Journal of Orthopaedic Research®

WILEY | ORS Orthopaedic Research Society

CONSENSUS

2025 International Consensus Meeting on Musculoskeletal Infection: Summary From the Biofilm Workgroup on Biofilm Formation, Persistence, and Host-Environment Interactions

Gowrishankar Muthukrishnan[1] | Débora C. Coraça-Huber[2] | Gerald J. Atkins[3] | Ahmad Abbaszadeh[4] | Armita A. Abedi[5] | Ezzuddin Abuhussein[6] | Joshua S. Bingham[7] | Kyle H. Cichos[8] | Tom Coenye[9] | Lorenzo Drago[10,11] | John Hamilton[12] | Noreen J. Hickok[13] | Ferdinando Iannotti[14] | Jessica Amber Jennings[6] | Louise Kruse Jensen[15] | Bingyun Li[16] | Mojieb Manzary[17,18] | T. Fintan Moriarty[19,20] | Katya McDonald[1] | Kohei Nishitani[21] | Nicholas Norton[22] | Ebru Oral[23] | Javad M. Parvizi[24] | Dina Raafat[25,26,27] | Kordo Saeed[22] | Imre Sallai[28] | Edward M. Schwarz[1] | Claudia Siverino[19] | Amita Sekar[29] | Jermiah Tate[6] | Margarita Trobos[30] | Andie Tubbs[6]

[1]Center for Musculoskeletal Research, Department of Orthopaedics, University of Rochester, Rochester, New York, USA | [2]Medical University Innsbruck, University Hospital for Orthopaedics and Traumatology, Innsbruck, Austria | [3]Center for Orthopedic and Trauma Research, Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia | [4]Joint Reconstruction Research Center, Tehran University of Medical Sciences, Tehran, Iran | [5]Department of Orthopaedic Surgery and Traumatology, University of Copenhagen, Copenhagen, Denmark | [6]Department of Biomedical Engineering, University of Memphis, Memphis, Tennessee, USA | [7]Mayo Orthopaedics, Mayo Clinic, Rochester, Minnesota, USA | [8]Hughston Clinic, Columbus, Georgia, USA | [9]Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium | [10]UOC Laboratory of Clinical Medicine with Specialized Areas, IRCCS MultiMedica Hospital, Milan, Italy | [11]Clinical Microbiology and Microbiome Laboratory, Department of Biomedical Sciences for Health, University of Milan, Milan, Italy | [12]Department of Orthopedic Surgery, Rush University Medical Center, Chicago, Illinois, USA | [13]Department of Orthopaedic Surgery, Sidney Kimmel Medical School, Thomas Jefferson University, Philadelphia, Pennsylvania, USA | [14]Department of Surgical and Medical Sciences and Translational Medicine, Sapienza University of Rome, Rome, Italy | [15]Research Group of Experimental Pathology, Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark | [16]Department of Orthopaedics, School of Medicine, West Virginia University, Morgantown, West Virginia, USA | [17]Department of Orthopedic Surgery, Johns Hopkins University, Baltimore, Maryland, USA | [18]Johns Hopkins Aramco Healthcare Center, Dhahran, Saudi Arabia | [19]AO Research Institute Davos, Davos, Switzerland | [20]Center for Musculoskeletal Infections (ZMSI), University Hospital Basel, Basel, Switzerland | [21]Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan | [22]University Hospital Southampton NHS Foundation Trust, Southampton, England | [23]Harvard Medical School and Massachusetts General Hospital, Harris Orthopaedic Laboratory, Boston, Massachusetts, USA | [24]International Joint Center, Acibadem Mehmet Ali Aydınlar University, Istanbul, Turkey | [25]Institute of Immunology, University Medicine Greifswald, Greifswald, Germany | [26]Center for Orthopaedics, Trauma Surgery and Rehabilitation Medicine, University Medicine Greifswald, Greifswald, Germany | [27]Department of Microbiology and Immunology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt | [28]Department of Orthopaedics, Semmelweis University, Budapest, Hungary | [29]Department of Orthopaedic Surgery, Harris Orthopaedics Laboratory, Boston, Massachusetts, USA | [30]Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Correspondence: Gowrishankar Muthukrishnan (Gowri_Shankar@urmc.rochester.edu) | Débora C. Coraça-Huber (Debora.Coraca-Huber@i-med.ac.at)

