MENTORS IN ORTHOPEDICS

BIOFILM-RELATED INFECTIONS: HOW TO IMPROVE LABORATORY DIAGNOSIS

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AUTHORS

Lorenzo Drago - University of Milan, Milan, Italy
Giarritiello Fabiana - University of Molise, Campobasso, Italy
Luigi Regenburgh De La Motte - UOC Laboratory of Clinical Medicine with Specialized Areas, IRCCS MultiMedica, Milan, Italy
Guenter Lob - DGOU, Berlin, Germany
Hazem Mohammad Ishaq Alkhawashki - Advanced Medical Center, Riyadh, Saudi Arabia
Carlo Luca Romanò - Studio Medico Cecca-Romano, Milan, Italy

SUMMARY

Background: Biofilm-associated infections in medical devices, such as prosthetic joints, pose significant clinical challenges. The bacterial extracellular matrix protects pathogens from host immunity and conventional diagnostics. Bacteria within biofilms often exist in a low-metabolic state, frequently resulting in false-negative cultures and delayed treatment for periprosthetic joint infection (PJI).

Objective: This article examines the efficacy of mechanical and chemical biofilm disruption techniques, specifically sonication and dithiothreitol (DTT) treatment, and evaluates their integration with molecular diagnostics to optimize pathogen recovery and identification in orthopedic settings.

Key Points: Diagnostic sensitivity is enhanced by disrupting the biofilm matrix to release bacteria. Sonication employs ultrasound vibrations to dislodge organisms from explanted hardware. Alternatively, DTT serves as a mucolytic reducing agent that cleaves disulfide bonds in the extracellular polymeric substance, facilitating bacterial release from both solid surfaces and biological fluids like synovial fluid. While culture remains the gold standard for susceptibility testing, molecular methods such as multiplex PCR and 16S rRNA sequencing provide critical data for fastidious or antibiotic-suppressed organisms. Current International Consensus Meeting (ICM) guidelines incorporate these advanced modalities into diagnostic algorithms. Emerging technologies, including metagenomics, metabolomics, and microfluidic biosensors, represent future directions for increasing diagnostic precision and speed.

Conclusion: Improving the detection of biofilm-mediated infections necessitates a transition from isolated culture methods to integrated diagnostic workflows. Utilizing sonication or DTT alongside molecular assays significantly increases microbiological yield, supporting more accurate clinical decision-making and improved patient outcomes in prosthetic joint revision surgery.

KEYWORDS

Prosthesis-Related Infections; Arthroplasty, Replacement, Knee; Arthroplasty, Replacement, Hip; Dithiothreitol; Sonication

INTRODUCTION

Infections involving biofilms are increasingly recognized as a major burden in clinical practice, particularly in the context of medical devices such as prosthetic joints, catheters, and heart valves. In these scenarios, bacteria organize themselves into complex communities attached to surfaces and encased in a self-produced matrix. This matrix protects the bacteria not only from the immune system but also from antibiotics and, crucially, from diagnostic methods traditionally used in microbiology laboratories. Diagnosing these infections is not straightforward. Standard cultures often yield negative results even when infection is present, and clinicians are left with uncertainty that can delay appropriate treatment. Therefore, improving the laboratory's ability to detect biofilm-associated infections is essential for better patient outcomes. Biofilm Infections Are Hard to Detect.One of the primary reasons biofilm infections are so elusive is that the bacteria embedded in the matrix are in a low-metabolic, often non-replicating state. This makes them much less likely to grow in conventional culture media. In addition, prior exposure to antibiotics further reduces the yield of standard cultures. This leads to a classic clinical paradox: a patient may present with signs of infection, but cultures from blood, tissue, or synovial fluid may come back negative. In such cases, unless the laboratory adopts specific protocols aimed at disrupting the biofilm, the true etiology of the infection may remain hidden.

BREAKING THE BIOFILM: SONICATION AND DTT

To overcome the challenge of poor culture sensitivity, several techniques have been developed to physically or chemically disrupt the biofilm matrix and release the bacteria into a form that can be more easily detected.

Sonication is perhaps the best-known technique in this context. It involves placing explanted devices—such as joint prostheses—into a sterile container filled with fluid and then exposing them to ultrasound waves (Figure 1). The vibrations break up the biofilm and release the embedded bacteria into the surrounding fluid, which is then cultured. This method was validated by Trampuz et al. [1] and confirmed by Portillo et al. [2].

