Review Article

A systematic review on current osteosynthesis-associated infection animal fracture models

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\textbf{ABSTRACT}

\textbf{Objective:} Osteosynthesis-associated infection is a challenging complication post fracture fixation, burdening the patients and the orthopaedic surgeons alike. A clinically relevant animal model is critical in devising new therapeutic strategies. Our aim was to perform a systematic review to evaluate existing preclinical models and identify their applications in aspects of animal selection, bacterial induction, fracture fixation and complications.

\textbf{Methods:} A systematic literature research was conducted in PubMed and Embase up to February 2020. A total of 31 studies were included. Information on the animal, bacterial induction, fracture fixation, healing result and complications were extracted.

\textbf{Results:} Animals selected included murine (23), rabbit (6), ewe (1) and goat (1). Larger animals had enabled the use of human-sized implant, however small animals were more economical and easier in handling. \textit{Staphylococcus aureus} (\textit{S. aureus}) was the most frequently chosen bacteria for induction. Bacterial inoculation dose ranged from $10^2$ to $10^4$ CFU. Consistent and replicable infections were observed from $10^4$ CFU in general. Methods of inoculation included injections of bacterial suspension (20), placement of foreign objects (8) and pretreatment of implants with established biofilm (3). Intramedullary implants (13), plates and screws (18) were used in most models. Radiological (29) and histological evaluations (24) in osseous healing were performed. Complications such as instability of fracture fixation (7), unexpected surgical death (5), sepsis (1) and persistent lameness (1) were encountered.

\textbf{Conclusion:} The most common animal model is the \textit{S. aureus} infected open fracture internally fixated. Replicable infections were mainly from $10^4$ CFU of bacteria. However, with the increase in antibiotic resistance, future directions should explore polymicrobial and antibiotic resistant strains, as these will no doubt play a major role in bone infection. Currently, there is also a lack of osteoporotic bone infection models and the pathophysiology is unexplored, which would be important with our aging population.

\textit{The translational potential of this article:} This systematic review provides an updated overview and compares the currently available animal models of osteosynthesis-associated infections. A discussion on future research directions and suggestion of animal model settings were made, which is expected to advance the research in this field.

\section*{Introduction}

Infections after fracture fixation is one of the most challenging complications in trauma surgery, which often leads to delayed healing, permanent function loss and even amputation of the affected limb [1]. In open fractures, wound contamination with microorganisms can reach as high as 65\%, with infection rates after osteosynthesis up to 30\%, resulting in prolonged recovery periods and hospitalisation [2]. \textit{Staphylococcus aureus} is the most common virulent microorganism causing osteosynthesis-associated infection and often forms a biofilm making the treatment difficult [3]. Recent studies show costs exceeding USD 108,000 per patient with reported treatment success rates ranging from 70\% to

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90% [4]. The current goal of treatment is therefore to eradicate infection, allow fracture healing and preserve function.

With the increasingly frequent use of internal fixation and high morbidity of osteosynthesis-associated infections, it is imperative to further investigate factors that affect bone stability during fracture healing and potential interventions. Major factors that play a role include the bacterial type and size of inoculum, fracture and fixation method, and wound size. With the heterogeneity in clinical settings, the use of an animal model would allow reproducible investigations that simulate these important clinical situations. Furthermore, it saves research costs [5] and most importantly, intervention must be well-validated in animal models before conducting clinical trials.

With current existing models, clinicians need to be aware which is most suitable for their use. The purpose of this systematic review was to identify and characterize rise the strengths and limitations of current fracture models for infection. An accurate simulation of the clinical setting would be crucial for the development of new therapies that would significantly benefit the patient and decrease healthcare costs.

Materials and methods

Search strategy

The PubMed and Embase databases (date last accessed 16 February 2020) were searched. The keywords used for the search criteria were “fracture*” AND “animal*” AND “infection*”.

Search criteria

The inclusion criteria were: (1) preclinical studies; (2) use of animal model; (3) fractures performed with fixation; and (4) study on fracture healing. The exclusion criteria were: (1) review papers; (2) conference/abstracts; (3) no analysis on fracture healing; (4) no fracture fixation performed; (5) no full-text literature: and (6) not in English language.

Selection of studies

Two independent reviewers performed the selection process on two databases. Each reviewer screened the titles and abstracts of each published study. Articles were selected based on the inclusion and exclusion criteria. A third reviewer resolved any disagreement upon group discussion.

Data extraction

For eligible studies, the two reviewers extracted information on: (1) animal used; (2) bacteria species, inoculum size and method of inoculation; (3) location and type of fracture, and fixation method; (4) radiological and/or histological evidence of fracture healing; (5) interventions used in current models; (6) additional parameters assessed; and (7) complications.

Data analysis

Due to the data heterogeneity in animal models and methodology, a qualitative review was performed.

Results

Results of the search

A total of 1142 and 1459 studies were identified from PubMed and Embase respectively (date last accessed 16 February 2020). All duplicate entries were removed, leaving 1895 records. Each title and abstract was reviewed and 1837 records were excluded based on inclusion and exclusion criteria. Upon detailed review of each study in full text, an additional 27 were excluded. 6 of these studies did not employ fracture fixation [6–11]; 10 did not perform a fracture [11–20]; 4 were not related to fracture healing [21–24] and 7 did not have analysis of fracture healing [25–31]. Our results show a total of 31 studies for our systematic review (Figure 1).

Characteristics of the papers

The 31 studies were published from 2002 to 2020. All studies were preclinical experiments performed in the mouse (9 studies) [32–38], rat (14 studies) [39–52], rabbit (6 studies) [53–58], goat (1 study) [59] and ewes (1 study) [60]. Fracture and fixation were performed and bacteria were inoculated in each study. Please refer to Table 1 for details.

Bacteria species, load and method of inoculation

All studies used S. aureus as the inoculum species, except for a rat model infected with Staphylococcus epidermidis [39]. The inoculum load ranged from $10^3$ to $10^{10}$ CFU. In 19 models, bacteria suspensions were induced with the aid of injectable devices to the fracture site [34,35,36,41,43,47,53,54,56,60–62] or throughout the medullary canal [39,44,47–49,51,52,59]. 3 studies carried out inoculation by immersing the fixation plates in bacterial suspension prior to fracture fixation [33,37,55]. 8 studies utilised a foreign carrier, including fibrillar collagen [32,40,45,46,50], colloidal clay [38] or hydrogel [36], which was placed into the bone defect for inoculation. 1 induced the bacteria through subcutaneous injection 48 h post-operatively [57]. Please refer to Table 1.