Received: 7 October 2025 | **Revised:** 8 December 2025 | **Accepted:** 18 December 2025

Keywords: biofilm | biofilm formation | biofilm persistence | host-pathogen interactions | musculoskeletal infection (MSKI) | osteomyelitis

ABSTRACT

Musculoskeletal infection (MSKI) remains a major problem after trauma and elective orthopedic surgery. Chronic MSKI is related to the formation of biofilm, which impairs diagnosis and effective treatments. Therefore, to understand and communicate global standards and best practices, the 2025 International Consensus Meeting (ICM) on MSKI created a Biofilm Section to address crucial aspects of

Gowrishankar Muthukrishnan and Débora C. Coraça-Huber are co-first authors to whom correspondence should be addressed.

biofilm biology pertaining to its mechanisms of drug resistance and immune evasion, and potential approaches to overcome them. This featured a 2-year process, with final voting and discussion on May 8–10, 2025, in Istanbul, Turkey. This Consensus Article is the effort of the Biofilm Basic Mechanisms Workgroup, which interpreted the results on ICM questions related to (1) the infectious microenvironment; (2) appropriate inocula in preclinical research; (3) biofilm behavior in infected tissues; and (4) synergy within biofilms and with other comorbidities. Collectively, we find that this field has the necessary research tools to discover the pathophysiology of orthopedic implant-associated biofilm development and maturation, perform clinically relevant studies in animal models, and elucidate mechanisms that allow opportunistic infections in compromised tissues and patients with other health issues.

1 | Introduction

Musculoskeletal infection (MSKI) remains a major source of morbidity following trauma and orthopedic procedures, with consequences that include fracture nonunion, implant failure, amputation, and premature mortality [1–4]. Despite improvements in asepsis and perioperative antibiotic prophylaxis, the absolute burden of MSKI is rising in parallel with the global volume of orthopedic surgical interventions and use in an aging, comorbid population [5–7]. While biofilm is widely regarded as the dominant barrier to eradication in implant-associated infections, fundamental gaps remain in our understanding of how biofilms form, persist, and interface with host tissues [7–10]. Clarifying these basic mechanisms is the prerequisite for designing preclinical models that accurately predict clinical performance and prioritizing suitable translational targets.

The 2025 International Consensus Meeting (ICM) on MSKI convened a Biofilm Workgroup to address high-priority knowledge gaps spanning preclinical science through clinical translation (https://www.icmortho.org; https://www.ors.org/2025-icm-on-mski/). This Consensus Article synthesizes findings and future directions for the "Basic Mechanisms" domain. The questions interrogate: whether an "immune proteome" signature exists in periprosthetic joint infection (PJI) (B3); what models of orthopedic infection are best to evaluate diagnostic technologies (B4); which pathogens should be evaluated in preclinical models (B8); intracellular survival of bacteria in osteoblasts and its role in disease propagation (B9–B10); minimum data elements to standardize orthopedic infection studies (B12); the centrality of biofilm itself as the main treatment challenge (B15); biofilm behavior in synovial fluid (B17); viable-but-non-culturable (VBNC) states in orthopedic contexts (B18); material-specific affinities (B20); regulation of extracellular DNA (eDNA) via autolysis in *Staphylococcus aureus* (B21); potential links between biofilms and bone tumors (B24); and polymicrobial synergy versus antagonism in biofilm formation (B29). Together, these questions aim to align preclinical inquiry with the pathophysiology that drives persistent MSKI and clinical failure [10–12].

2 | Methodology

The objective of the ICM 2025 Biofilm Section was to generate expert, actionable recommendations that sharpen preclinical research questions and harmonize best practices for study design and reporting. The Biofilm Section included experts in microbiology, immunology, biomedical engineering, infectious diseases, and orthopedic surgery. An international panel selected questions aligned with their expertise to (i) scope the relevant literature, (ii) identify recurrent methodological pitfalls, and (iii) propose consensus guidance. As in prior ICMs, recommendations were developed and voted on using Delphi methodology [11, 12].

Over 10 months, each question was assigned to a liaison who coordinated further delegates to perform structured reviews. Covidence was used to manage systematic reviews; searches incorporated appropriate MeSH terms and synonyms. Teams screened titles/abstracts, performed full-text review, and extracted key data elements (model characteristics, organisms, inocula, materials, endpoints, and analytic methods). Draft "Response with Rationale" documents were posted for all delegates at https://www.icmortho.org/documents for open comment. Author teams revised their responses based on feedback in preparation for in-person discussions and voting at the ICM (May 8–10 2025, Istanbul, Turkey).

