B15: "Are we certain that biofilms are the main challenge of treating implant-associated infection?"

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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[1].

Level of Evidence: Strong

Delegate Vote: Agree: [% vote], Disagree: [%], Abstain: [%]

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 an extracellular polymeric substances (EPS) composed of polysaccharides, proteins, and extracellular DNA (eDNA)[2-4]. *Staphylococcus* spp., particularly *S. aureus* and *S. epidermidis*, commonly cause IAIs due to their robust biofilm-forming capabilities[5-9]. 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)[3,10-12]. The *icaADBC* operon, responsible for synthesizing polysaccharide intercellular adhesin (PIA), contributes to the intercellular adhesion within biofilms[3,13-15]. However, biofilm formation can also occur through PIA-independent mechanisms, including accumulation-associated protein (Aap) or extracellular eDNA[3,16]. 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 barriers, and (3) persister/dormant cell variants tolerant to antibiotics[17-19]. For example, *S. aureus* biofilms exhibit MIC90 values up to 245 μg/mL for ciprofloxacin, far exceeding planktonic MICs (0.07-2.80 μg/mL)[20]. Similarly, *S. epidermidis* biofilms resist vancomycin and daptomycin, even at supratherapeutic doses[21-23]. Rifampin and doxycycline show improved biofilm penetration, though eradication typically requires prolonged high-dose regimens[24,25]. Antoci et al.

demonstrated that vancomycin-coated titanium alloys prevent *S. epidermidis* colonization but are ineffective against *Escherichia coli*, underscoring antibiotic specificity[26]. Some studies confirmed the clinical significance of biofilms, Sevensson Malchau et al. showed that patients with prosthetic joint infection (PJI) caused by strong biofilm-producing staphylococci had a fivefold greater risk of recurrent infection[27]. Morgenstern et al. linked strong biofilm production in *S. epidermidis* to higher osteomyelitis treatment failure rates[27], and Hagstrand Aldman *et al*. linked strong biofilm production in *S. lugdunensis* to PJI recurrence[28]. 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. [29-32].

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[33,34]. Neutrophils trapped within biofilms undergo NETosis, releasing eDNA that stabilizes the biofilm matrix[7,35]. Macrophages attempting phagocytosis often fail to penetrate biofilms and instead adopt anti-inflammatory phenotypes, further impairing bacterial clearance[34,36-38]. Biofilm persistence is strongly linked to chronic infections. In PJIs, biofilms survive despite aggressive surgical debridement and systemic antibiotics[27,39]. Animal models demonstrate that *S. aureus* biofilms on titanium (Ti) implants resist immune clearance for weeks, even in immunocompetent hosts[40,41]. 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[42]. 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[42,43]. The *mazEF* toxin-antitoxin system in *S. aureus* further enhances chronicity by promoting biofilm antibiotic tolerance and modulating virulence during acute phases[17,44]. Bell et al. reported that *S. aureus* biofilms induce PD-1 ligands and IL-1 receptor antagonist (IL-1RA), suppressing T-cell responses[33]. Nishitani et al. used a murine tibia model quantified biofilm maturation and observing peak bacterial loads 3-7 days post-implantation[40]. 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[45].

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[46,47]. 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[46]. Molecular techniques like PCR-mass spectrometry (Ibis T5000) and nuclease-based probes (e.g., AttoPolyT) enable rapid, culture-free detection[48,49]. For example, the AttoPolyT probe targets *S. aureus* nuclease activity, achieving 90% accuracy in synovial fluid samples[49,50]. More recently the use of isothermal microcalorimetry (IMC) to detect microorganisms in tissue samples and synovial fluid was evaluated[51,52] 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[50]. Zatorska et al. correlated elevated eDNA levels in clinical *S. aureus* isolates with greater amounts of biofilm formation, suggesting eDNA as a diagnostic marker[7].