Location, type of fracture and fixation

A variety of fracture models were utilised. Twenty studies performed fractures at the femoral shaft [32–41,44–47,50,52,55,57]; 7 at the tibial shaft [42,43,48,49,54,59,60]; and 2 at the humerus [53,56]. In 1 case, the fracture was created at the medial femoral condyle [58] and another included groups of different fracture location in their model design [51]. Twenty-seven studies adopted an open fracture model [32–35,37–41,43,45–48,50–60] while four were closed fractures [36,42,44,49]. Thirteen models used an intramedullary device [36,40–44,48–51,54,56,59], and 18 models used plate and screws [32–35,37–39,45–47,52,53,55–57,60] for fracture fixation. In 2 models, cerclage wires were used to stabilise the bone for additional support [47,57]. Please refer to Table 1.

Interventions and utilisation of the model

One frequent application of the bacterial-induced fracture was the investigation of therapeutic treatment. Thirteen studies used an antibiotic or antimicrobial material [32,34,36,37,44,48,50,52–54,59,60,62]. Two assessed the efficacy of osteoinductive agents [47,49]. One employed a lavage irrigation remedy [58]. One applied a hyperbaric oxygen therapy [38] and another used adenoviral gene therapy [57]. 1 study compared two fixation techniques in fracture healing with infection [56]. 5 studies adopted two or more therapeutic strategies in their investigation [40,45,46,51,62]. The remaining 8 articles reported findings on pathophysiology and did not involve any therapeutic intervention [33,35,39,41–43,55,61]. Please refer to Table 2.

Evidence of osseous healing

Of the 22 studies that exploit a therapeutic intervention, 13 reported both radiological and histological findings of osseous healing [32,36,44–50,52,53,57,59]. 8 did not include histological evaluation [34,38,40,51,54,58,60,62] and 2 without radiological assessment [37,61]. Based on these two assessments, it is shown that a number of treatments were effective in reversing osteolysis from bone infection, and promoting fracture healing. In a rabbit infection model, Ter Boo, G. demonstrated...
that a gentamicin grafted hyaluronic acid hydrogel could encourage periosteal callus formation at 28 days [53]. Another study used a lysostaphin loaded hydrogel [36]. The treatment resulted in significant higher bone and callus volume around the fracture site. Co-delivery of local and systematic vancomycin were shown to significantly reduce bone resorption in Inzana’s study [32]. Schear T.P. explored the possibility of N, N-dodecyl, methyl-polyethleneimine (PEI) derivatised coating on fixations [60]. More bridging callus were observed at one-month between the treatment and control groups. Complete fracture consolidation was achieved in 28 days using a lysostaphin-coated titanium implant on mice [34]. In a rat model that applied recombinant human bone morphogenetic protein-2 and systemic ceftriaxone, the bone defect healed securely at 12 weeks [45]. Furthermore, in a mice study investigating the effect of gentamicin or vancomycin infused calcium sulfate/hydroxyapatite (CAS/HA) insets, the mice with infection did not show a healing fracture gap [62]. Table 2 summarises the radiological and histological findings for all 31 studies.

**Additional parameters**

Clinical evaluation was included in 22 studies [32,37,39–43,45–57, 59,60]. Parameters of body temperature, body weight, evaluations for well-being and signs of infection, i.e. the presence of abscesses, purulent discharge and swelling, lameness and weight bearing were monitored clinically. Samples of soft tissue, synovial, bone samples, lavage and implants were used for bacteriology and documented in 22 studies [32,33, 35–39,41–44,48,50,52–58,60,62]. Scanning electron microscopy (SEM) [32,35,39,43,55,60], confocal laser scanning microscopy [43,55,59] and crystal violet staining [39] were applied to visualise dislodged bacteria and biofilm formation. One study employed a newer imaging technology where spatial signals of the bioluminescent bacteria on live animals could be captured over the observation period [32]. Haematology was investigated in 12 studies [34,36,38,39,42,51–54,56,59,62]. C-reactive protein (CRP) level and white blood cell (WBC) count were common parameters of assessment. The elevation of these blood markers is associated with systemic immune response to inflammation and serves as indicators for progression of bone infection in these models. One study applied a local therapeutic treatment and incorporated blood test for renal function to investigate on systemic toxicity [54]. Apart from histology and radiology, mechanical testing was included in 10 studies for additional assessment on fracture healing [36,41,42,45,46,48,49,53,56,59].

**Complications**

Twenty studies [32,38–45,47–49,53,55–60,62] reported adverse events and complications on animals, including loosening of fixation, persistent lameness, signs of septic infection and unexpected fracture due to extensive osteolysis. Animal deaths and failures during surgical operation are also common [39,42,45,48,49,53,62]. Premature euthanasia was required in some occasions. In a 16-week study, 19 of 64 rabbits were euthanised for humane reasons [57]. Another study showed that 21 of 72 mice died during experimental procedures, among which 9 were early euthanised because of non-weight bearing of the operated extremity in 3 mice, and non-controllable wound defects in 6 mice [62]. Only one rat and one rabbit model reported no complications where all animals tolerated well from the operations and intervention till end-point [46,54].

**Discussion**

Implant infection during the setting of fracture repair, is a challenging complication in orthopaedic trauma. Prolonged hospitalisation, repeated operations, and the demand of a multidisciplinary approach in management are all contributing factors to encumber patients and health workforce, along with high socio-economic burden [2]. Despite prophylactic administration of antibiotics, the incidence of fracture-related infection is still commonly encountered [63]. A recent review on preclinical models of fracture-related infection highlighted that only 6.7% of the studies included in their review combined the key features of having the presence of a fracture, delay before treatment and soft tissue damage [64]. A clinically relevant animal model is thus important to simulate these scenarios for establishing translational therapeutic strategies.