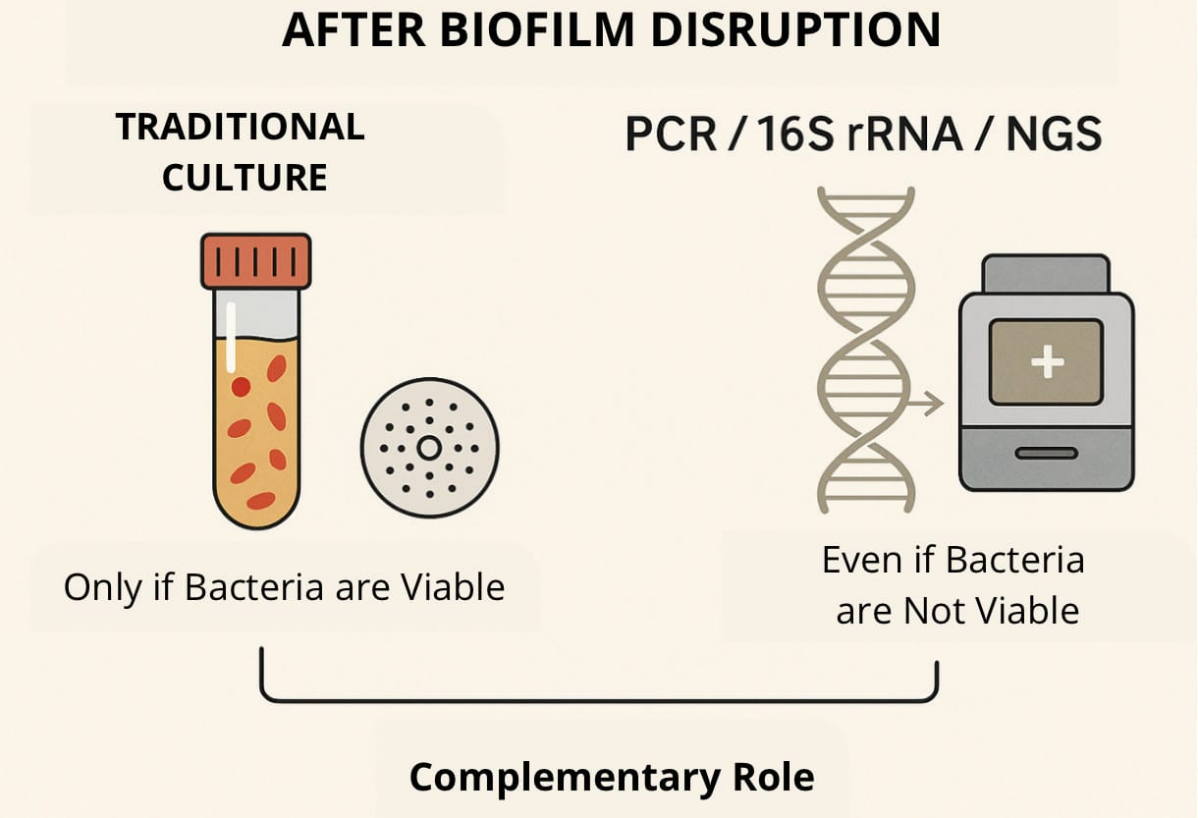

Figure 1 : Illustrates the sonication process and subsequent bacterial release for culturing.

An alternative to sonication is the use of dithiothreitol (DTT), a chemical agent capable of breaking disulfide bonds in the biofilm matrix. When applied to explanted devices or tissue samples, DTT can effectively disrupt the biofilm and release viable bacteria into solution. Its effectiveness was shown by Drago et al. [3] and later supported by Karbysheva, S et al. [4].

BIOFILMS IN BIOLOGICAL FLUIDS AND THE ROLE OF DTT IN DIAGNOSTIC ENHANCEMENT

Biofilm formation is not restricted to the surfaces of implanted devices; increasingly, evidence shows that bacteria can form biofilm-like aggregates directly in biological fluids such as synovial fluid, cerebrospinal fluid, and bronchoalveolar lavage. These aggregates are typically embedded in host-derived extracellular matrices such as fibrin or hyaluronic acid, making them difficult to detect with traditional culture methods. This phenomenon is particularly relevant in prosthetic joint infections (PJIs), where biofilm aggregates in synovial fluid can lead to false-negative cultures and underdiagnosis [5].

