

**ICM 2025 Question B15 “Are we certain that biofilms are the main challenge of treating implant-associated infection?”**

**Chao Xie, Tom Coenye, Margarita Trobos, Xin Qi, Leibnitz J Martinez, Hatem Bakr, Mark Smeltzer, Bingyun Li, Edward M Schwarz**

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**RESPONSE/RECOMMENDATION:** Yes, implant-associated infections are notoriously difficult to treat, often leading to chronic complications, implant failure, and high healthcare costs. Microbial biofilms are widely recognized as the primary challenge in managing these clinical infections, and preclinical studies have demonstrated that biofilm fulfills Koch's postulates as the etiologic factor in implant-associated infections<sup>1</sup>.

**Level of Evidence: Strong**

**Delegate Vote: Agree: [36/92% vote], Disagree: [1/3%], Abstain: [2/5%]**

**Rationale:** Implant-associated infections (IAIs) represent a major clinical challenge, frequently resulting in prolonged patient morbidity, implant failure, and substantial healthcare costs. To synthesize current insights, our systematic review process began with 1,522 articles identified through PubMed keyword searches. Following title screening, abstract review, and full-text evaluation, 135 articles were selected, with 80 primary research studies and systematic reviews ultimately meeting inclusion criteria. Among these, biofilm formation emerged as a dominant theme: 33 studies specifically investigated *Staphylococcus* species (notably *S. aureus* and *S. epidermidis*) and their role in biofilm development on orthopedic implants, underscoring its critical contribution to periprosthetic infections. Antibiotic resistance and tolerance were addressed in 16 studies, while 9 additional works proposed novel therapeutic strategies to combat these issues. Implant material design and its relationship to infection risk were explored in 9 studies, and 12 studies focused on unraveling the pathogenesis and molecular mechanisms driving bacterial infections. A subset of 8 studies analyzed bacterial behavior and host immune response interactions, supplemented by 5 studies examining inflammation and immune dynamics. Preclinical validation was provided by 8 studies employing *in vivo* infection models, and 6 works advanced diagnostic and identification methodologies. Finally, 5 studies addressed miscellaneous topics, collectively illustrating the breadth of research addressing IAIs.

**Biofilm formation and reduced antimicrobial susceptibility:** Biofilms are structured microbial communities embedded in extracellular polymeric substances (EPS) composed of polysaccharides, proteins, and extracellular DNA (eDNA)<sup>2-4</sup>. *Staphylococcus* spp., particularly *S. aureus* and *S. epidermidis*, commonly cause IAIs due to their robust biofilm-forming capabilities<sup>5-9</sup>. These pathogens adhere to implant surfaces and components of the host extracellular matrix via a wide array of surface adhesins such as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and secretable expanded repertoire adhesive molecules (SERAMs), autolysin/adhesins, wall teichoic acids (WTA) and lipoteichoic acids (LTA)<sup>3,10-12</sup>. The *icaADBC* operon, responsible for synthesizing polysaccharide intercellular adhesin (PIA), contributes to the intercellular adhesion within biofilms<sup>3,13-15</sup>. However, biofilm formation can also occur through PIA-independent mechanisms, including accumulation-associated protein (Aap) or extracellular eDNA<sup>3,16</sup>. Biofilms confer protection against antibiotics through multiple mechanisms: (1) reduced metabolic activity and reduced growth rates in cells from deeper layers, (2) EPS acts as a physical barrier, and (3) persister/dormant cell variants tolerant to antibiotics<sup>17-19</sup>. For example, *S. aureus* biofilms exhibit MIC<sub>90</sub> values up to 245 µg/mL for ciprofloxacin, far exceeding planktonic MICs (0.07-2.80 µg/mL)<sup>20</sup>. Similarly, *S. epidermidis* biofilms resist vancomycin and daptomycin, even at supratherapeutic doses<sup>21-23</sup>. Rifampin and doxycycline show improved biofilm penetration, though

eradication typically requires prolonged high-dose regimens<sup>24,25</sup>. Antoci et al. demonstrated that vancomycin-coated titanium (Ti) alloys prevent *S. epidermidis* colonization but are ineffective against *Escherichia coli*, underscoring antibiotic specificity<sup>26</sup>. Some studies confirmed the clinical significance of biofilms, Sevansson Malchau et al. showed that patients with periprosthetic joint infection (PJI) caused by strong biofilm-producing staphylococci had a fivefold greater risk of recurrent infection<sup>27</sup>. Morgenstern et al. linked strong biofilm production in *S. epidermidis* to higher osteomyelitis treatment failure rates<sup>27</sup>, and Hagstrand Aldman et al. linked strong biofilm production in *S. lugdunensis* to PJI recurrence<sup>28</sup>. Taha et al. reported that combining povidone-iodine with vancomycin reduce *S. aureus* in immature biofilms. This dynamic was visualized in real-time by Xie et al. through longitudinal intravital imaging of osteomyelitis<sup>29-32</sup>.