Different animal models have been described in the literature to study bone infection associated with fracture healing. In a previous study by Schear et al., a sheep model on OAI was selected to investigate the bactericidal properties of internal fixation coated with N, N-dodecyl,methyl-polyethylene imine [65]. An existing locking compression plate was used to stabilize the mid-diaphyseal fracture. In the infection group, 100% of the control animals had non-union of the fracture combined with consistent results in radiographic signs, soft tissue destruction, implant colonization and delayed fracture healing. This study highlighted the fundamental advantages of using large animals for OAI, since a larger bone size could facilitate both surgical fixation and evaluation with actual implants. A goat model was adopted by Tran et al. to evaluate the antimicrobial efficacy of silver-doped Ti/Siloxane coating on intramedullary nails [59]. The larger medullary canal and bone size of the animal had allowed the application of human scale implants in which modification of implants were not necessary. A higher antibiotic tolerance is another advantage of using large animals in infection studies [66]. However, only two goats were used in the study. Cost and ease of handling could be limitations of a larger sample size. On the other hand, rabbits, which are less expensive and
Table 1
Summary of the study characteristics.

| Source          | Animal                          | Bacteria species and concentration (CFU)                                                                 | Method of inoculation                                                                 | Site of Fracture                                      | Year | Osteotomy type (open/closed; bone defect/fracture) | Fixation technique                                      |
|-----------------|---------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------|------|-----------------------------------------------------|----------------------------------------------------------|
| Buren et al.    | BALB/c-mice                     | Staphylococcus aureus, 1 × 10^7/mL S. aureus, 1.35 × 10^9/mL                                            | Bacteria solution was inoculated to the osteotomy gap                                | Femoral diaphysis                                    | 2019 | Open fracture                                       | 4-hole plate and screw combination                        |
| Oezel et al.    | BALB/c mice                     | S. aureus, 1.35 × 10^9/mL                                                                             | Inoculation of the fracture gap with bacteria solution                              | Femoral diaphysis                                    | 2019 | Open fracture                                       | 6-hole titanium locking plate with locking self-tapping microscrews |
| Buren et al.    | BALB/c mice                     | S. aureus, 1.94 × 10^7                                                                                | Bacterial solution is induced to the fracture gap                                   | Femoral diaphysis                                    | 2018 | Open, fracture (0.22 mm)                             | 4-hole titanium locking plate with locking self-tapping microscrews 7 G needle |
| Cui et al.      | Sprague–Dawley rats             | S. aureus, concentration not specified                                                                  | Injection of bacterial solution through the intramedullary needle                   | Tibia fracture, femoral fracture, ulnar and radial fractures, diaphysis fracture and metaphyseal fracture | 2018 | Open, fracture                                       | Kirschner wires                                          |
| Helbig, L. et al.| Sprague–Dawley rats             | S. aureus, 10^3                                                                                      | Injected to the medullar cavity of the tibiae with a microlitre syringe              | Tibial diaphysis                                      | 2018 | Closed, fracture                                     | Kirschner wires                                          |
| Johnson et al.  | C57/B6 mice                     | S. aureus, 1.55 ± 0.51 × 10^9/mL (UAMS-1); 3.43 × 10^8/mL (USA300) S. aureus, methicillin-resistant S. aureus, 10^4 | Bacteria is mixed with the hydrogel components and polymerised in situ over the fracture  | Femoral diaphysis                                    | 2018 | Closed, fracture                                     | 25 G needle                                               |
| Mills, R. et al.| Wistar rats                     | Staphylococcus aureus, mettichlin-resistant S. aureus; methichlin-resistant Staphylococcus epidermidis, 10^6 | Collagen sponge loaded with bacteria placed to the fracture site                     | Femoral diaphysis                                    | 2018 | Open, fracture                                       | Kirschner wires                                          |
| Rochford, E. T. J. et al. | C57BL/6 and BALB/c mice           | S. aureus, 9 × 10^7                                                                                   | Immersion of implant plates into bacteria suspension for 20 min and air-dried for 5 min | Femoral diaphysis                                    | 2018 | Open, fracture                                       | Surface polished titanium and oxygen plasma-treated polyetheretherketone (PEEK) plate and screws Kirschner wires |
| Shiels et al.   | Sprague-Dawley rats             | S. aureus, 10^7                                                                                      | Bacteria solution is applied to the intramedullary (IM) canal and incubated for 2 min prior to placement of the experimental K-wire. | Tibial diaphysis                                      | 2018 | Open, fracture                                       | Kirschner wires                                          |
| Shiels et al.   | Sprague-Dawley rats             | S. aureus, 10^7                                                                                      | Via a collagen prewetted with bacterial solution placed into the defect              | Femoral diaphysis                                    | 2018 | Open, bone defect (2.58 ± 0.005 mm)                  | Radiolucent plate, affixed with K-wires                   |
| Ter Boo, G. J. et al. | New Zealand white rabbits                                    | S. aureus, 2.0 × 10^5                                                                                   | Injections of bacterial suspension into the empty screw hole overlapping the osteotomy and on the head of adjacent proximal and distal screws | Humeral diaphysis                                    | 2018 | Open, fracture                                       | Locking plate                                             |
| Lv Zhou et al.  | New Zealand white rabbits       | S. aureus, 10^5/mL                                                                                     | Inoculated into the medullary cavity of the fracture edges                           | Tibial diaphysis                                      | 2017 | Open, fracture                                       | Stainless steel wires                                   |
| Zhang, X. et al.| New Zealand White rabbits       | S. aureus, 10^5/mL                                                                                     | Steel implant was placed with 5 mL of bacterial solution and incubated for 48 h at 37 °C. The plate was then rinsed and taken for surgery. | Femoral diaphysis                                    | 2017 | Open, fracture                                       | Stainless steel plate                                    |
| Lovati, A. B. et al. | Wistar rats                     | S. epidermidis, 10^4, 10^5, 10^6                                                                     | The bacterial suspension was injected into the femoral defect and the suspension was allowed to spread throughout the medullary canal. | Femoral diaphysis                                    | 2016 | Open, bone defect (1 mm)                             | Stainless steel plate                                    |
| Arens, D. et al.| New Zealand White rabbits       | S. aureus, 6 × 10^2–6                                                                                 | Injections of bacterial suspension onto the central screw hole overlapping the osteotomy and to the humeral diaphysis | Humeral diaphysis                                    | 2015 | Open, fracture (0.45 mm)                             | Locked plate/a custom designed interlocked intramedullary nail |
| Source         | Animal                | Bacteria species and concentration (CFU) | Method of inoculation                                                                 | Site of Fracture | Year | Osteotomy type (open/ closed; bone defect/fracture) | Fixation technique                              |
|---------------|-----------------------|---------------------------------------|--------------------------------------------------------------------------------------|------------------|------|---------------------------------------------------|-----------------------------------------------|
| Bilgili, F. et al. | Sprague-Dawley rats  | S. aureus, 1 × 10^5/mL                | adjacent proximal and distal screws holes with a pipet                                | Femoral diaphysis | 2015 | Open, fracture                                     | Kirschner wire                                |
| Helbig, L. et al. | Sprague-Dawley rats  | S. aureus, 10^4                       | As opaque solution, injected into fracture area *(according to an established model)* | Tibial diaphysis  | 2015 | Closed, fracture                                   | Titanium Kirschner wire                        |
| Inzana, J. A. et al. | BALB/cj mice         | S. aureus, 8.0 × 2.9 × 10^8 per fibrillar collagen sheet | Loaded onto a fibrillar collagen sheet placed into the bone defect                    | Femoral diaphysis | 2015 | Open, bone defect (0.7 mm)                         | Titanium coated- polyether ether ketone plate, titanium screws |
| Rochford, E. T. J. et al. | CS7BL/6 mice        | S. aureus, 9 × 10^7 per implant       | Immersion of implant plates into bacteria suspension for 20 min and air-dried for 5 min | Femoral diaphysis | 2015 | Open, bone defect (0.44 mm)                        | Titanium plates and screws                     |
| Schindeler, A. et al. | Wistar rats           | S. aureus, 10^4                       | The bacterial suspension was loaded in a collagen carrier and packed into the defect.*| Femoral diaphysis | 2014 | Open, bone defect (0.22 mm)                        | Stainless locking plate                        |
| Windolf, C. D. et al. | BALB/c mice          | S. aureus, 1.94 × 10^5/mL             | Inoculated into the fracture gap                                                     | Tibial diaphysis  | 2013 | Open, fracture                                     | Stainless steel alloy intramedullary nail with interlocking screws |
| Tran, N. et al. | Goat                  | S. aureus, 2 × 10^5/mL                | Injection into the medullary canal at the fracture site                               | Femoral diaphysis | 2013 | Open, bone defect (0.22 mm)                        | Titanium locking plate                        |
| Windolf, C. D. et al. | BALB/c mice          | S. aureus, 10^4                       | Injection into the fracture gap with a micropipette                                   | Tibial diaphysis  | 2011 | Open, fracture                                     | Kirschner wire                                |
| Schena, T. P. et al. | Dorset-cross ewes    | S. aureus, 10^6, 10^8, 10^10/mL       | Via a temporary indwelling silastic catheter inserted into the osteotomy site        | Femoral diaphysis | 2011 | Open, fracture (0.6 mm)                            | Locking compression plate                      |
| Robinson, D. A. et al. | Sprague-Dawley rats  | S. aureus, 10^5                        | injected into the medullary cavity via a polypropylene catheter                        | Femoral diaphysis | 2010 | Closed, fracture                                  | Stainless steel intramedullary pins            |
| Chen, X. et al. | Sprague-Dawley rats  | S. aureus, 10^4                       | The bacterial suspension was loaded in a collagen carrier and packed into the defect. | Femoral diaphysis | 2007 | Open, bone defect (6 mm)                           | Polycrystal plate, Kirschner wires             |
| Southwood, L. L. et al. | New Zealand White rabbits | S. aureus, 10^7/mL              | Percutaneous injection (48 h after surgery)                                          | Femoral diaphysis | 2003 | Open, bone defect (10 mm)                          | Stacked-cuttable bone plates, cortical screws, cerclage wire |
| Caprice, P. A., Jr. et al. | New Zealand White rabbits | S. aureus, 5 × 10^5/mL             | Via a colloidal clay in a syringe injected into the fracture site                     | Medial femoral condyle | 2002 | Open, fracture                                     | Single screw                                   |
| Chen, X. et al. | Sprague-Dawley rats  | S. aureus, 10^5                        | Injected into the opening of the medullary canal on both ends of the defect with a syringe needle | Femoral diaphysis | 2002 | Open, bone defect (6 mm)                           | Polycrystal plate, Kirschner wires, cerclage wire |

