

ICM 2025 Question B11: “How should antimicrobial properties of an orthopaedic titanium implant be evaluated in animal and clinical studies?”

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RESPONSE/RECOMMENDATION: The antimicrobial properties of orthopaedic titanium implants should be evaluated using a combination of *in vitro*, animal, and clinical studies to ensure comprehensive assessment of safety and efficacy. Animal models provide crucial insights into the biological interactions of implants with host tissues and pathogens, while human studies validate clinical applicability. To effectively evaluate the antimicrobial properties of new orthopaedic titanium implants, it is recommended to use a combination of well-established animal models, appropriate bacterial species, standardized inoculation doses, and comprehensive analytical techniques.

LEVEL OF EVIDENCE: Moderate. While there is substantial evidence supporting the evaluation methods, variations in study design, bacterial strains, and outcome measures necessitate further standardization to strengthen clinical translation.

DELEGATE VOTE: Agree: [42/98% vote], Disagree: [0/0%], Abstain: [1/2%]

RATIONALE: Eligibility criteria: A systematic literature review was conducted of the PubMed and Web of Science databases. Due to the immense number of scientific articles on this topic (2747 studies screened), this review only included studies published from 2015 onwards, focusing on animal models and human studies that evaluate the effectiveness and safety of antimicrobial implants made of titanium (Ti) and its alloys. Review articles, studies restricted to purely *in vitro* models, or focusing on non-orthopaedic devices or biomaterials other than Ti were excluded. Included interventions were antibacterial coatings, nanomaterials, surface modifications, and localized drug delivery systems applied to the implants to prevent bacterial colonisation. Excluded interventions were antimicrobial treatments not part of the implant itself, such as systemic or local antibiotics, debridement treatments, *in situ* sonication, and electrical stimulation. Included outcomes were reduction in biofilm formation, bacterial adhesion, infection rates, antibiotic resistance, bacterial viability, inflammatory response, histological analysis, clinical outcomes, and functional outcomes. Studies that only assessed biocompatibility or osseointegration were excluded.

Introduction: Ti implants are widely used in orthopaedics due to their excellent biocompatibility, mechanical strength, and corrosion resistance. However, implant-associated infections remain a significant challenge, necessitating the development and evaluation of antimicrobial strategies. While *in vitro* models provide initial insights into antimicrobial efficacy, their relevance is limited due to the absence of complex host factors such as immune responses and tissue integration. This review provides an overview of the recent animal and human models used to evaluate the antimicrobial properties of orthopaedic Ti implants. Of the 51 studies analysed, 42 utilized animal models and 9 were human clinical studies (2 randomized controlled trials, 6 cohort studies, and 1 case-control study).

Methodological considerations:

***In vitro* models** are essential for preliminary screening of antimicrobial properties before progressing to *in vivo* studies. *In vitro* models allow for controlled experimentation on bacterial adhesion, biofilm formation, and antimicrobial efficacy under standardized conditions. Common *in vitro* techniques include colony forming unit (CFU) counting, live/dead staining, crystal violet staining, and imaging using confocal laser-scanning microscopy (CLSM) and scanning electron microscopy (SEM). *In vitro* models provide valuable insights into the mechanisms of action of antimicrobial strategies and help refine experimental conditions for subsequent *in vivo* studies. Animal models and human studies both play crucial roles in evaluating the antimicrobial properties of orthopaedic Ti implants.

Animal models of orthopaedic-device related infection (ODRI) offer controlled environments and the ability to perform invasive procedures, providing valuable insights into the implant's effectiveness in preventing infections. The most frequently used animal species in the studies are rats, rabbits, and mice, selected for their cost-effectiveness and translational relevance to human bone physiology. For instance, Sprague-Dawley rats, New Zealand White rabbits, and C57BL/6 mice are commonly employed due to their manageable size and well-characterized immune responses. In addition, sheep and minipigs were also used. The most common sample size per experimental group was $n=6$ for rabbits,¹⁻⁵ $n=5-10$ for rats and mice,⁶⁻⁸ and $n=7$ for sheep and minipigs.^{9; 10} The inoculum (CFU and volume) used to induce infection must be appropriate for the animal species and body region, and determined through preliminary pilot studies. Too large an inoculum can cause sepsis and death, while too small a dose may be cleared rapidly. The time required for infection establishment varies by species and differs from clinical cases. Factors such as bacterial strain virulence and growth phase influence the inoculum concentration needed. The choice of bacterial species depends on whether the model targets acute (for example, *Staphylococcus aureus*) or chronic infections (such as, coagulase-negative staphylococci). Reflecting its clinical relevance in implant-associated infections, *S. aureus* was the predominant bacterial species used to induce infections (32 studies), with strains such as ATCC 25923 and Xen29 being most common. The inoculation doses to induce infections mimicking clinical conditions vary, with typical doses being 10^6-10^8 CFU/ml for rabbits and 10^6 CFU/ml for rats. Most animal models have so far utilized an initial inoculum of planktonic bacterial cultures. Introducing mature biofilms grown on an implant as the initial inoculum in animal models could further optimise antimicrobial treatment strategies.¹¹ In addition, bone remodelling kinetics differ between animals and humans, affecting study duration. To enhance clinical relevance in weight-bearing applications, implant placement should allow weight-bearing movement. The types of implants used include rods, screws, nails, pins, and plates, often placed in the femur or tibia to simulate clinical scenarios.¹²⁻²¹ Typical outcomes measured include bacterial viability, infection rates, clinical outcomes, inflammatory response, and reduction in biofilm formation. Evaluation time-points range from 1 week to several months, depending on the study design. For instance, Zhou *et al.* (2017) evaluated infection reduction and clinical outcomes over 8 weeks in a rabbit model.⁵

