic osteomyelitis model using a midshaft chevron osteotomy followed by 3×10^8 CFU of S aureus bacterial inoculation and intramedullary nail placement, it was determined that intramedullary nail fixation may not be appropriate in all models, as it may stimulate virulence, and thus interfere with the efficacy of antibiotic treatment. The use of external fixation was suggested for future studies.[97] Another research group seemingly took this advice when designing their model, in which a titanium locking compression plate was used to stabilize an osteotomy. Another novelty in this protocol was the introduction of 2.5 mL of 10^6 CFU/mL of S aureus using a catheter at the site of the osteotomy.[98] The reproducibility of this model was further validated in a follow-up study evaluating a novel N,N-dodecyl,methyl-polyethylenimine (PEI) coating on the same titanium locking compression plates. All of the control (untreated) animals successfully developed osteomyelitis, indicating the reliability of this model.[15] Another ovine-based study utilizing orthopedic plates modeled open fracture type IIIB.[99] To contaminate the stainless steel fixation plates, S aureus was allowed to reach a biofilm state in vitro. Then, it was attached to a polyetheretherketone (PEEK) membrane containing 2.07×10^9 to 5.05×10^9 CFU, which was placed on the stainless steel plate. All five sheep that received the plates contaminated by a biofilm developed infection, while none of the five sheep that received plates contaminated with planktonic bacteria developed infection.[99]

4 | CONCLUSION

The current treatment regimen of osteomyelitis involves long-term antibiotic therapy, along with surgical debridement if warranted. However, this regimen is often challenged by a rise in antibiotic resistance, infection recurrence, and difficulty in eradicating the original infection. The use of animal models for osteomyelitis research allows for development and refinement of therapeutics in order to combat this complex disease. A wide variety of reproducible animal models exist across multiple species, including those focusing on post-traumatic infection, implant-based infection, and hematogenous seeding of bacteria. As these models are further developed to better reflect the human manifestation of osteomyelitis, the therapeutics and orthopedic hardware tested via these routes will show a higher margin of safety and efficacy when transitioned to human medicine.

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