* indicates modification performed by the authors to the original model.
### Table 2
Summary of the study characteristics.

| Source               | Intervention                                                                 | Radiological/histological evidence of osseous healing                                                                 | Parameters assessed                                                                 | Complication                                      |
|----------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|---------------------------------------------------|
| Büren et al.         | NA                                                                            | (Histology) At 4 weeks postoperatively, the callus formation in infection group was smaller compared with the control group; Mice in infection group showed no complete osteotomy consolidation. In control group, 1 of 16 mice showed complete osteotomy consolidation and 12 showed partial consolidation. | Histology Microbiological analysis                                                  | No case fatalities due to surgery or anaesthesia |
| Oezel et al.         | Gentamicin or vancomycin infused calcium sulfate/hydroxyapatite (CAS/HA) insets | (X-ray) All mice with infection did not show a healing fracture gap, independently of the application of calcium sulfate/hydroxyapatite (CAS/HA) insets or antibiotics; Mice of the groups infected solo, CAS/HA and CAS/HA-G had similar destruction of the bone, mice of the CAS/HA-V group suggest that vancomycin infused insets show a less distinct bone destruction; | X-ray Microbiological analysis Haematology (polymorphonuclear (PMN) leucocytes, interleukin (IL-6)) | 21 of 72 mice died during experimental procedures |
| Buren et al.         | Hyperbaric oxygen therapy                                                     | (X-ray) All mice in the control and treatment group showed a healing fracture gap. The infection group had the same bone healing score as the controls but showed a greater individual heterogeneity and non-union number. | X-ray Microbiological analysis Haematology (AP, procollagen type I N propeptide (PINP)) Cytokine analysis | 18 of 120 mice died during the experimental procedures. Reasons were not specified. |
| Cui et al.           | Masquelet induced membrane therapy: (1) vancomycin, mixed with poly(methyl methacrylate) (PMMA) bone cement, placed locally in bone defect (2) morselised cancellous bone grafting | (X-ray) Primary bone healing was achieved in 50 rats with an average healing time of 15 ± 1.56 weeks. | X-ray Haematology (tumour necrosis factor alpha (TNF-α), white blood cell (WBC), C-reactive protein (CRP)) Clinical Microbiological analysis (methodology not specified) | Not specified |
| Helbig, L. et al.    | rhBMP-7, rhBMP-2, intramedullary injection five weeks post-fracture           | (micro-computed tomography (CT)) The sterile group showed complete bridging of the fracture gap. The infection and treatment groups showed increased hypertrophic callus formation. Callus formation showed no differences between the two treatments. (histology) Staining of the cartilage also showed no gross differences in healing between sterile control fractures and fractures treated with lysostaphin-delivering hydrogels. | X-ray, Micro CT Histology Mechanical test Clinical Microbiological analysis | Of the rats, 3 died due to complications with general anaesthesia, 2 were sacrificed due to postoperative infected haematomata; 5 were excluded due to technical problems during preparation of the tibiae |
| Johnson et al.      | Lysostaphin, loaded in hydrogel injected to osteotomy gap                    | (micro-CT) The infection group shows no callus formation, presence of bone resorption and reactive bone formation around the fracture site. The sterile group showed robust fracture callus. The treatment group showed significant bone healing with a significantly higher bone and callus volume. (histology) Staining of the cartilage also showed no gross differences in healing between sterile control fractures and fractures treated with lysostaphin-delivering hydrogels. | X-ray, Micro CT Histology Cytokine analysis Haematology (antilysoptaphin antibody and liver enzyme test) Mechanical testing Microbiological analysis | Not specified |
| Mills, R. et al.     | BMP-2, CSA-90, loaded in collagen sponge placed to the fracture site         | (micro-CT) The methicillin-resistant Staphylococcus aureus (MRSA) infected treatment group showed an increased bone volume around the fracture site compared to the untreated MRSA group. Maximal ectopic bone formation was achieved with 500 mg CSA-90 and 10 mg bone morphogenic protein-2 (BMP-2). (X-ray) 19 of the 20 infected fracture achieved a modified radiographic union scale in tibial fractures (RUST) score consistent with fracture union. No difference was identified between the methicillin-resistant Staphylococcus epidermidis (MRSE) treated and untreated groups with all fractures achieving fracture union. (histology) Inflammatory cell debris and pus are clearly visible around the non-united fractures of the non-treated and delayed treatment groups. Evolving bony architecture was seen in the co-treatment group. | X-ray, micro CT Histology Clinical Microbiological analysis | number not specified |
| Rochford, E. T. J. et al. | NA                                                                             | (histology) By Day 7, a minimal to moderate granulocytic to necrotizing myelitis, sometimes | X-ray Histology | Not specified |