During the meeting, statements with ambiguity or contention were edited in session to promote clarity and achieve broader agreement. With regard to the proposed response, delegates voted to (1) agree, (2) disagree, or (3) abstain. Outcomes followed predefined thresholds: (a) Simple majority (50.1%–59%): No Consensus; (b) Majority (60%–65%): Weak Consensus; (c) Super Majority (66%–99%): Strong Consensus; (d) 100%: Unanimous Consensus. The 13 questions summarized here specifically address the evaluation of basic mechanisms underpinning biofilm formation, persistence, and interaction with the host, as well as preclinical model standardization. Consensus was reached without compulsion, undue influence, or process constraints. Full question-level recommendations, including downloadable PDFs with responses, consensus votes, and post-meeting rationales, are available at https://www.ors.org/2025-icm-on-mski/. Following voting at ICM 2025, the authors of this manuscript were assigned to the Biofilm Basic Mechanisms Workgroup and tasked with highlighting the most important questions on this topic, interpreting the recommendations and voting results, and providing insights on the way forward. The following represents this work product, and the results are summarized in Table 1.

3 | Results and Discussion

Question B3 asked "Is there an immune proteome in PJI?" The delegates unanimously (100% agreement) answered that there is an immune proteome in PJI, and our understanding of this proteome continues to grow and evolve. Novel high-throughput approaches paired with more targeted protein investigations have indicated that there is an immune proteome in PJI compared to aseptic revisions. This proteome involves the synovial fluid, local tissues, implant, and peripheral blood. While discrepancies exist between compartments, interleukin-6

TABLE 1 | Summary table of the questions for ICM2025 and the consensus agreement.

Question	Consensus
B3: Is there an immune proteome in PJI?	Unanimous Consensus
B4: What is (are) the best preclinical model(s) of orthopedic infection for the evaluation of diagnostic technologies?	Strong Consensus
B8: What crtical pathogens should be routinely evaluated in preclinical models to study important questions in orthopedic infection research?	Strong Consensus
B9: Can bacteria survive in the intracellular space of osteoblasts?	Strong Consensus
B10: Do intracellular bacteria play a role in propagating MSKI?	Strong Consensus
B12: What is (are) the minimum data set for studying orthopedic infections?	Strong Consensus
B15: Are we certain biofilms are the main challenge of treating implant-associated infection?	Strong Consensus
B17: Does biofilm form in the synovial fluid?	Strong Consensus
B18: Does the concept of viable but non-culturable (VBNC) apply to orthopedic infections?	Unanimous Consensus
B20: Does biofilm have different affiniites for different surfaces?	Strong Consensus
B21: What key regulatory mechanisms control the release of extracellular DNA (eDNA) from *S. aureus* during autolysis, which significantly contributes to the structural integrity of biofilm?	Unanimous Consensus
B24: Are biofilms carcinogenic or associated with bone tumors?	Strong Consensus
B29: In the case of polymicrobial infection, do all pathogens participate synergistically in biofilm formation or is there any antagonistic influence by some organisms?	Strong Consensus

(IL-6) was the most commonly elevated cytokine in PJI compared to aseptic revisions. The composition of the proteome also varied based on the detection method used for each study, primarily either LC-MS or bead-based/ELISA assays. As more advanced and unbiased techniques evolve and become more readily available, so too will the understanding of this proteome and how it can be used for diagnosis, treatment, and ultimately prevention of PJI in the future.

Question B4 queried "What is (are) the best preclinical model (s) of orthopedic infection for the evaluation of diagnostic technologies?" The absence of a definitive diagnostic is highlighted by the fact that a perennial outcome of MSKI ICMs has been an updated definition of PJI, which remains a high priority with robust research activity. Delegates agreed that the "best" model depends on the specific hypothesis tested and animal welfare considerations. In animal models, translational potential, ethical approval, model design, and statistical success metrics should be predetermined and declared. To evaluate new diagnostics, current industry standards are receiver operating characteristic (ROC) curve analysis of the new technology compared to clinical cultures, clinical signs and symptoms, or other FDA-approved diagnostics of orthopedic infection. Additionally, endpoints should be valid, and diagnosis should involve quantitative microbiological, radiological, serological, histological, and clinical observations. Ultimately, less invasive mechanisms for infection surveillance to reduce animal morbidity and numbers are important goals, and although validated In Silico and In Vitro models to assess diagnostics of orthopedic infection do not currently exist, these technologies are rapidly emerging and may need to be considered in the near future. This recommendation received overwhelming ICM agreement (93%) with three abstaining votes.