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[53]. Rifampin and doxycycline are preferred for staphylococcal biofilms due to penetration into EPS[24,25,27,54]. However, resistance emerges rapidly if used as monotherapy. Combinations like rifampin + vancomycin or daptomycin + linezolid show synergy in static and dynamic biofilm models[55-57].
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 [58,59]
3) Local Therapies: (i) Antimicrobial Coatings: Vancomycin-modified titanium and chitosan-infused surfaces reduce biofilm adhesion[26,60,61]. (ii)Biofilm Disruptors: DNase and EDTA degrade eDNA and chelate metal ions, respectively, weakening biofilm structure[35,62,63]. (iii)Electromagnetic Induction: A portable device heating Ti implants to 70°C reduced S. aureus biofilms by 3-6 $\log_{10}$ CFU[64].
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[65]. Sun et al. developed simvastatin-hydroxyapatite coatings that inhibit biofilm formation and enhance osteogenesis in rat models[66]. Ding et al. developed a nanoparticle system that disrupts bacterial iron metabolism, enhancing the effectiveness of cefiderocol against *P. aeruginosa* biofilms[67]. Biomaterial modifications, such as vancomycin-povidone-iodine coating[29] and camel peptides, show efficacy against staphylococcal biofilms[68]. Non-antibiotic interventions, including electromagnetic heating[64] and biofilm-focused clinical guidelines, highlight multidisciplinary solutions[69].

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

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 mimic *in vivo* scenarios where host-bacteria interactions, shear stress, and nutrient gradients are relevant, improving translational validity[71,72]. 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[73,74].
2) Personalized Medicine: Genomic profiling of biofilm-related genes (e.g., ica, agr) could guide therapy[9,13]. Chen et al. identified fnbA and clfA as predictors of *S. aureus* PJI severity[75].
3) Biomaterial Innovations: Magnesium Alloys: Degradable magnesium implants induce localized alkalization, inhibiting *P. aeruginosa* biofilms[76]. Liquid-Infused Surfaces (LIS): Chitosan-conjugated LIS coatings repelled bacteria while promoting osteoblast adhesion[60].
4) Adjunctive Strategies include immunotherapy: Anti-staphylococcal vaccines targeting biofilm antigens (e.g., PNAG) are in preclinical trials[77]. Saeed et al. outlined ICM consensus guidelines, prioritizing biofilm-targeted research[78]. MacConnell et al. reviewed novel irrigation systems (e.g., XPERIENCE™) combining pulsatile lavage with antimicrobials[47,69,79].

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 75 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[80].

References:

1. Ehrlich GD, Arciola CR. From Koch's postulates to biofilm theory. The lesson of Bill Costerton. *Int J Artif Organs*. Oct 2012;35(10):695-9. doi:10.5301/ijao.5000169
2. Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials*. 2012;33(26):5967-82. doi:10.1016/j.biomaterials.2012.05.031
3. Campoccia D, Montanaro L, Ravaioli S, Pirini V, Cangini I, Arciola CR. Exopolysaccharide production by Staphylococcus epidermidis and its relationship with biofilm extracellular DNA. *Int J Artif Organs*. 2011;34(9):832-9. doi:10.5301/ijao.5000048
4. Kavanaugh JS, Flack CE, Lister J, et al. Identification of Extracellular DNA-Binding Proteins in the Biofilm Matrix. *mBio*. 2019;10(3)doi:10.1128/mBio.01137-19
5. Hellmark B, Söderquist B, Unemo M, Nilsdotter-Augustinsson Å. Comparison of Staphylococcus epidermidis isolated from prosthetic joint infections and commensal isolates in regard to antibiotic susceptibility, agr type, biofilm production, and epidemiology. *Int J Med Microbiol*. 2013;303(1):32-9. doi:10.1016/j.ijmm.2012.11.001
6. Sánchez A, Benito N, Rivera A, et al. Pathogenesis of Staphylococcus epidermidis in prosthetic joint infections: Can identification of virulence genes differentiate between infecting and commensal strains? *J Hosp Infect*. 2020;doi:10.1016/j.jhin.2020.04.026
7. Zatorska B, Groger M, Moser D, Diab-Elschahawi M, Lusignani LS, Presterl E. Does Extracellular DNA Production Vary in Staphylococcal Biofilms Isolated From Infected Implants versus Controls? *Clin Orthop Relat Res*. 2017;475(8):2105-2113. doi:10.1007/s11999-017-5266-0
8. Yu S, Jiang B, Jia C, et al. Investigation of biofilm production and its association with genetic and phenotypic characteristics of OM (osteomyelitis) and non-OM orthopedic Staphylococcus aureus. *Ann Clin Microbiol Antimicrob*. 2020;19(1):10. doi:10.1186/s12941-020-00352-4
9. Santos INM, Kurihara MNL, Santos FF, et al. Comparative Phenotypic and Genomic Features of Staphylococci from Sonication Fluid of Orthopedic Implant-Associated Infections with Poor Outcome. *Microorganisms*. 2022;10(6)doi:10.3390/microorganisms10061149
10. Mirzaei B, Faridifar P, Shahmoradi M, et al. Genotypic and phenotypic analysis of biofilm formation Staphylococcus epidermidis isolates from clinical specimens. *BMC Res Notes*. 2020;13(1):114. doi:10.1186/s13104-020-04965-y
11. Foster CE, Kok M, Flores AR, et al. Adhesin genes and biofilm formation among pediatric Staphylococcus aureus isolates from implant-associated infections. *PLoS One*. 2020;15(6):e0235115. doi:10.1371/journal.pone.0235115
12. Giormezis N, Kolonitsiou F, Foka A, et al. Coagulase-negative staphylococcal bloodstream and prosthetic-device-associated infections: the role of biofilm formation and