(continued on next page)
| Source | Intervention | Radiological/histological evidence of osseous healing | Parameters assessed | Complication |
|--------|--------------|------------------------------------------------------|---------------------|--------------|
| Shiels et al. | Cefazolin, systemic; Chlorhexidine, N(3-Sulfopropyl)-N-methacryloxy ethyl-N,N-dimethyl ammonium betaine, coated on implant | (radiographical) An evident mitigation of osteolysis and increased radiographic union were seen in the intervention group compared to the unmodified control group. (histology) The intervention group showed signs of bone formation, the control group exhibited signs of active bone resorption. | X-ray, micro CT Histology Clinical Microbiological analysis Mechanical test | 8 out of 161 were excluded and euthanised (2: uncontrollable oedema; 3: sequestration of the K-wire leading to destabilisation of the operative tibia; 1: post-operative torsional destabilisation of the tibia; 1: K-wire rupturing through the posterior cortex of the tibia) |
| Shiels et al. | Rifampin, vancomycin, topical, powder form | (X-ray) Rifampin-reduced radiographic indications of infection compared to the control empty and vancomycin group. (histology) Presence of bacteria was seen within both the control and vancomycin groups with large abscesses and necrotic bone. The rifampin group showed signs of healthy new bone formation. (X-ray, histology) Group receiving the intervention showed new callus information and no necrotic tissue was observed 28 days post-operative. In the non-inoculated control group, a large amount of periosteal callus was formed around the osteotomy gap at 28 days. | Microbiological analysis X-ray Histology Clinical Haematology (WBC count) | Not specified |
| Ter Boo, G. J. et al. | Gentamicin, loaded in a biodegradable thermoresponsive poly(N-isopropyl acrylamide) grafted hyaluronic acid hydrogel injected to the osteotomy gap and over the locking plate | Clinical X-ray Mechanical testing Histology Microbiological analysis Haematology (CRP, WBC count, serum gentamicin level) | 5 out of 45 euthanised before end-point due to symptoms of cardiac arrest and a fracture of the operated bone |
| Lv Zhou et al. | Tobramycin, coated on K-wires with poly(D-L-lactide) (PDLLA) | (X-ray) At day 56, 5 out of 6 in the intervention group and all 6 rabbits in the non-inoculated control group showed healed fractures. | Microbiological analysis Haematology (Blood, urea, nitrogen (BUN), CRP, CR, Hb) Clinical Histology X-ray Urology | All rats recovered well from the operation and survived till end-point. |
| Zhang, X. et al. | NA | (micro- CT) At day 21, cortical bone in the infected group showed obvious corrosion and absorption, for control group it remained intact. Significant bone callus formation was observed around the fracture site in the control group. | Clinical Histology Haematology (WBC count) | All rats survived till end-point; clear instability of the plates and screws was observed in the infected group. |
| Lovati, A. B. et al. | NA | (micro-CT) 103 methicillin-resistant Staphylococcus epidermidis (MRSE) group: 67% showed a fracture healing less than 75% and displayed mainly fibrous non-union; 105 MRSE group: 83% showed a fracture healing less than 75% with absence of bony healing; 108 MRSE group: all samples showed a fracture healing less than 75% and displayed non-union extended across the entire bone. Some animals in the control group showed a well-organised bone callus and remodelling. (histology) Fractures in the control group appeared closed with a great amount of new bone formation in a remodelling phase. 103 MRSE group: incomplete bone healing characterised by a great formation of fibrovascular tissue; 105 MRSE group: missing of cortical bridging with non-union establishment; 108 MRSE group: massive cortical and endosteal osteolysis | Micro CT Haematology (WBC count) Microbiological analysis Clinical | 1 was excluded for radiographic evaluation due to mechanical loss of the proximal screws followed by a fracture dislocation from surgical inaccuracy. |
| Arens, D. et al. | NA | (histology) At day 70, the plate fixation infected group resulted in periosteal osseous new bone formation around the plate; the nail group displayed some endosteal bone formation and bony integration of the implant. In the non-inoculated groups, complete osteotomy closure was observed at 10 weeks. (X-ray) At day 70, the osteotomy gap is still visible in the infected group. All rabbits in the non-inoculated nail group displayed callus bridging between the proximal and distal diaphysis. | X-ray Histology Mechanical testing Haematology (CRP, WBC count) Clinical Microbiological analysis | 7 out of 61* were euthanised (6 unexpected fracture, in which 3 had implant failure; 1 persistent lameness post surgery); a small number of rabbits showed proprioceptive deficits on the operated leg due to stress to the radial nerve during surgery. |
| Bilgili, F. et al. | NA | (X-ray) At 42 days, complete bony union was found in the control group, whereas the infection group showed only initial stages of bony union. (histology) At 42 days, evidence of bone healing was observed by 42 days in the control group but not in the infection group. | Clinical X-ray Histology Mechanical testing Microbiological analysis | One out of 75 died, loosening around the implant was observed in the infection group |

