MENTORS IN ORTHOPEDICS

THE SAMPLE'S JOURNEY IN ORTHOPEDIC IMPLANT INFECTIONS : FROM THE OPERATING ROOM TO THE MICROBIOLOGY LAB

https://doi.org/10.71165/xjme-5qej

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SUMMARY

Background: Implant-related infections (IRIs) are significant complications in orthopedic surgery, often involving microbial biofilms on biomaterial surfaces. These structured communities complicate diagnosis, leading to false-negative results and substantial healthcare costs associated with suboptimal management.

Objective: This review delineates standardized intra-operative sampling procedures and evaluates physical and chemical biofilm-targeted pretreatment strategies designed to enhance microbiological diagnostic sensitivity and specificity.

Key Points: Accurate diagnosis requires adherence to structured protocols, such as the World Association against Infection in Orthopaedics and Trauma guidelines, prioritizing sequential collection of synovial fluid, tissue, and hardware. Maintaining a cold chain at 4°C and utilizing sterile, hermetic containers are essential for preserving pathogen viability. To liberate biofilm-embedded bacteria, validated pretreatment techniques include sonication of hardware and chemical elution using dithiothreitol (DTT). Sonication utilizes low-frequency ultrasound to disrupt the extracellular matrix, while DTT cleaves disulfide bonds. Microbiological analysis should incorporate blood culture bottle systems and extended 14-day incubation periods to detect fastidious organisms. Quantitative thresholds, typically ≥50 CFU/mL, help distinguish infection from contamination. Molecular diagnostics, including polymerase chain reaction and next-generation sequencing, serve as adjuncts in culture-negative scenarios but require careful clinical correlation to avoid misinterpretation of non-viable microbial DNA.

Conclusion: Optimizing the diagnostic pathway through standardized sampling and antibiofilm pretreatment reduces diagnostic errors. Multidisciplinary collaboration is essential for accurate pathogen identification, supporting antimicrobial stewardship and improving clinical outcomes in the management of orthopedic infections.

KEYWORDS

Prosthesis-Related Infections; Biofilms; Arthroplasty, Replacement; Sonication; Dithiothreitol

INTRODUCTION

Implant-related infections (IRIs) represent one of the most feared complications in orthopedic and trauma surgery [1]. These infections can affect joint prostheses, internal fixation devices, and other biomaterials, often leading to prolonged treatments, multiple surgeries, and poor functional outcomes [2]. One of the main challenges in diagnosing these infections lies in the behavior of the causative microorganisms, which frequently reside within biofilms — structured communities that adhere to implant surfaces and resist both antibiotics and immune responses [3],[4],[5].

Correct identification of the responsible pathogens is fundamental to plan adequate antimicrobial therapy and surgical strategies [6]. However, microbiological diagnosis is highly dependent on the quality of the sample collection and handling process. The pre-analytical phase — often underestimated — includes all steps from intra-operative sampling to laboratory processing, and each step carries a risk of error that may compromise the final diagnosis [7].

In this review, we describe the current procedures for intra-operative sampling and biofilm-targeted pretreatment strategies prior to culture analysis. We emphasize the critical impact of false negatives (missed infections) and false positives (contaminants misidentified as pathogens), both of which can lead to inappropriate therapy, extended hospital stays, unnecessary surgeries, and increased costs. Analysis estimated that a single false negative in prosthetic joint infection can incur more than €49.000 in additional patient costs, while a single false positive may generate over €8.500 in unnecessary treatments and follow-up expenses [8]. The following sections cover best practices for sampling, handling and transport of explanted materials, chemical biofilm disruption techniques, and workflow optimizations to minimize diagnostic errors. The goal is to provide a clear guide to support clinicians, microbiologists, and healthcare teams in optimizing diagnostic accuracy in implant-related infections.

