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SPECIAL ISSUE ARTICLE

The 2023 Orthopedic Research Society's international consensus meeting on musculoskeletal infection: Summary from the in vitro section

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Abstract

Antimicrobial strategies for musculoskeletal infections are typically first developed with in vitro models. The In Vitro Section of the 2023 Orthopedic Research Society Musculoskeletal Infection international consensus meeting (ICM) probed our state of knowledge of in vitro systems with respect to bacteria and biofilm phenotype, standards, in vitro activity, and the ability to predict in vivo efficacy. A subset of ICM delegates performed systematic reviews on 15 questions and made recommendations and assessment of the level of evidence that were then voted on by 72 ICM delegates. Here, we report recommendations and rationale from the reviews and the results of the internet vote. Only two questions received a ≥90% consensus vote, emphasizing the disparate approaches and lack of established consensus for in vitro modeling and interpretation of results. Comments on knowledge gaps and the need for further research on these critical MSKI questions are included.

KEYWORDS

antimicrobial, biofilm, in vitro models, international consensus meeting (ICM), musculoskeletal infection (MSKI)

For affiliations refer to page 516.

1 | INTRODUCTION

There is a general need for standard testing of novel antimicrobial biomaterials to (i) facilitate comparison between studies, (ii) allow unequivocal assessment of antimicrobial functionality, and (iii) define success measures for progression from preclinical to clinical development. However, such standard testing methods are currently not available.

Antimicrobial technologies are usually first characterized with in vitro model systems that are created based on basic microbiological considerations, medical device properties, and ultimately the biological environment. The 2018 International Consensus Meeting (ICM) on Musculoskeletal Infection had a breakout group for questions associated with biofilms,[1] yet critical gaps in our knowledge remain. Throughout the ICM process, the In Vitro Section of the Orthopedic Research Society (ORS) Musculoskeletal Infection (MSKI) 2023 ICM focused on bacteria and biofilm phenotype, standards, and the ability to predict in vivo efficacy. The results highlight the implant environment, implant surface properties, pathogenic species, and drug delivery mechanisms that can limit the value of in vitro testing, and which would substantially benefit the community if consensus opinion could be established.

2 | METHODOLOGY

Led by Chairs of the ORS ICM on MSKI, Drs. Fintan Moriarty, Edward Schwarz, Antonia Chen, Noreen Hickok, Kordo Saeed, and Thomas Schaer, MSKI experts were recruited and the framework defined. *In vitro* Section co-chairs, Noreen Hickok, Bingyun Li, Ebru Oral, and Sebastian Zaat recruited additional delegates, identified and refined questions, assigned two to three experts per question to identify and analyze the best available data for in vitro models of MSKI, and finally reviewed all submitted text before combining them into a single document. The groups of two to three delegates subsequently recruited additional co-authors at their own discretion.

Data analysis for each question focused on laboratory evaluations, although animal and clinical data were used to give context. The individual groups researched and refined the questions, including a literature review that was presented as a 1–2-page summary that indicated the search strategy, a reworded question, an assessment of the strength of the evidence, and a recommendation based upon the number and rigor of the studies. After the unsuccessful attempt at voting at ORS2023, delegates were asked to vote online having been supplied with the questions, review process, answers, rationales, and references for them to review. The delegates (72 responses) voted to: (1) agree, (2) disagree, or (3) abstain, on each response during the online voting and the voting results were rated as: (a) Simple majority (50.1%–59%): No Consensus; (b) Majority (60%–65%): Weak Consensus; (c) Super Majority (66%–99%): Strong Consensus; and (d) Unanimous (100%): Unanimous Consensus. The online voting levels of agreement are summarized here and a complete list of all 65 questions with recommendation and rationale (https://ors-org.s3.amazonaws.com/wp-content/uploads/2023/06/ORS-MSKI-RIG-ICM-Final.pdf) and the voting results from the 72 delegates (https://ors-org.s3.amazonaws.com/wp-content/uploads/2023/06/ORS-MSKI-ICM-Vote.pdf) are available on the ORS website.