Question B8 queried "What critical pathogens should be routinely evaluated in preclinical models to study important questions in orthopedic infection research?" The delegates overwhelmingly agreed (98%) with the recommendation that, in addition to prevalent pathogens like *S. aureus* and *E. coli*, other microorganisms, particularly Gram-negative bacilli, *Enterobacterales*, and *C. acnes*, should also be assessed. This rationale is based on a systematic review of six studies, which identified a range of pathogens as causative agents of disease, including less commonly studied species, such as *S. pseudointermedius* and various fungi. Notably, while most preclinical models have focused on common pathogens, further research into less common organisms is necessary to ensure a comprehensive understanding of the full spectrum of pathogens involved in orthopedic infections and therapeutic development. Until then, a multidisciplinary approach involving orthopedic surgeons and MSKI specialists is crucial in managing these infections.

Question B9 asked "Can bacteria survive in the intracellular space of osteoblasts?" The question considered the evidence of stable intracellular infections of osteoblast-lineage cells, from osteoprogenitors to mature osteocytes. Stable infection was defined as evidence of viable pathogens inside viable host cells for at least 24 h post-infection. The majority of delegates (98% in favor, 2% opposed) agreed that this occurs. Several case reports of chronic osteomyelitis have identified intracellular pathogens, including obligate intracellular organisms, in osteocytes and osteoblasts as being the likely cause of infection chronicity. Additionally, a multitude of In Vitro studies have demonstrated the intracellular persistence of numerous facultative and obligate intracellular pathogenic species in both well-characterized osteoblast and osteocyte cell lines and primary osteoblasts and osteocytes. Pathogens, including *S. aureus*, coagulase-negative staphylococci, *Brucella* species, *Porphyromonas gingivalis*, Mycobacterium species and *Chlamydia pneumoniae*, were all found to be capable of infecting and persisting in viable osteoblast lineage cells for experimental periods ranging from 24 h to 28 days.

Question B10 asked "Do intracellular bacteria play a role in propagating MSKI?" This question extended the findings of

Question B9. The majority of delegates (98% in favor, 2% opposed) agreed that this occurred. Strong evidence supports the notion that both facultative and obligate intracellular pathogens can invade host cells, survive within them, and contribute to the development of chronic or recurrent infections. *S. aureus* is the most extensively studied species in this context, demonstrating persistence in various musculoskeletal cell types, including osteoblasts, osteocytes, macrophages, and osteoclasts, which can last for 21 days or longer. Clinical and preclinical studies further establishing connections between intracellular bacteria and chronic/recurrent infections and antimicrobial resistance are much needed. Mechanisms of bacterial escape and the triggers for pathogenic reactivation remain under investigation. These unresolved issues indicate a need to improve the diagnosis of intracellular infections and for ongoing research to better inform therapeutic strategies against persistent MSKI.

Question B12 asked "What is (are) the minimum data set for studying orthopedic infections?" This question highlighted the variability in reporting registry data, which leads to challenges in comparing studies. The expert recommendation was that a minimum data set should include a sample size estimation, patient characteristics, index operative factors, diagnostic criteria, operative and medical treatment factors, and postoperative outcomes to maximize generalizability and comparability across orthopedic literature (Table 2). To increase the accuracy of clinical data across different studies, it is reasonable to propose a minimum sample size of 300 patients per comparison group. This will increase statistical power and reduce the likelihood of type II errors. Another consideration is host optimization, as it is an important preoperative factor to prevent socioeconomic disparities in healthcare utilization and enhance recovery from orthopedic postoperative complications and infections [13]. At a minimum, patient age, sex, body mass index, surgical history, and comorbidities should be recorded. Optimally, inclusion of socioeconomic factors through a comprehensive approach, such as the Area Deprivation Index, is also recommended. Surgical factors, including indication, operative time, blood loss, transfusion, and hospital stay influence the development of orthopedic infection and should be reported. Diagnostic criteria for infection are also valuable to report, and the recommendation of experts is to use the 2018 ICM Criteria or the 2021 European Bone and Joint Infection Society criteria for hip and knee PJI [14, 15]. Standardizing and reporting outcome measures for treatment success or failure remains challenging. A minimum of 1-year follow-up is recommended, but up to 5-year follow-up would improve outcome tracking. Patient-reported outcome measures (PROMs) are now considered important for assessing treatment success, but do not always give a complete picture of infection treatment success. The "clinical relevance ratio" is a new way to analyze PROMs and can help show how many patients had meaningful

TABLE 2 | Criteria for the minimum data set required to study orthopedic infections.