SAMPLING: CURRENT INTRA-OPERATIVE PRACTICES

Accurate intra-operative sampling underpins reliable microbiological diagnosis in IRIs by balancing two objectives: maximizing pathogen recovery (high sensitivity) and minimizing contamination (high specificity).

Sampling must follow a strict, stepwise protocol. The World Association against Infection in Orthopaedics and Trauma (WAIOT) “10-rules” procedure, reported in Figure 1, begins with synovial fluid sampling by fine needle joint aspiration prior to surgery and/or, at surgery, immediately after skin incision—thus avoiding the risk of dragging skin flora into the joint—followed by immediate transfer of the fluid into sterile containers or blood culture bottles. Any purulent exudate at the incision site is likewise collected and sent for culture. Next, a biopsy of the synovial membrane or joint capsule is obtained using sterile instruments for each specimen [9],[10].

Figure 1 : The 10 WAIOT golden rules.

Once tissue sampling is complete, prosthetic components or osteosynthesis devices are explanted, usually from 3 to 6 samples [7]. Before handling the device, the surgical team should change gloves and switch to a fresh instrument set. The removed hardware is placed directly—never resting on the operative field or trays—into dedicated sterile, hermetically sealed transport containers [11],[12]. Finally, periprosthetic tissues, bone sequestra or osteolytic fragments at the implant–bone interface are sampled in the same manner. Each specimen—fluid, soft tissue, implant, bone—must be collected with sterile instruments and immediately sealed [13] amcli.it.

The Italian Society of Orthopaedics and Traumatology (SIOT) guidelines reinforce these principles, emphasizing gloves and instrument changes between each sampling step and strict avoidance of any intermediate handling or surface contact [https://old.giot.it/wp-content/uploads/2018/04/04_Art_LINEE_Guida-1.pdf]. The Italian Association of Clinical Microbiologists (AMCLI) pathway mirrors this sequence—synovial fluid first, then capsule, then implant components, and finally periprosthetic tissue or bone—providing detailed instructions on container choice, labeling, and rapid transfer to the microbiology laboratory [amcli.it].

Adherence to these recommendations (schematized in Figure 2), dedicated tools per specimen, strict asepsis, immediate sealing, and a clear sampling order, lays the groundwork for reliable downstream transport, storage, pretreatment, and culture, thereby reducing both false negatives and false positives.