3 | RESULTS

We summarize considerations associated with the 15 questions listed, with their recommendations and responses in Supporting Information: Table S1. In summarizing conclusions, we have grouped questions into areas of similar or related content.

3.1 | Methodology: In vitro development—What constitutes efficacy and how is it measured?

Four questions focused on predicting antimicrobial efficacy, especially in the transition from an in vitro to an in vivo setting.

Question 38 focused on reduction in bacterial numbers associated with antimicrobial treatments (including device-associated, local delivery, or other modalities) and noted that the in vitro models were very limited and far from reflecting the in vivo situation. The delegates recommended the review by Moriarty et al.[2] The assumption is that if bacterial numbers are sufficiently reduced, the residual bacteria may be cleared by the immune system. It was noted that it is unknown how mere reduction in bacteria would translate in vivo, or if complete eradication is in fact necessary. *It was suggested that a minimum of 2–3 log reduction should be sought (86% agreed) to demonstrate antibacterial efficacy*. In review of this summary, some noted that the mix of persisters and nondormant cells in clinical biofilms may require complete eradication in vitro to achieve reductions in morbidity/mortality in vivo.

Question 41 focused on reduction of bacterial colonization on surfaces and evaluated studies that included in vitro measurements integrated with in vivo outcomes that achieved ≥3 log colony forming units (CFU) decrease. The animal models were predominantly mice and rats with some rabbit models. Under those conditions, the limited studies supported the idea that *a 1.5 log reduction in CFU/mL on antimicrobial surfaces in vitro could result in a minimum 3 log reduction in an animal model (62.5% agreed)*. It was noted that most of the surfaces examined were "elution" surfaces, where the antibacterial activity was based on the eluted concentration of antibacterial drugs/agents. In review, some suggested caution in the generalizability of this recommendation to various device types, antimicrobials and pathogens in vivo. The authors noted the need for (1) more accurate reporting of reductions obtained in vitro (whether determined in the same report or previously) when engaged in in vivo studies, and (2) the need for more studies that examine both in vitro and in vivo reductions in the same series of studies.

Question 43 asked "Should multiple outcome measures be used to determine antimicrobial efficacy in vitro?" The group concluded that while measurement of CFU is the gold standard in microbiology,

its low throughput and issues surrounding detection of bacteria in challenging situations (e.g., in biofilm or in vivo where bacteria can be viable but nonculturable) can limit its value. The use of multiple orthogonal measurements (spectroscopic, reagent-based assays, reporter assays, microscopy, and qRT-PCR) are able to reduce artifacts and performance issues. *There was 97% agreement with the recommendation that multiple outcome measures should be used*.

Question 44 addressed bacterial inoculum concentrations used in in vitro testing for antimicrobial efficacy. The authors noted that inoculum size can alter the effect size and that an eluting surface may have different criteria than a noneluting surface. Especially for eluting surfaces, multiple test methods including elution profile and pharmacologic profiles, should be considered. *It was recommended that there is no universal starting inoculum (85% agreement)*. Overall, the need for standardized in vitro and in vivo models, as well as the need for studies that include both in vitro and in vivo assessments were emphasized.

3.2 | Methodology: Toward standard testing

In this section, questions 35, 42, and 46 investigated negative and positive controls, microbial test species and strains, and sterilization, respectively. Question 49 queried the development of regulatory standards for novel antimicrobial technologies.

Question 35 addressed the issue of general[izable] negative and positive controls to be included for antimicrobial testing. The wide diversity of surface types (surface-associated antibacterial and surface elution technologies) has resulted in a diverse field of tests for their performance without well-established positive or negative controls for benchmarking. It is recommended that unmodified material surfaces be incorporated as negative controls into biofilm experiments, independent of the underlying mechanism of action of the modified surface. Positive controls, that is, controls with high antimicrobial activity, are often difficult to define and standardize, and are not often reported. It may be an option to define such controls, preferably via commercially available and certified materials, for specific medical device categories separately. *The recommendation ("Unknown") and the voting (67% agreement) indicate that this is a largely unresolved question*.