Clinical Studies often employ cohort or randomized controlled trial (RCT) designs. The populations studied include patients undergoing orthopaedic surgeries with increased infection risk, such as those with fractures or joint replacements. Similar to the animal studies, clinical studies evaluated antibiotic-loaded coatings, silver coatings, and other antimicrobial strategies. For instance, gentamicin-coated intramedullary nails and silver-coated mega prostheses have been investigated for their efficacy in preventing infections. Among the 9 human studies evaluated, all included clinical outcome measurements, such as patient recovery rates and implant success rates, 6 measured infection rates, and 4 assessed functional outcomes, such as mobility and pain levels.²²⁻³⁰ Evaluation time-points range from several months to years, 1 year being a common follow-up timepoint. For example, DeMeo *et al.* (2023) reported infection rates and clinical outcomes over a follow-up period of 34.41 ± 9.46 months,²² and Kawano *et al.* (2023) reported clinical and functional outcomes for 5 years.²⁴

Outcome evaluation and analytical techniques: Commonly measured **outcomes** for evaluating the antimicrobial properties of Ti orthopaedic implants in animal and clinical studies include: (1) **Bacterial viability**, which assesses the proportion of live versus dead bacteria to determine the implant's antimicrobial effectiveness (killing or inhibition). Common methods include live/dead staining, microbiological cultures, metabolic assays, ATP quantification, and qPCR targeting viable bacteria. (2) **Bacterial adhesion** quantifies the initial bacterial attachment to the implant surface, indicating how well the implant prevents colonization. Measurement methods include CFU counting after surface detachment by sonication and vortexing, fluorescence microscopy, crystal violet staining, and SEM imaging. (3) **Reduction in biofilm formation** measures the implant's ability to prevent or reduce mature biofilms, which are hard to treat. Methods for assessing biofilm formation include crystal violet staining for biofilm biomass quantification, CLSM, SEM imaging, and CFU counting from biofilm dispersal. (4) **Infection rates** track the incidence and severity of infections post-implantation to assess the clinical

effectiveness of antimicrobial strategies. Methods for measuring infection rates include clinical observation, microbiological cultures, imaging (X-ray, MRI), and infection scoring systems. (5) **Clinical outcomes** assess patient health and implant success, including recovery rates and complication rates, implant integration, and overall health outcomes following implantation. Measurement methods include clinical examination, patient-reported outcomes (including pain, quality of life, imaging, and complication tracking). (6) **Inflammatory response** monitors local and systemic inflammatory markers to detect infections or adverse reactions caused by the implant. Methods for assessing inflammatory response include blood tests, histological analysis, and immunohistochemistry. Fewer studies include (7) **functional outcomes** to evaluate the implant's impact on physical function, ensuring it does not impair performance.^{5;} ²⁶ Measurement methods include mobility tests, range of motion assessments, pain scales, and functional scoring systems. (8) **Antibiotic resistance** evaluates whether bacteria exposed to the implant develop resistance to antibiotics over time, which could compromise long-term antimicrobial effectiveness. Measurement methods include antibiotic susceptibility testing (MIC), detection of resistance genes, and whole-genome sequencing. Interestingly, only one of the studies evaluated antibiotic resistance after exposure to the implant.²² Recent technological advances have significantly enhanced the evaluation of antimicrobial properties in orthopaedic implants. Technologies such as 3D-printing, bacterial genomic sequencing, and real-time *in vivo* imaging have improved preclinical models, making them better at mimicking clinical infections and evaluating antimicrobial materials. These advances enable more accurate and detailed assessments of how implants interact with bacteria and the host immune system. Among the evaluated animal studies, the most frequently employed analytical techniques were: (1) **histology** (90%) to examine tissue samples for signs of infection, inflammation, and bone integration (H&E, Gram, Giemsa, and ALP/TRAP staining were commonly used); (2) **CFU counting** (67%) to quantify the number of viable bacteria on the implant surface and surrounding tissues; (3) **radiographic imaging (X-ray)** (36%) to evaluate bone healing, implant integration, and signs of infection; (4) **haematological analyses** (29%) of inflammatory markers (white blood cell counts (WBC), C-reactive protein (CRP), TNF- α , and IL-6) to assess the animal's inflammatory response to the implant; (5) **micro-computed tomography (micro-CT)** (19%) to provide detailed 3D images of the bone and implant interface; (6) **microscopy** for the visualisation of biofilms on implants or tissues (**SEM** (17%), **CLSM** combined with Live/Dead® BacLight™ staining (7%)); and (7) **bacterial bioluminescence** imaging using IVIS (*in vivo* imaging system) for bacterial tracking (17%).^{31; 32} These methods provide quantitative and qualitative assessments of ODRI and host response. Among the 9 human studies evaluated, outcomes were typically evaluated by: (1) **Bacterial culturing** of tissue biopsies,^{22; 29} (2) **radiographic imaging** for detection of osseointegration, implant stability, and signs of implant loosening and failure;^{22; 24-26} (3) **haematological analyses** including complete blood count (CBC) paired with serum analysis,²³ and (4) the use of various **scoring/classification systems** (such as Harris Hip Score (HHS),²³ Gustilo–Anderson classification for open fractures,²² Japanese Orthopaedic Association (JOA) score for spine,²⁴ ASEPSIS wound healing score,²⁵ Lower Extremity Functional Scale (LEFS),²⁷ Short Assessment of Patient Satisfaction (SAPS),²⁷ and Implant failure modified classification by Henderson *et al.*)³⁰