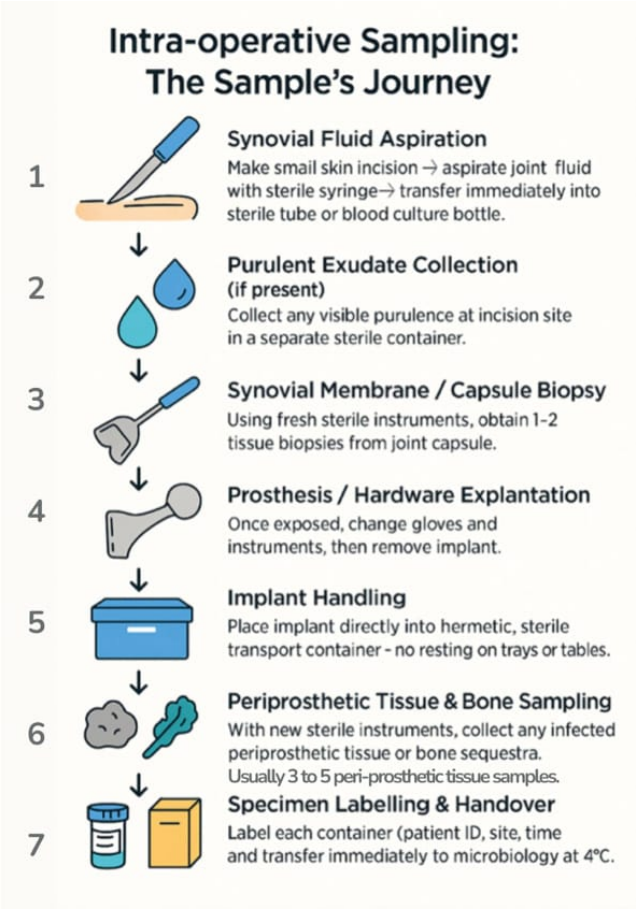

Figure 2. Intra-operative Sampling Workflow Stepwise guide to the "sample's journey" in implant-related infections: (1) synovial fluid aspiration via small incision; (2) collection of purulent exudate if present; (3) synovial membrane or joint capsule biopsy; (4) prosthesis or hardware explant following glove and instrument change; (5) direct placement of the implant into hermetic transport containers; (6) periprosthetic tissue and bone sampling with fresh instruments; and (7) specimen labeling and immediate transfer to the microbiology laboratory at 4 °C.

HANDLING, STORAGE, AND TRANSPORT OF SAMPLE

Proper handling and transport of intra-operative specimens are critical to preserving pathogen viability and preventing overgrowth of contaminants. As soon as each sample is collected, it should be sealed in its dedicated sterile, hermetic container in the operatory room [World Health Organization, 2012. Guidance on Regulations for Transport of Infectious Substances 2013-2014, World Health Organization. Switzerland. Retrieved from https://coilink.org/20.500.12592/crmmz2 on 25 May 2025. COI: 20.500.12592/crmmz2.]. Clear labeling directly on the container—indicating patient identifiers, anatomical site, date and time of collection, and collector's initials—ensures accurate tracking A standardized request form, detailing relevant clinical information such as recent antibiotic therapy and the patient's comorbidities, must accompany every specimen [14].

Tissue, bone, and explanted implant components should be kept at 4 °C from collection until they reach the laboratory, if transit exceeds 2 hours. [15],[16]. If immediate transport is not possible, refrigerated storage at 4°C for up to 24 hours is acceptable, though longer delays increase the risk of bacterial death and false-negative cultures [17],[18],[19]. Synovial fluid specimens, when inoculated directly into blood-culture bottles in the

operating room, may be held at ambient temperature and processed according to the manufacturer's incubation protocol. Freezing any clinical material is strictly discouraged, as ice crystal formation damages cellular structures and compromises culture yield [20].

For transport, specimens must be placed in rigid, secondary container and clearly separated from any non-clinical items. Courier services or laboratory porters should be notified in advance of time-sensitive shipments to prevent unintended delays. Upon arrival at the microbiology laboratory, each batch of samples is checked against its request form, and a chain-of-custody log is signed to document receipt. Any deviations—such as temperature excursions or prolonged transit times—are recorded promptly.

By ensuring immediate sealing and labeling, strict temperature maintenance, rapid delivery, and meticulous documentation, the integrity of the sample "journey" is preserved, maximizing culture sensitivity and reducing diagnostic errors (Figure 3).