Question 42 investigated whether a panel of *Staphylococcus aureus* (methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus* [MRSA]), *Staphylococcus epidermidis*, Group B Streptococci, *Escherichia coli, Pseudomonas aeruginosa*, *Cutibacterium acnes*, and *Candida albicans* strains would sufficiently capture the minimum required strains to claim universal antimicrobial efficacy when considering a novel prevention technology. These strains included important representatives of Gram-positive, Gram-negative, aerobic and anaerobic bacteria, and yeast (*Candida*). Comparison of surface antimicrobial activities within and between studies should be facilitated by using "standard" species and strain set. The species selected should be a minimal set but provide appropriate coverage of the micro-organisms causative of orthopedic infections. Moreover, the strains should be representative, available through curated, certified channels such as the American Type Culture Collection and should include antibiotic resistant strains such as MRSA. Finally, because of the possibility of fungal overgrowth, the possibility for inclusion of these species was raised. *In question 42, the recommendation ("Unknown") and the voting (69% agreement) indicate that this is a largely unresolved question*.

Question 46 asked if the in vitro sterilization method should be the same as that used in vivo. *The recommendation is "Yes" and 93% of the voters agreed*. Sterilization can affect the physical, chemical, and mechanical properties of the intended implants or materials, which is critical for softer materials such as polymers, resorbable materials, antibacterial constructs, and metals.[3,4] The choice of sterilization technique is preferably determined by its impact on clinical performance and commercial viability. For example, if a sterilization technique would negatively or unpredictably affect device antimicrobial performance, then it would be unacceptable clinically. From the perspective of moving a new antimicrobial technology toward commercialization, it would make sense to determine sterilization viability as early as possible, as it pertains to device performance.

Question 49 examined whether existing International Safe Transit Association (ISTA)/American Society for Testing and Materials (ASTM) standards can be used for constructing biofilm models on medical devices. *The recommendation is "No," with 80% agreement*. The ASTM International has implemented standardized methods, guidelines, and specifications for the accurate and reproducible formation of biofilms and testing of antimicrobial substances, which are however mainly used for environmental biofilms. Four types of biofilm devices have been addressed: the drip flow reactor and rotating disk reactor to evaluate biofilm formation in a continuous flow under low and medium shear stress; the Calgary Biofilm Device (CBD; ASTM E2799-17) and the Centers for Disease Control (CDC) biofilm reactor (ASTM E2562-17, ASTM E3161-18, ASTM E2871-19) for evaluation of disinfectants; and the colony biofilm model to grow and quantify *Bacillus subtilis* biofilms (ASTM E3180-18).[5] An ASTM symposium on "Antimicrobial combination devices" in 2020 held a session on methods for quantifying biofilms and methods for assessing antimicrobial efficacy in biofilm eradication.[6,7] Despite these efforts, there remains an unmet need to standardize methods and techniques for the evaluation of clinically relevant biofilms. The existing ASTM standard test methods may serve as a model to derive such methods.

3.3 | Methodology: Biofilm characterization

Question 39 and **Question 48** examined whether there were methods to determine minimum biofilm eradication concentrations (MBEC) and whether there was a standard method to remove bacteria from surfaces.