(continued on next page)
| Source | Intervention | Radiological/histological evidence of osseous healing | Parameters assessed | Complication |
|--------|--------------|------------------------------------------------------|---------------------|--------------|
| Helbig, L. et al. | NA | (micro-CT) The infected group showed clearly reduced consolidation of the fractures at day 35. Fracture gap was not bridged for all rats in the group. Fracture in the non-infect group were bridged completely in 9 of 10 animals at day 35. | Micro CT Haematology (blood count, leucocytes count, C-reactive protein (CRP)) Clinical Microbiological analysis Mechanical testing | 2 out of 22 died due to anaesthesia immediately after operation |
| Inzana, J. A. et al. | Vancomycin, IV systemic; local, loaded in a PMMA spacer tied into the defect using a nylon suture | (X-ray, micro-CT) The resorbed bone volume was significantly reduced in groups receiving systemic and local antibiotics on day 14. (histology) A large amount of new reactive bone had formed on the periosteal surface of the control femurs, no osteogenic response was observed on the infected femurs on day 14. | Clinical Histology, X-ray, Micro CT Microbiological analysis (bacterial culture, scanning electron microscopy (SEM), Bioluminescent imaging) | None of the mice died during study, but one infected mouse sustained a fracture between days 10 and 14, which likely resulted from dramatic thinning of the cortex. Implant failure was seen in the placebo group from micro-CT scans. |
| Rochford, E. T. J. et al. | Silver, coated on intramedullary nails | (X-ray, histology) Osteotomy gap clearly not healed in infected animals on day 35. In non-infected animals, complete healing was observed on day 35. | Clinical X-ray Histology Microbiological analysis Flow cytometry analysis Reverse transcription quantitative polymerase chain reaction (RT-qPCR) Cell stimulation and cytokine quantification | Not specified |
| Schindeler, A. et al. | Cationic steroid antibiotic bone morphogenetic protein 2, loaded in a collagen sponge disc placed circumferentially around the fracture site | (micro-CT) Increase in callus tissue volume was more pronounced in the rhBMP-2/CSA90 group. (histology) tartrate-resistant acid phosphatase (TRAP) staining revealed no obvious alterations in osteoclasts for the treatment group, although bone nodules were highly heterogeneous, having undergone substantive remodelling. | Clinical X-ray, Micro CT Histology | Did not specify number of unexpected deaths, but mention some were culled due to loss of intramedulary fixation |
| Windolf, C. D. et al. | Lysostaphin, coated on titanium discs | (X-ray) The intervention group showed clear signs of fracture healing by 14 days and complete fracture consolidation by 28 days. Fracture healing could not be observed at any time point in the group receiving control plates. | X-ray Haematology (PMNs, leukocyte count) IL-6 quantification in lavage Microbiological analysis X-ray, Micro CT Histology Haematology (WBC count, neutrophil and lymphocyte level, silver level) | Not specified |
| Tran, N. et al. | Silver, coated on intramedullary nails | (X-ray) Non-union of bone is seen in all groups at 35 days. (histology) Neither animal of the groups formed a bridging callus that filled the osteotomy gap at 35 days. | Clinical Mechanical testing Microbiological analysis Silver level testing in organs | Implant loosening was absent in all animals at 35 days |
| Windolf, C. D. et al. | NA | (X-ray, histology) Infected mice showed a significantly reduced in bony healing at 7, 14 and 28 days, when compared to non-infected mice. All femora from non-infected mice showed early fracture healing by day 7 and complete fracture consolidation by day 28. (histology) Clear signs of infected non-union without any bony bridging of the fracture site. (micro-CT) The treatment group showed more bridging callus formation at one month post-operative than the control group. (histology) Compared to the control group, there is a significantly lower histology score consistent with improved bone healing and absence of infection in the treated animals. | X-ray Histology Microbiological analysis Cellular and IL-6 quantification in lavage | Not specified |
| Alt, V. et al. | NA | (X-ray, micro-CT) All infected animals of the group showed persistence of the osteotomy gap and other signs of infected non-union. Complete bony bridging of the osteotomy gap was observed in the control animals at day 42. (histology) Clear signs of infected non-union without any bony bridging of the fracture site. (micro-CT) The treatment group showed more bridging callus formation at one month post-operative than the control group. (histology) Compared to the control group, there is a significantly lower histology score consistent with improved bone healing and absence of infection in the treated animals. | Clinical X-ray, Micro CT Histology Microbiological analysis | All animals survived and completed the study, the infection group showed clear instability of the fracture site |
| Schauer, T. P. et al. | N,N-dodecylmethylpolyethyleneimine-derivatised coating on fixations | (histology) Compared to the control group, there is a significantly lower histology score consistent with improved bone healing and absence of infection in the treated animals. | Clinical X-ray, Micro CT Histology Microbiological analysis | Unstable osteotomy was observed in the control group at the time of explant. |
| Robinson, D. A. et al. | Ceftriaxone, systemic, subcutaneous injection | (X-ray) The fracture callus did not bridge the fracture site, but a small amount of new bone formation was observed at 21 days with intervention. Images of the control group were characterised by the formation of a normal bridging callus. (histology) Specimen of the intervention group contained a bridging callus that was composed primarily of new bone, similar to the control group. | X-ray Histology Microbiological analysis | 2 of 30 were euthanised due to incisional dehiscence and self-mutilation. |

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intermediate in size, have allowed the study of local antibiotics [53,54], implant devices [56] and the use of pulsatile lavage irrigation systems [58]. With the development of smaller implants and better fixation techniques, it has provided more possibilities of small animal experiments in recent years. Although a Haversian system is absent in the bone structure of rodents, their bone remodelling mechanism using resorptive cavities remains similar to that of human [67]. Furthermore, the use of small animals is less costly and easy to handle. In respect to their immune functions, however, the immune genes of rodents are thought to be more phylogenetically distant to humans than rabbits and some larger animals [68–70]. Mice engraved with humanized immune systems could be a possible solution if all facets of pathogenesis in clinical infections are warranted [71]. In our reviewed literature, murine models had been utilised to explore a range of osteo-inductive agents [40,45–47,49,51] and antibiotic strategies [32,36,40,44–46,48,52] in combating infection on the course of bone healing. Whether these interventions conducted in small animals can be extrapolated to clinical practice, however will require further investigations for delivery dosage in clinical trials. Clinicians and researchers should consider their individual study needs, surgical skills and resources, and are encouraged to select a lower-order animal initially.