distribution of adhesin and toxin genes. *J Med Microbiol*. 2014;63(Pt 11):1500-1508. doi:10.1099/jmm.0.075259-0
13. Post V, Wahl P, Uçkay I, et al. Phenotypic and genotypic characterisation of Staphylococcus aureus causing musculoskeletal infections. *Int J Med Microbiol*. 2014;304(5-6):565-76. doi:10.1016/j.ijmm.2014.03.003
14. Lora-Tamayo J, Senneville É, Ribera A, et al. The Not-So-Good Prognosis of Streptococcal Periprosthetic Joint Infection Managed by Implant Retention: The Results of a Large Multicenter Study. *Clin Infect Dis*. 2017;64(12):1742-1752. doi:10.1093/cid/cix227
15. Pesset CM, Fonseca COD, Antunes M, et al. Biofilm formation by Staphylococcus pseudintermedius on titanium implants. *Biofouling*. 2024;40(1):88-97. doi:10.1080/08927014.2024.2320721
16. Zaborowska M, Tillander J, Brånemark R, Hagberg L, Thomsen P, Trobos M. Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants. *J Biomed Mater Res B Appl Biomater*. 2017;105(8):2630-2640. doi:10.1002/jbm.b.33803
17. Ma D, Mandell JB, Donegan NP, et al. The Toxin-Antitoxin MazEF Drives Staphylococcus aureus Biofilm Formation, Antibiotic Tolerance, and Chronic Infection. *mBio*. 2019;10(6)doi:10.1128/mBio.01658-19
18. Manasherob R, Mooney JA, Lowenberg DW, Bollyky PL, Amanatullah DF. Tolerant Small-colony Variants Form Prior to Resistance Within a Staphylococcus aureus Biofilm Based on Antibiotic Selective Pressure. *Clin Orthop Relat Res*. 2021;479(7):1471-1481. doi:10.1097/corr.0000000000001740
19. Urish KL, DeMuth PW, Kwan BW, et al. Antibiotic-tolerant Staphylococcus aureus Biofilm Persists on Arthroplasty Materials. *Clin Orthop Relat Res*. 2016;474(7):1649-56. doi:10.1007/s11999-016-4720-8
20. Babushkina IV, Mamonova IA, Ulyanov VY, Gladkova EV, Shpinyak SP. Antibiotic Susceptibility of Staphylococcus aureus Plankton and Biofilm Forms Isolated in Implant-Associated Infection. *Bull Exp Biol Med*. 2021;172(1):46-48. doi:10.1007/s10517-021-05328-8
21. Koch JA, Pust TM, Cappellini AJ, et al. Staphylococcus epidermidis Biofilms Have a High Tolerance to Antibiotics in Periprosthetic Joint Infection. *Life (Basel)*. 2020;10(11)doi:10.3390/life10110253
22. Reiter KC, Villa B, Paim T, de Oliveira CF, d'Azevedo PA. Inhibition of biofilm maturation by linezolid in meticillin-resistant Staphylococcus epidermidis clinical isolates: comparison with other drugs. *J Med Microbiol*. 2013;62(Pt 3):394-399. doi:10.1099/jmm.0.048678-0
23. Daffinee KE, Piehl EC, Bleick C, LaPlante KL. Eradication of Staphylococcus epidermidis within Biofilms: Comparison of Systemic versus Supratherapeutic Concentrations of Antibiotics. *Antimicrob Agents Chemother*. 2023;67(6):e0010823. doi:10.1128/aac.00108-23
24. Mandell JB, Orr S, Koch J, et al. Large variations in clinical antibiotic activity against Staphylococcus aureus biofilms of periprosthetic joint infection isolates. *J Orthop Res*. 2019;37(7):1604-1609. doi:10.1002/jor.24291
25. Okae Y, Nishitani K, Sakamoto A, et al. Estimation of Minimum Biofilm Eradication Concentration (MBEC) on In Vivo Biofilm on Orthopedic Implants in a Rodent Femoral Infection Model. *Front Cell Infect Microbiol*. 2022;12:896978. doi:10.3389/fcimb.2022.896978
26. Antoci V, Jr., Adams CS, Parvizi J, et al. The inhibition of Staphylococcus epidermidis biofilm formation by vancomycin-modified titanium alloy and implications for the treatment of