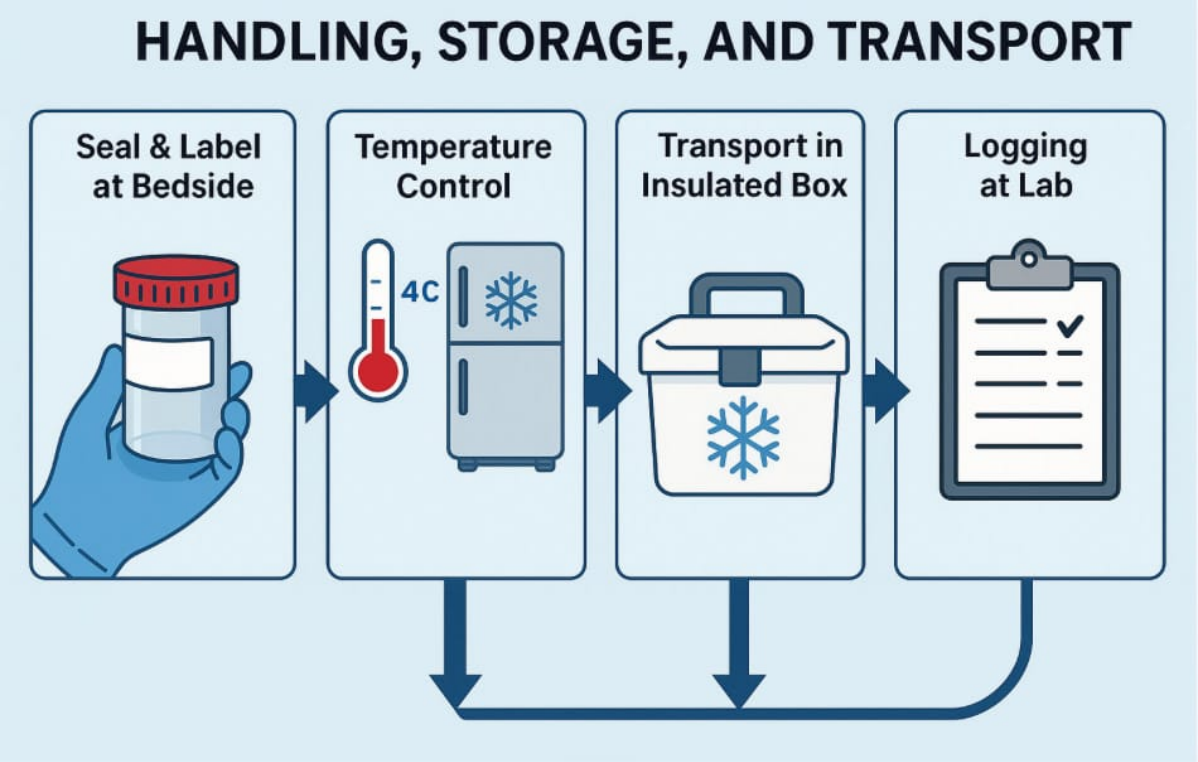

Figure 3. Handling, Storage, and Transport Workflow Infographic summarizing the post-collection phase: (1) bedside sealing and labeling of each specimen in sterile, hermetic containers; (2) maintenance of tissue, bone, and implant samples at 4 °C (up to 24 h) and ambient transport for inoculated blood-culture bottles; (3) placement in rigid, insulated secondary boxes with cold packs; and (4) logging and chain-of-custody documentation upon arrival in the microbiology laboratory.

PHYSICAL OR CHEMICAL ANTIBIOFILM PRETREATMENT TECHNIQUES

Routine cultures of biofilm- and implant-related infections may fail or yield false culture-negative results in a significant number of patients, with figures ranging from 5% to 42% in Prosthetic Joint Infections. (PJIs) [21]; to

increase the sensitivity, pretreatment of samples have been proposed to liberate biofilm-embedded bacteria. Validated antibiofilm strategies include sonication and chemical biofilm debonding with dithiothreitol (DTT).

Physical antibiofilm pretreatment with Sonication

Implanted components are submerged in sterile fluid and exposed to low-frequency ultrasound waves (typically 40–50 kHz for 5–10 minutes) [22]. Cavitation disrupts the extracellular polymeric matrix, releasing bacteria into suspension for culture [23],[24]. The SIOT recommends sonication exclusively for explanted hardware (excluding cement), with quantitative thresholds (≥50 CFU/mL sonicate or ≥200 CFU/mL concentrated sonicate) indicating infection [25]. Advantages include wide availability in high volume orthopedic surgery centers and proven efficacy [26]; limitations are equipment cost, operator dependency, maintenance of the sonicator, potential bacterial damage if parameters are not strictly controlled [27], and the need to manually sort the sample, a step that can itself introduce cross-contamination. Moreover, phenotypic changes of some pathogen following sonication have been reported [28]. It is also worth noting that some well-known experts did not find a superiority of sonication over tissue samples collected according to strict rules [29].