\textbf{S. aureus} is the most common organism in bone infection [72]. As demonstrated in our reviewed studies, the bacteria can form a potent biofilm and can cause osteolysis [55,56], periostal reaction [55] and fracture non-union [35,43,56] which are frequently encountered in clinical bone infection. Therefore, most infection studies used \textit{S. aureus} as the organism of choice. However, although Staphylococcus infection is often encountered, polymicrobial infections are also common and often cause complications [63]. Retrospective studies in health settings have reported that 21%-31% of cultured positive, osteomyelitis cases were polymicrobial [63,73,74]. Animal models that mimic this situation would also closely resemble the clinical settings of bone infection associated with open fracture [75,76]. These models are lacking and future studies should have development in this area.

The inoculum load ranged from $10^2$ to $10^{10}$ CFU in the current literature. In 2 dose responsive studies, it was found that a bacterial inoculum of less than $10^4$ CFU resulted in unsuccessful and inconsistent infection [39,56]. The low infection rates was consistent to pilot work associated with open fracture [39,41]. In overview, there is no consensus in the literature to indicate a standardized threshold or inoculum dose for the creation of a bone infection model, which may be variable due to the animal species, bacterial strain of individual studies and a number of factors such as fracture type and location [77]. Clinicians should consider the severity of bacterial infection required for the specific clinical scenario they wish to mimic.

| Source | Intervention | Radiological/histological evidence of osseous healing | Parameters assessed | Complication |
|--------|--------------|------------------------------------------------------|---------------------|--------------|
| Chen, X. et al. | Ceftriaxone, systemic, IM injection; recombinant human bone morphogenetic protein-2 (rhBMP-2), local, loaded in a collagen sponge and packed into fracture site | (micro-CT) Greatest amount of new bone formation which consistently and securely connected the ends of the defects occurred in group receiving both interventions at 12 weeks. (histology) Defects in animals treated with the rhBMP-2 and antibiotic exhibited the greatest amount of newly mineralised callus. Only minimal amount of new bone appeared to form within the defect or bridged the outside of the defects in the control group at 12 weeks. | X-ray, Micro CT Mechanical testing Histology Clinical Microbiological analysis | 3 out of 127 animals were excluded (2 at the time of anaesthetic administration, and 1 due to a femoral fracture that occurred during the placement of K-wires. |
| Chen, X. et al. | Ceftriaxone, systemic, IM injection; recombinant human osteogentic protein-1 (rhOP-1), local, loaded in a collagen carrier and packed into fracture site | (micro-CT, histology) Volume and areas of newly mineralised callus within the defect and bridging the outside of the defect increased with time after debridement and was greater with antibiotic treatment than without antibiotic treatment at 12 weeks after debridement in the defects that had been treated with higher dose of rhOP-1. | X-ray, Micro CT Mechanical testing Histology Microbiological analysis Clinical | No complication, the animals tolerate well from the operation and debridement. No signs of lameness, draining sinus or clinical symptoms indicative of systemic infection was observed during the study period. |
| Southwood, L. L. et al. | Adenoviral transfer of the bone morphogenetic protein-2 (Ad-BMP-2) gene, percutaneous injection | (X-ray) Rabbis in the intervention group had initial and bridging-caluse at earlier times than the control group. (histology) There was a trend that the intervention group has a higher grade for new bone at 14 and 28 days than the control group, but not statistically significant. | Microbiological analysis Clinical Histology X-ray | 19 out of 64 were euthanised before end-point for humane reason. No complications was mentioned in the paper |
| Caprise, P. A., Jr. et al. | High-pressure pulsatile lavage irritation | (micro-radiographs) Statistically significant difference in the amount of new bone formation postoperative day 14 in intervention group than other groups. (histology) No significant difference in new bone formation between the bulb syringe group and HPPL treatment. | Microbiological analysis Histology Micro-angiophotographs | 5 out of 40 died or euthanised before end-point due to symptoms of sepsis |
| Chen, X. et al. | Osteogenic protein-1 (OP-1), local, mixed with collagen and formed a mixture packed into the fracture site | (X-ray, histology) Bone formation inside and outside the defects with either dose of OP-1 at 63 days, were significantly greater than the untreated groups. | X-ray Histology Microbiological analysis Clinical | Loss of fixation was seen in some infected animals at 14 days. None of the infected animals exhibited a draining sinus, loss of weight commensurate with systemic involvement of infection, of limp of lameness during activity. |
Inoculums primarily using planktonic [34,35,39–44,47,53,54,56,57,59,60] and biofilm [55] bacteria were featured in our reviewed models. The majority of studies favor the use of planktonic inoculum, in which, bacteria were grown by batch culture, prepared as suspension and induced to the fracture site with an injectable device. In Zhang’s model, fixation devices were incubated in bacterial solution overnight and washed with phosphate-buffered saline (PBS) prior to fracture fixation [55]. The pretreatment allowed biofilm formation on the implant surface beforehand. Rochford et al. also performed a similar model, but the immersion time was shortened to 20 min followed by 5-min air-drying of the implants [33,37]. Other models employed the use of foreign objects that were soaked or premixed with bacteria solution, and placed in the fracture [32,36,40,45,46,50,52,58]. However, biofilm formation was not intended in these studies. Compared to planktonic bacteria, biofilm bounded bacterial cells are more likely to escape assault from the host immune defense [78] and antibiotics modalities [79,80]. Zhang’s model is thus more suitable in chronic infection models with established biofilm formation. The inoculation method was also claimed to be a more clinically relevant model [81] as a majority of the bacteria in the natural ecosystems reside as biofilm phenotype [82]. Theoretically, wound surface is more prone to contamination of biofilm bounded bacteria in orthopaedic trauma. However, given that both planktonic and biofilm had successfully produced consistent grade and highly reproducible infection models in our reviewed studies, either inoculation technique can be used to meet different research needs.