periprosthetic infection. *Biomaterials*. 2008;29(35):4684-90. doi:10.1016/j.biomaterials.2008.08.016
27. Svensson Malchau K, Tillander J, Zaborowska M, et al. Biofilm properties in relation to treatment outcome in patients with first-time periprosthetic hip or knee joint infection. *J Orthop Translat*. 2021;30:31-40. doi:10.1016/j.jot.2021.05.008
28. Hagstrand Aldman M, Thompson O, Påhlman LI. Biofilm formation is associated with poor outcome in prosthetic joint infections caused by Staphylococcus lugdunensis. *Infect Dis (Lond)*. 2023;55(5):328-332. doi:10.1080/23744235.2023.2180534
29. Taha M, Arulanandam R, Chen A, Diallo JS, Abdelbary H. Combining povidone-iodine with vancomycin can be beneficial in reducing early biofilm formation of methicillin-resistant Staphylococcus aureus and methicillin-sensitive S. aureus on titanium surface. *J Biomed Mater Res B Appl Biomater*. 2023;111(5):1133-1141. doi:10.1002/jbm.b.35220
30. Xie C, Ren Y, Weeks J, et al. Longitudinal intravital imaging of the bone marrow for analysis of the race for the surface in a murine osteomyelitis model. *J Orthop Res*. Mar 2024;42(3):531-538. doi:10.1002/jor.25716
31. Davidson DJ, Spratt D, Liddle AD. Implant materials and prosthetic joint infection: the battle with the biofilm. *EFORT Open Rev*. 2019;4(11):633-639. doi:10.1302/2058-5241.4.180095
32. Jørgensen NP, Meyer RL, Dagnæs-Hansen F, Fuursted K, Petersen E. A modified chronic infection model for testing treatment of Staphylococcus aureus biofilms on implants. *PLoS One*. 2014;9(10):e103688. doi:10.1371/journal.pone.0103688
33. Bell RD, Cann EA, Mishra B, et al. Staphyloccocus aureus biofilm, in absence of planktonic bacteria, produces factors that activate counterbalancing inflammatory and immune-suppressive genes in human monocytes. *J Orthop Res*. 2024;42(11):2582-2592. doi:10.1002/jor.25919
34. Josse J, Valour F, Maali Y, et al. Interaction Between Staphylococcal Biofilm and Bone: How Does the Presence of Biofilm Promote Prosthesis Loosening? *Front Microbiol*. 2019;10:1602. doi:10.3389/fmicb.2019.01602
35. Campoccia D, Ravaioli S, Mirzaei R, Bua G, Daglia M, Arciola CR. Interactions of Neutrophils with the Polymeric Molecular Components of the Biofilm Matrix in the Context of Implant-Associated Bone and Joint Infections. *Int J Mol Sci*. 2023;24(23)doi:10.3390/ijms242317042
36. Ricciardi BF, Muthukrishnan G, Masters E, Ninomiya M, Lee CC, Schwarz EM. Staphylococcus aureus Evasion of Host Immunity in the Setting of Prosthetic Joint Infection: Biofilm and Beyond. *Curr Rev Musculoskelet Med*. 2018;11(3):389-400. doi:10.1007/s12178-018-9501-4
37. Coraça-Huber DC, Kreidl L, Steixner S, Hinz M, Dammerer D, Fille M. Identification and Morphological Characterization of Biofilms Formed by Strains Causing Infection in Orthopedic Implants. *Pathogens*. 2020;9(8)doi:10.3390/pathogens9080649
38. Lamret F, Varin-Simon J, Six M, et al. Human Osteoblast-Conditioned Media Can Influence Staphylococcus aureus Biofilm Formation. *Int J Mol Sci*. 2022;23(22)doi:10.3390/ijms232214393
39. Carli AV, Bhimani S, Yang X, et al. Quantification of Peri-Implant Bacterial Load and in Vivo Biofilm Formation in an Innovative, Clinically Representative Mouse Model of Periprosthetic Joint Infection. *J Bone Joint Surg Am*. 2017;99(6):e25. doi:10.2106/jbjs.16.00815