Sample size	Need for a power analysis to determine the appropriate sample size
Patient characteristics	Age, sex, body mass index
	Relevant surgical history
	Host comprising factors (e.g., diabetes, autoimmune disease, immunosuppressive medication, renal disease, malnutrition)
	Extremity comprising factors (e.g., soft tissue loss, need for plastic surgery, vascular insufficiency)
	Socioeconomic factors (e.g., area deprivation index)
Index operative factors	Surgical indication (e.g., elective *vs.* traumatic)
	Operative time, length of stay
	Estimated blood loss, need for transfusion
	Type of implant used (e.g., primary *vs.* revision components)
	Delayed wound healing, extended antibiotic prescription
Diagnostic criteria	Time of infection onset
	Serological laboratory studies (ESR, CRP)
	Synovial fluid analysis (if applicable), microbiological data
Operative and medical treatment factors	Implant retained, removed, or exchanged (i.e., molecular components, temporary spacer, or real implants)
	Local antimicrobial delivery method
	Systemic antimicrobial therapy (agent and duration)
Postoperative outcomes	Minimum 1-year clinical follow-up
	General health and condition-specific PROMs
	Definition of treatment success: infection control with no continued antimicrobial therapy, infection control with suppressive antimicrobial therapy, retained spacer, need for reoperation, and death

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PROMs, patient-reported outcome measures.

improvements after treatment [16]. This question received votes from both the general session in the hip and knee category and in the biofilm section. The biofilm section delegates voted 80% in agreement, 7% in disagreement, and 13% abstained. The hip & knee section voted 84.6% in agreement, 7.7% in disagreement, and 7.7% abstained.

Question B15 inquired "Are we certain that biofilms are the main challenge of treating implant-associated infection?" Delegates reached a strong consensus, with 92% voting "agree," 3% "disagree," and 5% "abstain" that biofilms, particularly those formed by *S. aureus* and *S. epidermidis*, drive antibiotic resistance, immune evasion, and chronicity. These findings were supported by both clinical and preclinical evidence. Key implications of this question include the need for improved diagnostics (e.g., sonication, molecular assays), development of biofilm-targeted therapies, and innovations in implant materials. Debate remains over exceptions, such as non-biofilm pathogens, small-colony variants, and strain-specific differences, highlighting that biofilm is not the sole pathogenic mechanism. Overall, biofilms represent the central barrier to effective treatment, but ongoing research must address variability and explore personalized and adjunctive strategies.

Question B17 asked "Does biofilm form in the synovial fluid?" Based on a strong level of evidence, 97% of delegates agreed that suspended biofilm aggregates, particularly of *Staphylococcus* species, can form in synovial fluid both In Vitro and In Vivo. This finding may have significant clinical implications, as these aggregates exhibit reduced antimicrobial susceptibility, potentially contributing to the persistence and treatment resistance of PJIs. While most studies on this topic have focused on *S. aureus*, other PJI pathogens, including other Gram-positive bacteria (e.g., coagulase-negative staphylococci like *S. epidermidis*, and *C. acnes*), Gram-negative bacteria (e.g., *Enterobacterales* and *P. aeruginosa*), and fungi (e.g., *Candida* spp.), have also demonstrated the capability of forming biofilm-like aggregates in synovial fluid. However, the evidence for these additional species remains largely confined to In Vitro studies, and in general, In Vivo evidence for biofilm formation in synovial fluid remains limited. *S. aureus* biofilm aggregates form via binding to fibrinogen, fibronectin, and hyaluronic acid in synovial fluid; the ability to bind to these key synovial fluid components may allow stronger association of bacteria with tissues. Variability in experimental models complicates direct comparisons between results obtained in different studies, and further research is needed to standardize methodologies and better understand the clinical impact of these non-surface-attached biofilms in the management of PJI.