**Biofilms actively suppress host immune responses to establish chronic infections.** *S. aureus* biofilms secrete virulence factors such as phenol-soluble modulins (PSMs) and leukocidins, which impair neutrophil chemotaxis and promote immunosuppressive IL-10 production<sup>33,34</sup>. Neutrophils trapped within biofilms undergo NETosis, releasing eDNA that stabilizes the biofilm matrix<sup>7,35</sup>. Macrophages attempting phagocytosis often fail to penetrate biofilms and instead adopt anti-inflammatory phenotypes, further impairing bacterial clearance<sup>34,36-38</sup>. Biofilm persistence is strongly linked to chronic infections. In PJIs, biofilms survive despite aggressive surgical debridement and systemic antibiotics<sup>27,39</sup>. Animal models demonstrate that *S. aureus* biofilms on Ti implants resist immune clearance for weeks, even in immunocompetent hosts<sup>40,41</sup>. A “race for the surface” co-culture study, showed that when *S. aureus* establishes a biofilm, it disrupts macrophage function and induces cell death through upregulation of FcγR and TLR-2 receptors, NF-κB signaling, and NOX2-mediated reactive oxygen species production<sup>42</sup>. This leads to a persistent biofilm phenotype with upregulated *clfA*, *icaA*, and *sarA*, and downregulated *agrA*, *hld*, and *lukAB*. The upregulation of *lipA* supports intracellular survival. Clinically, this results in bacterial colonization of the implant and persistence of intracellular bacteria in periprosthetic tissues, contributing to chronic infection<sup>42,43</sup>. The *mazEF* toxin-antitoxin system in *S. aureus* further enhances chronicity by promoting biofilm antibiotic tolerance and modulating virulence during acute phases<sup>17,44</sup>. Bell et al. reported that *S. aureus* biofilms induce PD-1 ligands and IL-1 receptor antagonist (IL-1RA), suppressing T-cell responses<sup>33</sup>. Nishitani et al. used a murine tibia model and quantified biofilm maturation, observing peak bacterial loads 3-7 days post-implantation<sup>40</sup>. Morales-Laverde et al. identified strain-specific expression of adhesion genes (e.g., *clfA*, *fnbA*) that modulate biofilm formation by enhancing bacterial adherence and aggregation, and influence host immune responses by evading immune detection and promoting chronic infection<sup>45</sup>.

**Detecting biofilm-associated bacteria presents significant diagnostic challenges:** Conventional culture methods often struggle to detect bacteria inside biofilms due to their sessile nature and low metabolic activity<sup>46,47</sup>. However, sonication of explanted implants improves sensitivity by dislodging biofilm bacteria, as demonstrated in a landmark study where sonicate-fluid cultures detected 78.5% of PJIs versus 60.8% with tissue cultures<sup>46</sup>. Molecular techniques like PCR-mass spectrometry (Ibis T5000) and nuclease-based probes (e.g., AttoPolyT) enable rapid, culture-free detection<sup>48,49</sup>. For example, the AttoPolyT probe targets *S. aureus* nuclease activity, achieving 90% accuracy in synovial fluid samples<sup>49,50</sup>. More recently the use of isothermal microcalorimetry (IMC) to detect microorganisms in tissue samples and synovial fluid was evaluated<sup>51,52</sup>. The accuracy of IMC was found to be at least as good as culture, but IMC delivers results much faster. Weaver et al. used whole-genome shotgun sequencing to uncover polymicrobial biofilms in PJIs, challenging traditional monoculture paradigms<sup>50</sup>. Zatorska et al. correlated elevated eDNA levels in clinical *S. aureus* isolates with greater amounts of biofilm formation, suggesting eDNA as a diagnostic marker<sup>7</sup>.

## **Therapeutic strategies**

1) **Antibiotics:** Susceptibility testing methods, minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC), are being explored to guide therapy in PJI<sup>53</sup>. Rifampin and doxycycline are preferred for staphylococcal biofilms due to penetration into EPS<sup>24,25,27,54</sup>. However, resistance emerges rapidly if used as monotherapy. Combinations like rifampin + vancomycin or daptomycin + linezolid show synergy in static and dynamic biofilm models<sup>55-57</sup>.