Question 39 asked if there is a best in vitro method for assessing MBEC. It was noted that there are many conditions that are used to form biofilms, usually developed for nonmusculoskeletal indications

and that MBEC determination is highly dependent on the specific method, surface, and medium. Crucial methodological variables to consider and report[8] include the choice of bacterial species and strains (clinical isolates vs. laboratory-acclimated), inoculum preparation and quantitation, and conditions for biofilm formation, antibiotic challenge, and recovery (time, media, pH, temperature, fluid dynamics, etc.). These variables also control biofilm maturity which can affect MBEC results. Thus, relative biofilm maturity can be assessed functionally by measuring MBECs for an antibiotic whose activity is known to vary with biofilm maturity. Additional variables are introduced in methods for quantitation of bacterial survival. Finally, the purpose of standardized methods is to be able to predict clinical efficacy. To the best of our knowledge, no correlations exist between MBEC values for antimicrobial agents assessed by different methods and their efficacy in treatment of orthopedic device infections, nor have in vivo studies to address this question been performed. This would be a very valuable area of future studies to help decide the best standard method(s) for MBEC testing. Thus, *a recommendation of "No" was given, with 82% agreement with the recommendation*.

Question 48 addressed if there is a standard method to detach and quantify bacteria attached to surfaces. The question was explored in two parts. 1. For test coupons, sonication was the most common method for recovering bacteria while other enzymatic or chemical methods were also available. 2. For recovery from a tissue around an explant, homogenization was the most common. *Based on this research, it was concluded that there was not a standard method for detaching and quantifying surface bacteria (82% agreement).*

3.4 | Conceptual: Understanding concepts in bacterial tolerance and pathogen evolution

Questions 36, 37, and **45** were questions regarding the understanding of bacterial tolerance and pathogen evolution and testing concepts to match this understanding. While it is widely acknowledged that "biofilms" are associated with higher tolerance for drugs and are thought to result in greater treatment challenges, in vivo data on the characterization of bacterial tolerance are scarce.[9] Changes in antibiotic susceptibility have been attributed to the presence and changes in the biofilm matrix, changes in bacterial metabolism, increases in efflux pump activity, and alterations of antibiotic target and bacterial membrane permeability to antibiotics.

Question 36 asked if antibiotic tolerance is an indicator for the presence or maturity of a biofilm. There was *strong consensus (79% agreement) with the "No" recommendation*. Laboratory studies support that biofilm maturity increases generally with age for cultures up to 3 weeks.[10] The degree of tolerance is dependent on the antimicrobial agent, the species, the treatment exposure time and the model system. Thus, since the degree of tolerance depends not only on the age of the biofilm but many other factors,[11] it was recommended that a single measurement is not sufficient to determine whether a biofilm is mature.

Question 37 asked if drug clearance and protein binding could be modeled in vitro to predict drug efficacy. Caution was recommended when predicting in vivo antimicrobial susceptibility using in vitro platforms, especially those that lack the factors that mimic the in vivo environment in the host. Specifically, to predict the concentration of given drugs in the plasma and local tissues of interest, one or two-compartment pharmacokinetic/pharmacodynamic (PK/PD) models with variations for route of administration and/or clearance mechanism are commonly used and are necessary as part of the development of new antibiotic drugs.[12,13] There were no PK/PD models for antibiotics using intraarticular administration. Furthermore, there is very little information on biofilm characteristics in vivo. However, the authors concluded that such modeling, given the appropriate information, could be used to predict in vivo efficacy, and *recommended that the question be answered "yes." (75% agreement)*.

Question 45 asked if small colony variants (SCV) or persisters should be detected in clinical samples. Although both persisters and SCV refer to bacteria populations that persist in the presence of antibiotic stress, persisters revert to wild-type populations upon culture in the absence of antibiotics. Auxotrophy is associated with SCV while tox/anti-tox mechanisms are generally associated with the persister phenotype. SCV, which are detectable by morphology and colony size, have been reported to constitute 2%–20% of clinical populations[14] while persisters are difficult to detect in clinical isolates.[15,16] Several methods involving colony size and differential staining are in development for the detection of persisters.[16,17] While there is currently no correlation between the presence of SCV and difference in treatment outcomes,[18] persisters in general are associated with chronic infections which have shown lower responses to antibiotic therapy. *The recommendation, based on current evidence was "Unknown" and 72% agreed*. There is a clear gap in the tools to detect persisters in clinical scenarios due to their reversion to wild-type characteristics in culture, and the general lack of standard methods/definitions for detecting SCVs and persisters. Finally, because the antibiotic concentrations to which the bacterial populations are exposed are crucial in determining bacterial fate, the modeling of accurate antibiotic concentrations in vitro with simulated in vivo factors is important and needs further research.