Antimicrobial strategies: The reviewed studies employed various antimicrobial strategies, most often including antibiotic-loaded coatings (such as gentamicin, vancomycin),^{33; 34} antiseptic-loaded coatings (including silver,^{23; 24; 28} iodine,³⁰ chlorhexidine³⁵⁻³⁷), or antimicrobial peptides (AMPs).^{6; 38; 39} These antimicrobial agents were often loaded in hydrogels, nanoparticles, or nanotubes bound to the Ti implant surface. Most of the strategies demonstrated efficacy in reducing bacterial colonization and preventing osteomyelitis. For example, gentamicin-coated implants demonstrated significant reductions in infection rates and improved clinical outcomes in both animal and human studies.^{27; 40} Similarly, silver-coated implants demonstrated effective antimicrobial properties and reduced bacterial colonization without significant adverse effects.^{41; 42} Localized drug delivery systems, using biodegradable polymers and nanomaterials,⁴³⁻⁴⁵ provided sustained release of antimicrobial agents, effectively reducing biofilm formation and infection rates.

In **animal studies**, various antimicrobial strategies have been evaluated for their effectiveness in preventing infections associated with Ti orthopaedic implants. For instance, the use of hydroxypropyltrimethyl ammonium chloride chitosan coatings on Ti exhibited significant infection control and reduced bone destruction.⁴⁶ Similarly, polymeric nanofiber coatings loaded with antibiotics significantly reduced infection rates and biofilm formation while enhancing implant integration.³³ The combination of gentamicin and vancomycin in fluorine- and phosphorus-doped nanotubular oxide layers effectively decreased biofilm density without compromising implant integration.⁴⁷ Other studies highlighted the benefits of surface modifications, such as the incorporation of AMPs and angiogenic sequences, which reduced infection rates and inflammatory markers while improving vascularization and osseointegration.⁴⁸ Additionally, the use of oligo-ampicillin hydrogels prevented bacterial colonization and osteomyelitis,⁴⁹ and an AMP-based coating (OP-145) significantly reduced bacterial colonization and infection signs.⁵⁰ Various other strategies, including silver coatings, hierarchical TiO₂ nanotubes loaded with cinnamaldehyde, and antibiotic-loaded hydrogels, also demonstrated promising results in reducing infection rates, biofilm formation, and inflammatory responses.^{1; 51; 52}

Human studies have also explored the efficacy of different antimicrobial coatings on orthopaedic implants. For example, gentamicin-coated nails were effective in curing infections, promoting bone healing, and yielding good patient-reported outcomes.²⁷ Similarly, antibiotic-loaded hydrogel coatings significantly reduced fracture-related infections, although some complications were noted.²² Silver-coated implants demonstrated lower infection rates and improved clinical outcomes in various studies.^{24; 29} The use of iodine-coated Ti implants revealed a lower infection incidence in both prevention and treatment cases, with no significant difference between one-stage and two-stage replacements.³⁰ In addition, antibiotic-loaded resorbable hydrogels significantly reduced surgical site infections without adverse events.²⁵

Conclusion: Most studies reviewed demonstrate a statistically and clinically significant reduction in infection rates and improvement in clinical outcomes with antimicrobial coatings on orthopaedic Ti implants. A multi-tiered approach incorporating *in vitro*, animal, and clinical studies is essential for evaluating the antimicrobial properties of orthopaedic Ti implants, ensuring safety and efficacy before clinical introduction. Recent technological advances have further improved the evaluation process, enabling more accurate and detailed assessments. Most animal models of ODRI use *S. aureus*, but future studies should also include other clinically relevant bacteria, including coagulase-negative staphylococci, a common cause of chronic infections. Future research should also focus on antibiotic resistance development, which can significantly compromise the long-term effectiveness of antimicrobial strategies in orthopaedic implants. This will enhance the overall success and sustainability of antimicrobial treatments in orthopaedic applications.

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