Chemical antibiofilm pretreatment with Dithiothreitol

Dithiothreitol (DTT) is a reducing agent that cleaves disulfide bonds within the biofilm matrix. Per standard protocols, tissue or explant samples are incubated in 0.1% DTT (≈25 mM) for 15 minutes at room temperature, then vortexed and cultured [30]. According to SIOT guideline, DTT pretreatment can be applied both to prosthetic material and periprosthetic tissue, as shown by various studies [31]. Evidence demonstrates that DTT treatment increases sensitivity—up to 85% in some series—without impairing microbial viability [6]. Advantages include its relative low-cost, the possible implementation in all hospitals without the need for specific equipments, and the applicability to all types of explanted materials (fluids, tissues, metallic and polymeric implants), making the procedure less operator-dependent, more streamlined, and reducing cross-contamination from manual handling. Care must be taken to respect concentration and contact-time parameters, as excessive exposure can have bactericidal effects [32].

To further standardize and secure this workflow and minimize contamination, completely closed systems, like MicroDTTect®, integrate DTT elution within a sterile, single-use, specifically designed closed cartridge : implants or tissues are loaded intra-operatively, sealed, transported and then processed without any open handling, yielding up to 98 % diagnostic sensitivity and specificity while minimizing hands-on time, contamination risk and logistical complexity [8].

The 2018 International Consensus Meeting (ICM) on hip and knee PJIs specifically endorses sonication and DTT technologies to minimize culture-negative cases [33].

Using physical disruption (sonication) or chemical biofilm dissolution (DTT or MicroDTTect®), laboratories can dramatically improve microbial recovery from both tissue and prosthetic materials (Figure 4). In the next section, we will review culture protocols and diagnostic considerations once samples have been pretreated.

METHOD	DESCRIPTION	KEY FEATURE(S)
Sonication 	Uses ultrasonic waves to physically disrupt biofilm structure, releasing bacteria for culture examination. 	• Effective physical disruption; requires ultrasonic device. • Multi-step procedure: contamination risk. • May induce phenotypic change in some microorganisms. • Suitable for implants.
Dithiothreitol (DTT) 	Chemical agent that reduces disulfide bonds in biofilm matrix, breaking it down chemically. 	• Chemical disruption targeting biofilm matrix. • Suitable for implants, tissues, organic fluids analysis.
MicroDTTect 	A closed-circuit system combining DTT chemical treatment with sample collection to disrupt biofilm and reduce contamination risk. 	• Chemical disruption targeting biofilm matrix. • Completely closed circuit reduces contamination risk. • Suitable for implants, tissues, organic fluids analysis.

Figure 4: Comparison of Antibiofilm Pretreatment Techniques. Infographic overview of three key biofilm disruption methods for implant-related infections.

MICROBIOLOGICAL CULTURE AND DIAGNOSTIC CONSIDERATIONS

Optimal sampling, transport, handling and antibiofilm pretreatment are key preliminary steps to cultural examination, that remains the diagnostic cornerstone for implant-related infections. However, accurate interpretation of culture results requires attention to technique, incubation conditions, and the clinical context.

Quantitative and Qualitative Culture

Explanted tissue and synovial fluid should be plated onto solid media and inoculated into blood-culture bottles (BCBs). BCB systems enable larger sample volumes, continuous growth monitoring, and in-built antibiotic neutralization—often yielding faster and more sensitive detection than agar alone [34],[35].

Release of bacteria by sonication fluid or DTT eluates must be quantified: recovery of at least 50 CFU/mL of sonicate or DTT eluate is considered significant, whereas lower counts or growth only in enrichment broths demand clinical correlation before labeling an infection. [36],[37],[38] [https://old.giot.it/wp-content/uploads/2018/04/04_Art_LINEE_Guida-1.pdf].