Dithiothreitol (DTT), a mucolytic and reducing agent, has emerged as a valuable tool in this context. It acts by breaking disulfide bonds within the extracellular polymeric substance (EPS) of the biofilm, as well as in the host-derived matrix, thereby releasing bacteria into suspension without compromising their viability. This chemical disruption improves the recovery of pathogens from samples such as synovial fluid, as schematized in Figure 2 [6].

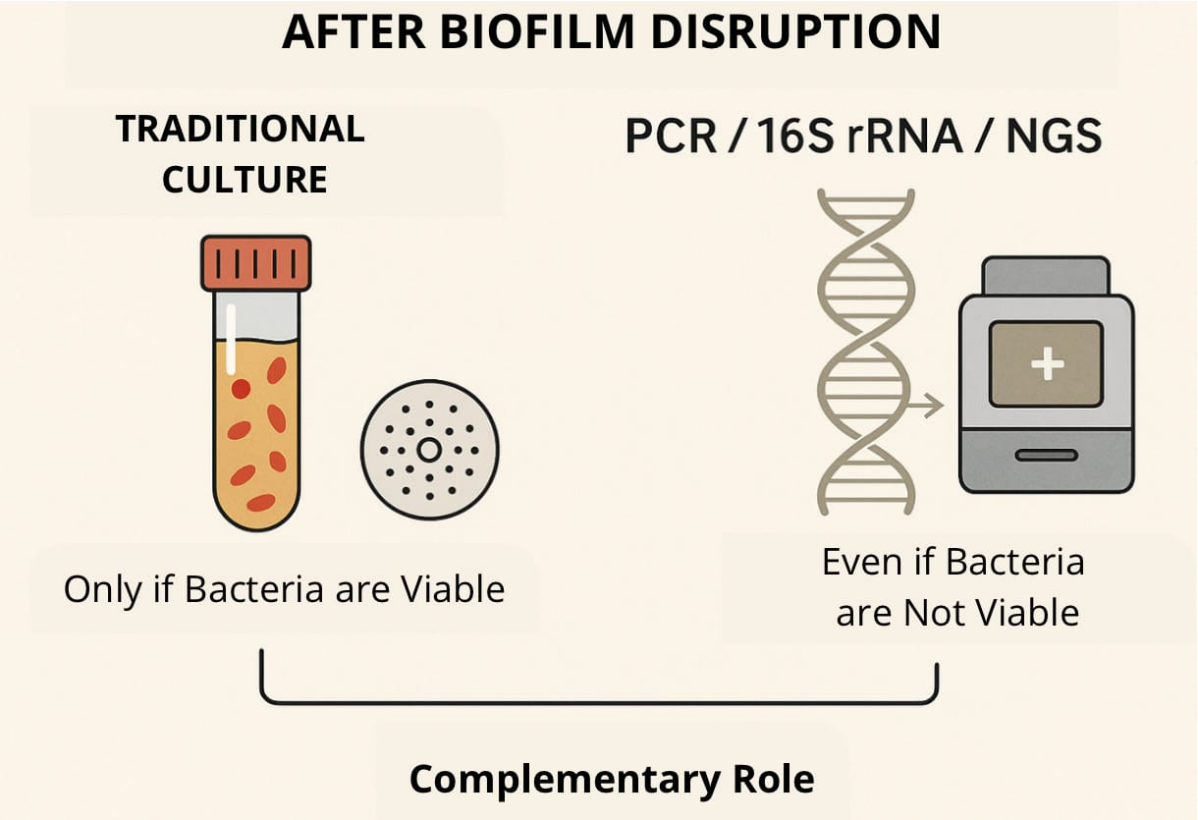

Figure 2 : Shows how biofilm in synovial fluid can be disrupted by DTT to improve pathogen recovery.

The ability of DTT to disaggregate biofilm structures in liquid matrices offers a practical and cost-effective enhancement to microbiological workflows in routine laboratories, and its application is expanding beyond orthopedic infections to include other biofilm-prone clinical contexts.

CULTURE VS. MOLECULAR TECHNIQUES: A COMPLEMENTARY ROLE

Once the biofilm has been disrupted, the next step is to detect and identify the bacteria. Traditionally, this has been done using culture methods, which remain the gold standard for antimicrobial susceptibility testing. However, culture has its limits—especially in patients pretreated with antibiotics, or when dealing with slow-growing or fastidious organisms.