Biomechanical factor is a major element in animal fracture models. Mechanically stable fixtures contribute to early callus formation, increased vascularity and tissue differentiation and thus shorten time course of osseous union [83]. In general implant devices such as intramedullary wires, metal plates and screws were shown to provide course of osseous union [83]. In general implant devices such as intramedullary wires, metal plates and screws were shown to provide course of osseous union [83]. Fixation systems should therefore be rigid since instability can greatly influence healing outcomes and cause further soft tissue damage [84]. For animal models, there have been controversies on the fixation stability of K-wires compared to plate and screws [85]. However, more recent studies have shown K-wire fixation are able to promote osseous healing within a timeframe similar to plate devices in bacterial inoculated models [56]. The use of the intramedullary nailing device in open tibia fractures is also commonly used in the clinical setting [86]. Other fixation techniques include polyether ether ketone (PEEK) plate, polyacetate plates and cerclage wire in the animal studies [32,37,45–47,57]. The PEEK material is recently FDA approved and used in orthopaedics and dental implants. Different from metallic implant fixture, its radioluency had allowed clearer visualisation of osteolysis and reactive bone formation [32,37]. Compared to traditional stainless and titanium, however, PEEK material is less stiff and has a similar bone modulus to human bone which makes it less attractive for long bone fixation [87,88]. For polyacetate plates, there were no comparison studies for this polymer with metal related to strength [45–47]. In animal studies, it is important to note that not all material represent a clinically relevant scenario. If a specific implant material is used, researchers could provide more information related to implant rigidity. Further studies on fixation rigidity in an infection model could also guide evolving new implant devices and materials in combating bone infection. In summary, researchers should select a fracture and fixation technique that best replicate the common clinical situations in devising translational therapeutics treatment.

A range of assessments was performed in the literature. According to Centre for Disease Control and Prevention/National Healthcare Safety Network’s (CDC/NHSN) diagnosis for bone infection, patients were clinically identified with osteomyelitis based on four major criteria: microbiological testing of blood and bone; anatomic and histological evaluation; localized signs and symptoms; and most importantly, radiographic examination supported by clinician correlation [89]. To closely replicate the clinical diagnosis on osteomyelitis, it is recommended that an animal model should make reference to the CDC/NHSN’s, in which radiological evaluation and assessment of at least one of the other three criteria should be performed. All reviewed literature had included these components and provided sound evidence. However, quality difference was observed among the literature evidence. In respect to microbiological evidence, tissue culture such as bone, soft tissue and synovial fluid should be preferred than swab culture. In clinical settings, a lack of concordance was seen in bacterial recovery of tissue biopsies and swab specimens [90–92]. Despite not encountered in our reviewed literature, swab culture is easily contaminated, leading to false positive result [93], and has a lower sensitivity in detecting biofilm-bounded bacteria [94]. Sonication of the implant to dislodge bacterial cells is also a viable approach in bacteria culture [2]. One study utilised bioluminescent microbe which enables longitudinal capturing of bacterial metabolic activity [32]. The technology was innovative; however results should be examined together with other microbiological assessment due to limited penetration depth [95]. When assessing bone healing complicating with infection, radiographical assessment could be achieved semi-quantitatively with scoring systems. Some grading systems included the radiographic union score foribia (RUST) [59,54], the Lane and Sandhu score [42,49] and one developed by An YH and Friedman RJ [49,60]. Additionally, a histological score system proposed by Büren et al. achieved high sensitivity and specificity for assessing the severity in OAI model in mice [61]. Some employed a self-defined rating scheme that was not validated [34,35,47,57]. A reason for lack of coherence in grading system used in the reviewed literature could be due to missing components of bony lysis, sequestration, periosteal reactions and implant instability. Establishing a rating scale specific in preclinical bone infection studies in the future could provide researchers consistency and accuracy in performing bone assessment. Regarding the preclinical studies of OAI, a recent review study by Moriarty et al. also show the basic principles as well as common errors for outcomes assessment [96].

Complications appear to be a concern in reviewed literature. Due to fracture dislocation, surgical inaccuracy, severe loss of fixation and septic infection, deaths were unavoidable. Before the experiment, researchers should intelligently design the study to answer the research question; have standardized protocols for anaesthesia and pain management; prepare details for materials and methods; ensure protocols meet criteria established for a “justified animal study”; design humane end-points with scientific output; and address confounding variables. A pilot study is also recommended for model validation and powering. During the experiment, skillful surgeons should perform animal surgeries and a complication rate of less than 10% should be targeted [97].

Clinically, OAI with biofilm formation remains an unresolved dilemma. In the human body, bacteria residing in the biofilm usually has high number of cells. In fact, it has been shown that a biofilm consisting of as few as 10^3 to 10^4 bacteria cells could be sufficient for causing valid wound contamination [81]. The components of biofilm including polysaccharides and proteins would secure its structural integrity and cause high resistance to antibiotics and host defense. Hence, it is difficult to eradicate the infection and achieve solid bone union even with long-term systemic antibiotic treatment. This was also confirmed by a recent review study of Croes et al., which suggested that there could be an inconsistency in the effect of systemic antibiotics on microbiological, clinical, and bone healing results in the context of latent infection [98].

To prevent or eradicate infection with biofilm formation, various strategies including Copper-bearing materials with biocidal anti-adhesive surface, antibiotic-coated implant, and hydrogel delivery of antibiotics and antimicrobial peptide have been proposed [99,100]. Based on current results, the use of systemic antibiotics in general improved bridging of the infected fracture. However, the most useful interventions were antibiotic-coated implants or the use of antibiotic loaded hydrogels, which allows effective fracture healing. The authors would therefore recommend the use of these interventions for potential translation into clinical use after further clinical trials.
There is an emerging need to develop new treatment strategies for osteosynthesis associated infection. In conclusion, most studies concentrate on S. aureus species, which is the most common bacteria in osteosynthesis-associated infections. Based on the fracture model, different interventions have been investigated on their efficacy in eradicating the bacteria. However, the emergence of antibiotic resistance organisms is a rising issue and more effort is needed to simulate other clinical scenarios of osteosynthesis associated infection. Furthermore, recently there has also been an increasing concern of an aging population. As immunity is strongly linked to estrogen deficiency, postmenopausal women are also at high-risk group for bone infection. An animal model that looks at fracture healing complicated with both bone infection and osteoporosis is thus worthwhile for future studies. Further studies on animal models should also investigate on poly-microbes and antibiotic resistant strains, as these will no doubt play a major role in bone infections.

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Conflict of Interest

The authors have no conflicts of interest to disclose in relation to this article.

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