Question B18 asked "Does the concept of viable but non-culturable (VBNC) apply to orthopedic infections?" Delegates unanimously agreed (100% agreement) that the concept of VBNC is relevant in orthopedic infections. Evidence from In Vitro studies, including those using clinical isolates from PJIs, has demonstrated that pathogens such as *S. aureus*, *S. epidermidis*, *S. lugdunensis*, and *P. aeruginosa* can enter the VBNC state under stress conditions such as antibiotic pressure, nutrient depletion, and anoxia when in a biofilm [17–22]. These cells can persist for prolonged periods, evade detection, survive treatment-like conditions, and resuscitate under favorable conditions, contributing to treatment failure and the recurrence of orthopedic infections. While evidence supports a link to culture-negative infections, key challenges remain in clinically validating these findings and developing accurate diagnostics and therapies to target dormant bacterial populations.

Question B20 asked "Does biofilm have different affinities for different surfaces?" Delegates strongly supported the assertion that biofilm exhibits different affinities for distinct surfaces (97% yes, 3% abstain). Bacterial adhesion and biofilm development on the implant surface are influenced by a combination of host factors, microorganism-specific characteristics, exposure time, and implant-related surface properties such as wettability, topography, micro- and nanoscale roughness, surface free energy, material, and charge. Substantial evidence suggests that increased implant surface roughness at the micro- and macrometer scale promotes bacterial adhesion, while surface peaks and valleys provide shelter from shear stresses. Nanoscale roughness, on the other hand, seems to reduce adhesion points. Furthermore, peaks with diameters close to bacterial cells exert mechanical stress on the bacterial membrane, resulting in its rupture. Surface chemistry also directly affects biofilm formation. There are differing opinions among authors regarding the effectiveness of various surface types in preventing bacterial colonization. Some argue that hydrophilic, highly hydrated, and uncharged surfaces are effective barriers, while others contend that hydrophobic surfaces with low surface free energy, such as cobalt chrome, inhibit bacterial growth.

Affinity arises from the complex interaction between surface properties and bacterial physicochemical properties. The same biomaterial can interact differently with various bacteria since each bacterial strain has different affinities for distinct surfaces. In light of these factors, establishing a universally valid correlation or identifying a surface that consistently prevents biofilm formation remains challenging. Despite this, multiple studies suggest that ceramic, cobalt-chromium, and titanium alloys outperform polyethylene, stainless steel, and Polyether-ether-ketone (PEEK) in preventing biofilm formation. The majority of evidence is derived from In Vitro or preclinical models, raising concerns regarding the influence of host immunity, tissue response, and the dynamic biological environment. Enhanced translational research on bacterial surface affinities is essential for developing biofilm-resistant implant designs.

Question B21 asked "What key regulatory mechanisms control the release of extracellular DNA (eDNA) from *S. aureus* during autolysis, which significantly contributes to the structural integrity of biofilm?" The release of eDNA contributes significantly to the structural integrity, growth, and immune-evasive properties of *S. aureus* biofilms in orthopedic infections. This process is primarily mediated by autolysis via murein hydrolase, encoded by the *atl* gene, and modulated by the *cidABC* and *lrgAB* operons, which regulate cell death and lysis. Additional mechanisms, such as those involving phosphodiesterase and thermonuclease, also contribute to eDNA release, albeit independent of autolysis. The delegates voted unanimously (100% agreement) with these proposed mechanisms. The scientific context underscores the crucial role of eDNA in biofilm stability, offering potential therapeutic targets for bacterial eradication. Although there is a strong consensus regarding the underlying mechanisms, unresolved issues remain regarding the role of environmental factors like oxygen and glucose levels in modulating eDNA release. Furthermore, the influence of subinhibitory antibiotic concentrations on eDNA production within biofilms warrants further investigation for clinical implications in treating MSKI.

Question B24 asked "Are biofilms carcinogenic or associated with bone tumors?" Delegates voted 81% in agreement, 2% in disagreement, and 17% abstaining that biofilms have a role in carcinogenesis. The oncogenic potential of biofilm has been studied extensively in colorectal cancers, where In Vitro and animal studies have shown both pathogen-specific and biofilm-related effects on cancer progression. Microbiome analyses have revealed changes in the local microbiome associated with a wide range of tumor types. Specific pathogens have been implicated in altering the tumor microenvironment, including *F. nucleatum, S. typhi*, and *S. epidermidis*. Proposed oncogenic mechanisms include the induction of an inflammatory environment and specific products of bacterial metabolism. While several cancers show strong associations with biofilm activity, there is a lack of data specific to bone tumors. Questions such as the microbiome of primary bone cancers and the oncogenic potential of pathogens specific to orthopedic infections remain unresolved.