2) **Phage therapy:** Phages disrupt biofilms by lysing bacterial cells and degrading EPS and are being used for a range of difficult-to-treat infections. The use of phages for treating PJI has recently been reviewed<sup>58,59</sup>

3) **Local therapies:** (i) Antimicrobial coatings: Vancomycin-modified Ti and chitosan-infused surfaces reduce biofilm adhesion<sup>26,60,61</sup>. (ii) Biofilm disruptors: DNase and EDTA degraded eDNA and chelated metal ions, respectively, weakening biofilm structure<sup>35,62,63</sup>. (iii) Electromagnetic induction: A portable device heating Ti implants to 70°C reduced *S. aureus* biofilms by 3-6 log<sub>10</sub> CFU<sup>64</sup>.

4) **Immunomodulation:** The host pathways (e.g., JAK/STAT) may reduce biofilm virulence. Turner et al. found that sodium salicylate inhibits *S. aureus agr*, reducing toxin production<sup>65</sup>. Sun et al. developed simvastatin-hydroxyapatite coatings that inhibit biofilm formation and enhance osteogenesis in rat models<sup>66</sup>. Ding et al. developed a nanoparticle system that disrupts bacterial iron metabolism, enhancing the effectiveness of cefiderocol against *P. aeruginosa* biofilms<sup>67</sup>. Biomaterial modifications, such as vancomycin-povidone-iodine coating<sup>29</sup> and camel peptides, show efficacy against staphylococcal biofilms<sup>68</sup>. Non-antibiotic interventions, including electromagnetic heating<sup>64</sup> and biofilm-focused clinical guidelines, highlight multidisciplinary solutions<sup>69</sup>.

**Variability and exceptions:** While biofilms dominate IAIs, exceptions exist: 1) Non-biofilm pathogens: *Escherichia coli* colonizes implants via flagellar motility rather than biofilms<sup>26</sup>. Small-colony variants (SCVs): SCVs of *S. aureus* persist intracellularly, evading antibiotics and immune cells<sup>34</sup>. 2) Strain-specific differences: *S. epidermidis* PJI isolates often carry *icaADBC* and IS256, while commensal strains lack these genes<sup>6</sup>. Fernandes & Dias reported *Candida krusei* PJI, as a rare non-bacterial biofilm case<sup>70</sup>. ~~Hagstrand Aldman et al. linked strong biofilm production in *S. lugdunensis* to PJI recurrence<sup>28</sup>.~~

## **Clinical and research implications:**

1) Standardized models: Current *in vitro* models (e.g., microtiter plates) may lack physiological relevance. The use of host cells and proteins and dynamic models (e.g., CDC biofilm reactor) could be used to mimic *in vivo* scenarios where host-bacteria interactions, shear stress, and nutrient gradients are relevant, improving translational validity<sup>71,72</sup>. The use of synovial fluid-based biofilm models (using human, animal or synthetic synovial fluid) allows to study biofilm aggregates and can further increase physiological relevance<sup>73,74</sup>.

2) Personalized medicine: Genomic profiling of biofilm-related genes (e.g., *ica*, *agr*) could guide therapy<sup>9,13</sup>. Chen et al. identified *fnbA* and *clfA* as predictors of *S. aureus* PJI severity<sup>75</sup>.

3) Biomaterial innovations: Magnesium alloys: Degradable magnesium implants induce localized alkalization, inhibiting *P. aeruginosa* biofilms<sup>76</sup>. Liquid-infused surfaces (LIS): Chitosan-conjugated LIS coatings repelled bacteria while promoting osteoblast adhesion<sup>60</sup>.

4) Adjunctive strategies include immunotherapy: Anti-staphylococcal vaccines targeting biofilm antigens (e.g., PNAG) are in preclinical trials<sup>77</sup>. Saeed et al. outlined ICM consensus guidelines, prioritizing biofilm-targeted research<sup>78</sup>. MacConnell et al. reviewed novel irrigation systems (e.g., XPERIENCE™) combining pulsatile lavage with antimicrobials<sup>47,69,79</sup>.

**Conclusion:** Biofilms are the central challenge in IAIs, driving antibiotic resistance, immune evasion, and chronicity. While exceptions exist, such as non-biofilm-forming pathogens or intracellular persistence, the

preponderance of evidence from 80 studies underscores biofilm dominance. Advances in diagnostics (e.g., sonication, nuclease probes), therapies (e.g., phage-antibiotic combinations, biomaterials), and personalized approaches (e.g., genomic profiling) are critical to improving outcomes. Future research must prioritize standardized physiological *in vivo* models, rapid diagnostics, and clinical trials of innovative biofilm-targeting (including biofilm-disrupting) agents. As Giordano and Giannoudis aptly noted, "The battle against biofilm is a marathon, not a sprint," requiring multidisciplinary collaboration to translate laboratory breakthroughs into clinical success<sup>80</sup>.

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