3.5 | Conceptual: Host/bacteria/biomaterial interactions

Questions 40 and **47** investigated bacterial-host interactions.

Question 40 asked if the concept of the "race for the surface" was still valid. The race for the surface posited that a combination of proteins, proteoglycans and host cells would cover a biomaterial surface, protecting it against colonization by bacteria. The time, number, and type of cells present; the types of biomacromolecules in the local environment; and the surface properties of the implant play significant roles in determining, in the short term, the dominance of bacteria or host cells that colonize the implant.[19,20] The authors suggested that a race may not exist based on the literature that they reviewed. Host cells appear to colonize first and depending on the strain of bacteria, either protect or succumb to a bacterial challenge.

As such, the conclusion was that there was NOT a race for the surface, with 58% agreement.

Question 47 addressed whether there are any rigorous in vitro bone cell models that can be generalized for the study of intracellular bone infections. A systematic review approach was used to analyze in vitro models of various cell types relevant to bone infection. Cell types discussed included mouse primary osteoblasts, calvarial osteoblasts, and osteoclasts; osteocyte cell lines, human mesenchymal progenitor cells, osteocytes, and primary osteoblasts, human osteoblast and osteocyte-like cell lines, human chondrocytes, and synovial fibroblasts. It was concluded that *S. aureus*, at least, could exist intracellularly. The models, using monolayer cultures of cells, varied in fidelity, with greatest confidence in primary cell models. Furthermore, it was recommended that controls should include noninternalizing bacterial strains, a method (and demonstration thereof) for clearance of extracellular bacteria, and characterization of the time and multiplicity of infection dependence of the model. With these various controls, *it was concluded that there were rigorous models for studying bacterial internalization in osteoblastic lineage cells (85% agreement)*. It was noted that studies with other musculoskeletal cell types were not sufficiently rigorous.

4 | DISCUSSION

In vitro assessments are critical for understanding MSKI and for screening possible therapies. While many studies seek to define antimicrobial properties, these studies are limited, and the in vitro section responses highlight this resultant high degree of variability.

The questions that achieved the highest consensus (Q. 43, outcome measures [97%] and Q. 46, sterilization method [93%]) indicate that target system-dependence must be considered. These recommendations integrate with the "No" recommendation for Q. 49 (79%) that concludes that existing ISTA/ASTM methods are inadequate for medical biofilm models. Together, these emphasize further need for flexibility and better definition of critical conditions.[21]

Recommendations of 1.5–3 log reduction for designating antibacterial efficacy rely on relatively few studies and highlight the need for integrated in vitro/in vivo experimental design. Other questions indicate that biofilm controls are necessary, but defining bacterial inocula, antibiotic tolerance, biofilm maturation, MBEC conditions, strain choice and breadth, and even detachment methods can be highly variable with gaps in knowledge of the relevance of in vitro methods to in vivo outcomes.

Modeling of bacterial internalization into osteoblastic cells was supported. However, the concept of the competition between native cells and bacteria, that is, "race for the surface," was felt to be limited and that the many variables made the concept of a race inadequate. Overall, there was 58% agreement and 31% disagreement.

The presence of persisters and SCV may further drive the need for benchmarks. While it is clear that persisters are clinically important, the role of SCV is not clear. There were also some unanswered questions dealing with the acquisition of antibiotic resistance and altered virulence, which are compelling subjects requiring further exploration.

Overall, the questions addressed in this section highlighted the need for studies that include both in vitro determinations with in vivo outcomes, and the need for better characterization of the different variables that determine pathogen behavior.

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.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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