40. Nishitani K, Sutipornpalangkul W, de Mesy Bentley KL, et al. Quantifying the natural history of biofilm formation in vivo during the establishment of chronic implant-associated Staphylococcus aureus osteomyelitis in mice to identify critical pathogen and host factors. *J Orthop Res*. 2015;33(9):1311-9. doi:10.1002/jor.22907
41. Vidlak D, Kielian T. Infectious Dose Dictates the Host Response during Staphylococcus aureus Orthopedic-Implant Biofilm Infection. *Infect Immun*. 2016;84(7):1957-1965. doi:10.1128/iai.00117-16
42. Turner AB, Giraldo-Osorno PM, Douest Y, et al. Race for the surface between THP-1 macrophages and Staphylococcus aureus on various titanium implants with well-defined topography and wettability. *Acta Biomater*. Jan 1 2025;191:113-139. doi:10.1016/j.actbio.2024.11.013
43. Kates SL, Owen JR, Xie C, Ren Y, Muthukrishnan G, Schwarz EM. Vaccines: Do they have a role in orthopedic trauma? *Injury*. Nov 2024;55 Suppl 6:111631. doi:10.1016/j.injury.2024.111631
44. Mandell JB, Gish C, Cappellini AJ, et al. Methicillin resistant Staphylococcus aureus mazEF expression promotes infections by influencing cellular growth, antibiotic sensitivity, and formation of biofilms. *Sci Rep*. 2024;14(1):21269. doi:10.1038/s41598-024-70829-1
45. Morales-Laverde L, Trobos M, Echeverz M, Solano C, Lasa I. Functional analysis of intergenic regulatory regions of genes encoding surface adhesins in Staphylococcus aureus isolates from periprosthetic joint infections. *Biofilm*. 2022;4:100093. doi:10.1016/j.bioflm.2022.100093
46. Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med*. 2007;357(7):654-63. doi:10.1056/NEJMoa061588
47. Urish KL, DeMuth PW, Craft DW, Haider H, Davis CM, 3rd. Pulse lavage is inadequate at removal of biofilm from the surface of total knee arthroplasty materials. *J Arthroplasty*. 2014;29(6):1128-32. doi:10.1016/j.arth.2013.12.012
48. Stoodley P, Conti SF, DeMeo PJ, et al. Characterization of a mixed MRSA/MRSE biofilm in an explanted total ankle arthroplasty. *FEMS Immunol Med Microbiol*. 2011;62(1):66-74. doi:10.1111/j.1574-695X.2011.00793.x
49. Schoenmakers JWA, López-Álvarez M, FFA IJ, et al. A fluorogenic micrococcal nuclease-based probe for fast detection and optical imaging of Staphylococcus aureus in prosthetic joint and fracture-related infections. *Eur J Nucl Med Mol Imaging*. 2024;51(10):2988-2997. doi:10.1007/s00259-023-06499-4
50. Weaver AA, Hasan NA, Klaassen M, Karathia H, Colwell RR, Shrout JD. Prosthetic joint infections present diverse and unique microbial communities using combined whole-genome shotgun sequencing and culturing methods. *J Med Microbiol*. 2019;68(10):1507-1516. doi:10.1099/jmm.0.001068
51. Morgenstern C, Renz N, Cabric S, Maiolo E, Perka C, Trampuz A. Thermogenic diagnosis of periprosthetic joint infection by microcalorimetry of synovial fluid. *BMC Musculoskelet Disord*. Jun 3 2020;21(1):345. doi:10.1186/s12891-020-03366-3
52. Cichos KH, Ruark RJ, Ghanem ES. Isothermal microcalorimetry improves accuracy and time to bacterial detection of periprosthetic joint infection after total joint arthroplasty. *J Clin Microbiol*. Dec 19 2023;61(12):e0089323. doi:10.1128/jcm.00893-23
53. Tillander JAN, Rilby K, Svensson Malchau K, et al. Treatment of periprosthetic joint infections guided by minimum biofilm eradication concentration (MBEC) in addition to