This is where molecular methods come into play. Techniques like PCR, 16S rRNA sequencing, and multiplex PCR panels allow for the direct detection of bacterial DNA, even when the organisms are not viable (Figure 3). These methods have shown increased sensitivity in several studies, including work by Tsang et al. [7] and Tande et al. [8].

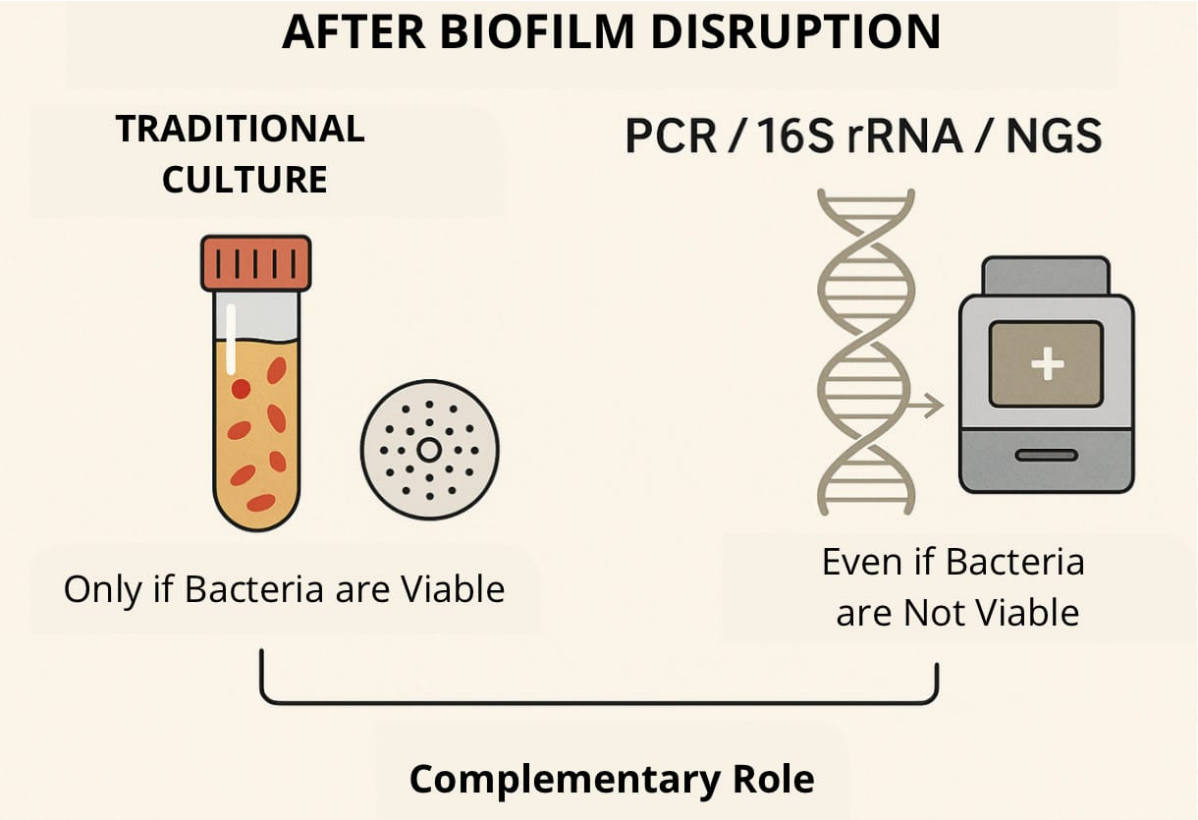

Figure 3 : Culture detects viable bacteria, while molecular methods identify pathogens even when non-viable. Combined use improves diagnostic accuracy.

PUTTING IT ALL TOGETHER: DIAGNOSTIC ALGORITHMS

An ideal diagnostic strategy for biofilm-associated infections should incorporate a combination of mechanical or chemical biofilm disruption techniques—such as sonication or dithiothreitol (DTT)—with both culture-based and molecular diagnostics. This integrated approach enhances diagnostic sensitivity and specificity by addressing the major limitations of each method when used alone. Biofilm disruption increases the release of viable bacteria that would otherwise remain embedded in the matrix, while molecular techniques offer the ability to detect non-cultivable or fastidious organisms and can provide results even when antibiotics have been administered prior to sampling.

Recent evidence supports the use of such multimodal workflows. For example, Portillo et al. demonstrated that combining sonication fluid cultures with periprosthetic tissue samples significantly increased the microbiological yield in prosthetic joint infections [2].