Question B29 asked "In case of polymicrobial infection, do all pathogens participate synergistically in biofilm formation or is there any antagonistic influence by some organisms?" Delegates voted 97% in agreement and 3% in disagreement that both synergistic and antagonistic effects occur depending on the species involved, with a limited level of evidence supporting this recommendation. Evidence, primarily from In Vitro studies, shows that while some pathogen pairs, such as C. albicans with S. aureus, enhance biofilm formation, antimicrobial tolerance, and virulence [23], others, such as S. aureus and P. aeruginosa, may initially compete before later developing synergistic colonization strategies [24] or exhibit antagonistic interactions [25]. Gram-positive organisms, particularly S. aureus, often outcompete Gram-negatives in bone or implant niches, but synergistic relationships can arise depending on the environment and infection stage [24, 25]. These findings have important implications for MSKI management, as polymicrobial interactions can alter pathogenicity, immune evasion, and, in some cases, antimicrobial susceptibility [23–25]. However, data specific to MSKI remain limited, and further In Vivo research is needed to inform targeted treatment strategies for mixed-species biofilms. Most current investigations are constrained by reliance on In Vitro models, limited pathogen diversity, and insufficient representation of clinically relevant infection environments [23–25].

4 | Conclusion

These recommendations from the ICM 2025 illustrate the current state of knowledge regarding biofilms and their role in MSKI. Specifically, this review highlighted the complexity of pathogen behavior, the need for robust preclinical models, and the importance of multidisciplinary approaches to improve patient outcomes. Multiple recommendations underscored the need for In Vivo and clinical studies for promising In Vitro findings. Filling in these knowledge gaps regarding biofilm is crucial to the development of better diagnostics and therapeutics.

Author Contributions

All authors participated in data generation (identification of the research questions and voting on their priority), contributed to the writing, and have read and approved the final submitted manuscript.

References

1. A. J. Tande and R. Patel, "Prosthetic Joint Infection," *Clinical Microbiology Reviews* 27 (2014): 302–345.

2. W. T. Obremskey, W. J. Metsemakers, D. R. Schlatterer, et al., "Musculoskeletal Infection in Orthopaedic Trauma: Assessment of the 2018 International Consensus Meeting on Musculoskeletal Infection," *Journal of Bone and Joint Surgery* 102 (2020): e44.

3. W. Metsemakers, M. Morgenstern, M. A. McNally, et al., "Fracture-Related Infection: A Consensus on Definition From an International Expert Group," *Injury* 49 (2018): 505–510.

4. S. Kurtz, K. Ong, E. Lau, F. Mowat, and M. Halpern, "Projections of Primary and Revision Hip and Knee Arthroplasty in the United States From 2005 to 2030," *Journal of Bone and Joint Surgery-American Volume* 89 (2007): 780–785.

5. M. Sloan, A. Premkumar, and N. P. Sheth, "Projected Volume of Primary Total Joint Arthroplasty in the U.S., 2014 to 2030," *Journal of Bone and Joint Surgery* 100 (2018): 1455–1460.

6. W. J. Metsemakers, T. F. Moriarty, M. Morgenstern, et al., "The Global Burden of Fracture-Related Infection: Can we do Better?," *Lancet Infectious Diseases* 24 (2024): e386–e393.

7. J. W. Costerton, P. S. Stewart, and E. P. Greenberg, "Bacterial Biofilms: A Common Cause of Persistent Infections," *Science* 284 (1999): 1318–1322.

8. E. A. Masters, B. F. Ricciardi, K. L. M. Bentley, T. F. Moriarty, E. M. Schwarz, and G. Muthukrishnan, "Skeletal Infections: Microbial Pathogenesis, Immunity and Clinical Management," *Nature Reviews Microbiology* 20 (2022): 385–400.

9. H. Y. Liu, E. L. Prentice, and M. A. Webber, "Mechanisms of Antimicrobial Resistance in Biofilms," *npj Antimicrobials and Resistance* 2 (2024): 27.