minimum inhibitory concentration (MIC): protocol for a prospective randomised clinical trial. *BMJ Open*. Sep 15 2022;12(9):e058168. doi:10.1136/bmjopen-2021-058168
54. Lamret F, Colin M, Mongaret C, Gangloff SC, Reffuveille F. Antibiotic Tolerance of Staphylococcus aureus Biofilm in Periprosthetic Joint Infections and Antibiofilm Strategies. *Antibiotics (Basel)*. 2020;9(9)doi:10.3390/antibiotics9090547
55. Siala W, Rodriguez-Villalobos H, Fernandes P, Tulkens PM, Van Bambeke F. Activities of Combinations of Antistaphylococcal Antibiotics with Fusidic Acid against Staphylococcal Biofilms in In Vitro Static and Dynamic Models. *Antimicrob Agents Chemother*. 2018;62(7)doi:10.1128/aac.00598-18
56. Kebriaei R, Lehman SM, Shah RM, et al. Optimization of Phage-Antibiotic Combinations against Staphylococcus aureus Biofilms. *Microbiol Spectr*. 2023;11(3):e0491822. doi:10.1128/spectrum.04918-22
57. Lu Y, Cai WJ, Ren Z, Han P. The Role of Staphylococcal Biofilm on the Surface of Implants in Orthopedic Infection. *Microorganisms*. 2022;10(10)doi:10.3390/microorganisms10101909
58. Ferry T, Onsea J, Roussel-Gaillard T, Batailler C, Moriarty TF, Metsemakers WJ. Bacteriophage therapy in musculoskeletal infections: from basic science to clinical application. *EFORT Open Rev*. May 10 2024;9(5):339-348. doi:10.1530/EOR-24-0042
59. Yang S, Mukh AA, Abdelatif E, et al. Bacteriophage therapy as an innovative strategy for the treatment of Periprosthetic Joint Infection: a systematic review. *Int Orthop*. Nov 2024;48(11):2809-2825. doi:10.1007/s00264-024-06295-1
60. Villegas M, Zhang Y, Badv M, et al. Enhancing osseointegration and mitigating bacterial biofilms on medical-grade titanium with chitosan-conjugated liquid-infused coatings. *Sci Rep*. 2022;12(1):5380. doi:10.1038/s41598-022-09378-4
61. Jiang N, Wang BW, Chai YM, et al. Chinese expert consensus on diagnosis and treatment of infection after fracture fixation. *Injury*. 2019;50(11):1952-1958. doi:10.1016/j.injury.2019.08.002
62. Oduwole KO, Glynn AA, Molony DC, et al. Anti-biofilm activity of sub-inhibitory povidone-iodine concentrations against Staphylococcus epidermidis and Staphylococcus aureus. *J Orthop Res*. 2010;28(9):1252-6. doi:10.1002/jor.21110
63. Jacqueline C, Caillon J. Impact of bacterial biofilm on the treatment of prosthetic joint infections. *J Antimicrob Chemother*. 2014;69 Suppl 1:i37-40. doi:10.1093/jac/dku254
64. Enrique CG, Medel-Plaza M, Correa JJA, et al. Biofilm on total joint replacement materials can be reduced through electromagnetic induction heating using a portable device. *J Orthop Surg Res*. 2024;19(1):304. doi:10.1186/s13018-024-04785-x
65. Turner AB, Gerner E, Firdaus R, et al. Role of sodium salicylate in Staphylococcus aureus quorum sensing, virulence, biofilm formation and antimicrobial susceptibility. *Front Microbiol*. 2022;13:931839. doi:10.3389/fmicb.2022.931839
66. Sun T, Huang J, Zhang W, et al. Simvastatin-hydroxyapatite coatings prevent biofilm formation and improve bone formation in implant-associated infections. *Bioact Mater*. 2023;21:44-56. doi:10.1016/j.bioactmat.2022.07.028
67. Ding J, Wang X, Liu W, et al. Biofilm Microenvironment Activated Antibiotic Adjuvant for Implant-Associated Infections by Systematic Iron Metabolism Interference. *Adv Sci (Weinh)*. 2024;11(17):e2400862. doi:10.1002/advs.202400862