Similarly, studies using DTT have shown improved recovery rates of pathogens in both solid and liquid clinical matrices, especially in culture-negative scenarios [9].

Molecular assays, including multiplex PCR and 16S rRNA sequencing, have proven particularly useful when culture results are negative or inconclusive, further contributing to diagnostic clarity [7],[8].

Importantly, the most recent iterations of the diagnostic criteria for prosthetic joint infection—such as those developed by the International Consensus Meeting (ICM) in 2018, 2023 and updated in 2025—explicitly include the use of sonicate fluid culture and molecular diagnostics (e.g., PCR and next-generation sequencing) as part of

their scoring algorithms and supportive criteria. These guidelines underscore the value of combining clinical, laboratory, and microbiological data to improve diagnostic certainty and treatment decisions.

The implementation of standardized diagnostic algorithms that leverage biofilm-disruptive techniques and multiple detection platforms is increasingly seen as a best practice in microbiology laboratories dealing with device-associated infections. Such workflows require close interdisciplinary collaboration among surgeons, infectious disease specialists, and microbiologists, as well as adequate infrastructure and training to ensure proper sample collection, processing, and interpretation.

LOOKING AHEAD: FUTURE DIRECTIONS IN BIOFILM DIAGNOSTICS

As scientific and technological innovation accelerates, the field of biofilm diagnostics is poised for a profound transformation. The traditional reliance on culture methods is gradually being complemented—and in some cases challenged—by cutting-edge tools that offer the potential for faster, more sensitive, and more comprehensive pathogen detection.

One of the most promising areas is metagenomics, which allows for untargeted sequencing of all microbial DNA in a clinical specimen. Unlike targeted PCR, metagenomics does not require prior knowledge of the organism, making it particularly valuable in polymicrobial infections or cases with rare or fastidious pathogens. Preliminary studies have shown its ability to identify pathogens in culture-negative prosthetic joint infections and other implant-related infections, although cost, turnaround time, and data interpretation remain challenges to widespread adoption.

Closely related to this are proteomics and metabolomics, which focus on identifying specific bacterial proteins or metabolic signatures associated with biofilm presence and activity. These approaches may not only detect the presence of infection but also help characterize the physiological state of the pathogens—whether dormant, active, or resistant—thus providing valuable information for therapeutic decisions.

Another emerging frontier involves biosensors, which are engineered to detect microbial components or biofilm-specific markers in real time. These compact, point-of-care devices could one day allow clinicians to identify biofilm-related infections intraoperatively or even bedside, facilitating earlier and more targeted interventions.

Machine learning and artificial intelligence (AI) are also making inroads. By integrating complex data from laboratory tests, clinical parameters, and imaging, AI-driven algorithms can help predict the likelihood of biofilm infection, suggest optimal diagnostic workflows, or even flag atypical cases that warrant molecular testing.

Additionally, microfluidics—the manipulation of fluids in miniaturized channels—is being applied to develop lab-on-a-chip systems that combine biofilm disruption, DNA extraction, amplification, and detection in a single, automated platform. These technologies hold promise for reducing turnaround times and minimizing sample volume requirements, making them ideal for point-of-care settings. Beyond the technological landscape, the concept of diagnostic stewardship will become increasingly important. Just as antimicrobial stewardship ensures appropriate antibiotic use, diagnostic stewardship promotes the correct selection, timing, and interpretation of diagnostic tests. This is particularly critical in biofilm infections, where unnecessary or poorly interpreted tests can lead to overdiagnosis, inappropriate therapy, or missed infections.

Ultimately, the integration of these novel technologies into routine microbiology practice will depend on validation studies, cost-benefit analyses, and collaboration among clinicians, microbiologists, engineers, and data

scientists. With appropriate investment and interdisciplinary effort, the future of biofilm diagnostics promises to be not only more accurate and efficient, but also more personalized and clinically impactful.

CONCLUSION

Biofilm-related infections are difficult to detect with standard laboratory methods, but advances in biofilm disruption techniques—particularly sonication and DTT—are helping to close the diagnostic gap. When combined with both culture and molecular methods, these approaches significantly enhance our ability to identify pathogens that would otherwise remain hidden.

The future of biofilm diagnostics lies in integrated, multi-modal strategies that combine physical, chemical, and molecular techniques with clinical insight and emerging technologies. As laboratories adopt these innovations and clinicians become more aware of their utility, the diagnosis and management of biofilm infections will become more accurate, timely, and effective.

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