10. E. M. Schwarz, J. Parvizi, T. Gehrke, et al., "2018 International Consensus Meeting on Musculoskeletal Infection: Research Priorities From the General Assembly Questions," *Journal of Orthopaedic Research* 37 (2019): 997–1006.

11. I. R. Diamond, R. C. Grant, B. M. Feldman, et al., "Defining Consensus: A Systematic Review Recommends Methodologic Criteria for Reporting of Delphi Studies," *Journal of Clinical Epidemiology* 67 (2014): 401–409.

12. J. Parvizi, T. Gehrke, M. A. Mont, and J. J. Callaghan, "Introduction: Proceedings of International Consensus on Orthopedic Infections," *Journal of Arthroplasty* 34 (2019): S1–S2.

13. S. Tarabichi and J. Parvizi, "Prevention of Surgical Site Infection: A Ten-Step Approach," *Arthroplasty* 5 (2023): 21.

14. J. Parvizi, T. L. Tan, K. Goswami, et al., "The 2018 Definition of Periprosthetic Hip and Knee Infection: An Evidence-Based and Validated Criteria," *Journal of Arthroplasty* 33 (2018): 1309–1314.e2.

15. M. McNally, R. Sousa, M. Wouthuyzen-Bakker, et al., "The Ebjis Definition of Periprosthetic Joint Infection: A Practical Guide for Clinicians," *Bone & Joint Journal* 103 (2021): 18–25.

16. M. N. Orr, A. K. Klika, J. J. Gagnier, M. Bhandari, and N. S. Piuzzi, "A Call for a Standardized Approach to Reporting Patient-Reported Outcome Measures: Clinical Relevance Ratio," *Journal of Bone and Joint Surgery* 103 (2021): e91.

17. S. Pasquaroli, B. Citterio, G. Mangiaterra, et al., "Influence of Sublethal Concentrations of Vancomycin and Quinupristin/Dalfopristin on the Persistence of Viable but Non-Culturable Staphylococcus Aureus Growing in Biofilms," *Journal of Antimicrobial Chemotherapy* 73 (2018): 3526–3529.

18. S. Pasquaroli, G. Zandri, C. Vignaroli, C. Vuotto, G. Donelli, and F. Biavasco, "Antibiotic Pressure can Induce the Viable but Non-Culturable State in Staphylococcus Aureus Growing in Biofilms," *Journal of Antimicrobial Chemotherapy* 68 (2013): 1812–1817.

19. S. Pasquaroli, B. Citterio, A. Cesare, et al., "Role of Daptomycin in the Induction and Persistence of the Viable but Non-Culturable State of Staphylococcus Aureus Biofilms," *Pathogens* 3 (2014): 759–768.

20. K. Šuster and A. Cör, "Induction of Viable but Non-Culturable State in Clinically Relevant Staphylococci and Their Detection With Bacteriophage K," *Antibiotics (USSR)* 12 (2023): 311.

21. L. Kvich, B. Fritz, S. Crone, et al., "Oxygen Restriction Generates Difficult-To-Culture P. Aeruginosa," *Frontiers in Microbiology* 10 (2019): 1992.

22. Z. Qi, Z. Huang, and C. Liu, "Metabolism Differences of Biofilm and Planktonic Pseudomonas Aeruginosa in Viable but Nonculturable State Induced by Chlorine Stress," *Science of the Total Environment* 821 (2022): 153374.

23. C. E. Zago, S. Silva, P. V. Sanitá, et al., "Dynamics of Biofilm Formation and the Interaction Between Candida Albicans and Methicillin-Susceptible (MSSA) and -Resistant Staphylococcus Aureus (MRSA)," *PLoS One* 10 (2015): e0123206.

24. P. M. Alves, E. Al-Badi, C. Withycombe, P. M. Jones, K. J. Purdy, and S. E. Maddocks, "Interaction Between Staphylococcus Aureus and Pseudomonas Aeruginosa Is Beneficial for Colonisation and Pathogenicity in a Mixed Biofilm," *Pathogens and Disease* 76 (2018), https://doi.org/10.1093/femspd/fty003.

25. C. R. Armbruster, D. J. Wolter, M. Mishra, et al., "Staphylococcus Aureus Protein a Mediates Interspecies Interactions at the Cell Surface of Pseudomonas Aeruginosa," *mBio* 7 (2016): e00538-16.