68. Nowicka J, Janczura A, Pajączkowska M, et al. Effect of Camel Peptide on the Biofilm of Staphylococcus epidermidis and Staphylococcus haemolyticus Formed on Orthopedic Implants. *Antibiotics (Basel)*. 2023;12(12)doi:10.3390/antibiotics12121671
69. MacConnell AE, Levack AE, Brown NM. Biofilm and How It Relates to Prosthetic Joint Infection. *Orthop Clin North Am*. 2024;55(2):161-169. doi:10.1016/j.ocl.2023.10.001
70. Fernandes A, Dias M. The Microbiological Profiles of Infected Prosthetic Implants with an Emphasis on the Organisms which Form Biofilms. *J Clin Diagn Res*. 2013;7(2):219-23. doi:10.7860/jcdr/2013/4533.2732
71. Tunney MM, Dunne N, Einarsson G, McDowell A, Kerr A, Patrick S. Biofilm formation by bacteria isolated from retrieved failed prosthetic hip implants in an in vitro model of hip arthroplasty antibiotic prophylaxis. *J Orthop Res*. 2007;25(1):2-10. doi:10.1002/jor.20298
72. Lamret F, Lemaire A, Lagoutte M, et al. Approaching prosthesis infection environment: Development of an innovative in vitro Staphylococcus aureus biofilm model. *Biofilm*. 2023;5:100120. doi:10.1016/j.bioflm.2023.100120
73. Gilbertie JM, Schnabel LV, Hickok NJ, et al. Equine or porcine synovial fluid as a novel ex vivo model for the study of bacterial free-floating biofilms that form in human joint infections. *PLoS One*. 2019;14(8):e0221012. doi:10.1371/journal.pone.0221012
74. De Bleeckere A, van Charante F, Debord T, et al. A novel synthetic synovial fluid model for investigating biofilm formation and antibiotic susceptibility in prosthetic joint infections. *Microbiol Spectr*. Jan 7 2025;13(1):e0198024. doi:10.1128/spectrum.01980-24
75. Chen P, Sun F, Feng W, Hong H, Li B, Song J. Pathogenic characteristics of Staphylococcus aureus isolates from arthroplasty infections. *Int J Artif Organs*. 2021;44(3):208-214. doi:10.1177/0391398820948877
76. Rahim MI, Babbar A, Lienenklaus S, Pils MC, Rohde M. Degradable magnesium implant-associated infections by bacterial biofilms induce robust localized and systemic inflammatory reactions in a mouse model. *Biomed Mater*. 2017;12(5):055006. doi:10.1088/1748-605X/aa7667
77. Masters EA, Trombetta RP, de Mesy Bentley KL, et al. Evolving concepts in bone infection: redefining "biofilm", "acute vs. chronic osteomyelitis", "the immune proteome" and "local antibiotic therapy". *Bone Res*. 2019;7:20. doi:10.1038/s41413-019-0061-z
78. Saeed K, McLaren AC, Schwarz EM, et al. 2018 international consensus meeting on musculoskeletal infection: Summary from the biofilm workgroup and consensus on biofilm related musculoskeletal infections. *J Orthop Res*. 2019;37(5):1007-1017. doi:10.1002/jor.24229
79. Sequeira SB, Myntti MF, Lee J, Mont MA. An Overview of Research for the Application of a Novel Biofilm-Preventing Surgical Irrigation System for Total Joint Arthroplasty Procedures in Order to Reduce the Risk of Periprosthetic Infection. *Surg Technol Int*. 2024;44doi:10.52198/24.Sti.44.Os1780
80. Giordano V, Giannoudis PV. Biofilm Formation, Antibiotic Resistance, and Infection (BARI): The Triangle of Death. *J Clin Med*. 2024;13(19)doi:10.3390/jcm13195779