To capture slow-growing organisms (e.g., Cutibacterium acnes), incubation should extend to 14 days, with a subculture check at day 5 and a final readout at day 14 [13],[21],[39],[40],[41].

Culture-Negative Scenarios

When microbiological cultures remain negative despite a high clinical and intra-operative suspicion of infection, several steps are strongly recommended. Surface swabs should be avoided to reduce contamination risk and repeat sampling—preferably multiple tissue specimens and synovial fluid—should be performed immediately [42]. At the time of explantation, all removed materials (hardware, tissue, fluid) should undergo antibiofilm pretreatment—sonication of implants whenever feasible, or chemical elution with dithiothreitol (DTT) for both biotic and abiotic samples—and, where possible, inoculation directly into blood-culture bottles to maximize recovery [43]. Cultures must be incubated for more than 14 days when anaerobic or fastidious organisms are suspected [40]. Parallel testing using different media and methods can further enhance yield. Finally, if cultures remain sterile, molecular diagnostics—broad-range or targeted PCR and next-generation sequencing (NGS)—may be employed, albeit with caution, since these methods can detect non-viable microbial DNA and require careful interpretation in the clinical context [44].

Molecular and Rapid Diagnostics

Molecular assays can play a crucial adjunct role in culture-negative scenarios, after prior antibiotic exposure, or when fastidious or biofilm-embedded organisms are suspected. Broad-range PCR targeting the bacterial 16S rRNA gene offers an unbiased approach, capable of detecting unexpected or slow-growing pathogens directly from synovial fluid, sonication fluid, or DTT eluate [45]. Its main advantages are high sensitivity and the ability to uncover rare organisms; however, it carries a significant risk of false positives from contaminant or non-viable DNA and does not distinguish live from dead bacteria, necessitating rigorous laboratory controls.

Targeted multiplex PCR panels—such as FilmArray or custom real-time qPCR assays—provide results within hours and can simultaneously identify predefined pathogens and key resistance genes [46]. This rapid turnaround enables earlier, more focused antimicrobial therapy, but panels are inherently limited to the organisms and resistance markers they include and may miss atypical or emerging pathogens [47],[48].

Next-generation sequencing (NGS) and metagenomic techniques hold the promise of comprehensive, culture-independent profiling of bacteria, fungi, and resistance determinants in a single assay [49],[50]. These methods eliminate panel restrictions and can reveal a full spectrum of pathogens and resistance genes. However, their high cost, need for specialized equipment and bioinformatics expertise, and longer turnaround times currently preclude routine implementation in most clinical laboratories. As these technologies mature, decrease in cost, and become more standardized, they may gradually transition from specialized referral centers to broader diagnostic use [51].

Reporting and Interpretation

Laboratory reports must strike a balance between speed and accuracy. A preliminary report—issued around day 5—alerts clinicians to any initial growth, even at low levels, with an antibiogram pending. The final report, at the end of the incubation period, should classify isolates as:

- Definitive pathogens (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacterales at significant counts)
- Possible contaminants (e.g., coagulase-negative staphylococci, Cutibacterium spp. from a single specimen; annotate “possible contaminant—interpret clinically”)
- No growth

By integrating quantitative culture thresholds, antibiofilm-enhanced methods, extended incubation, and judicious molecular testing, laboratories can deliver nuanced, timely data that directly inform appropriate antimicrobial therapy and surgical decision-making in implant-related infections [9].

CRITICAL ISSUES AND COMMON PITFALLS

Even the best protocols can be undermined by errors at any stage of the diagnostic pathway. Below are the most frequent missteps—and their downstream consequences—that teams must vigilantly guard against:

By anticipating these pitfalls, schematically reported in Figure 5 —and embedding quantitative culture criteria, strict time-and-temperature controls, clear reporting language, and tight coordination between surgeons, microbiologists, and infectious-disease specialists—teams can minimize both false positives and false negatives, ensure appropriate therapy, and curb the rise of antibiotic resistance in implant-related infections.

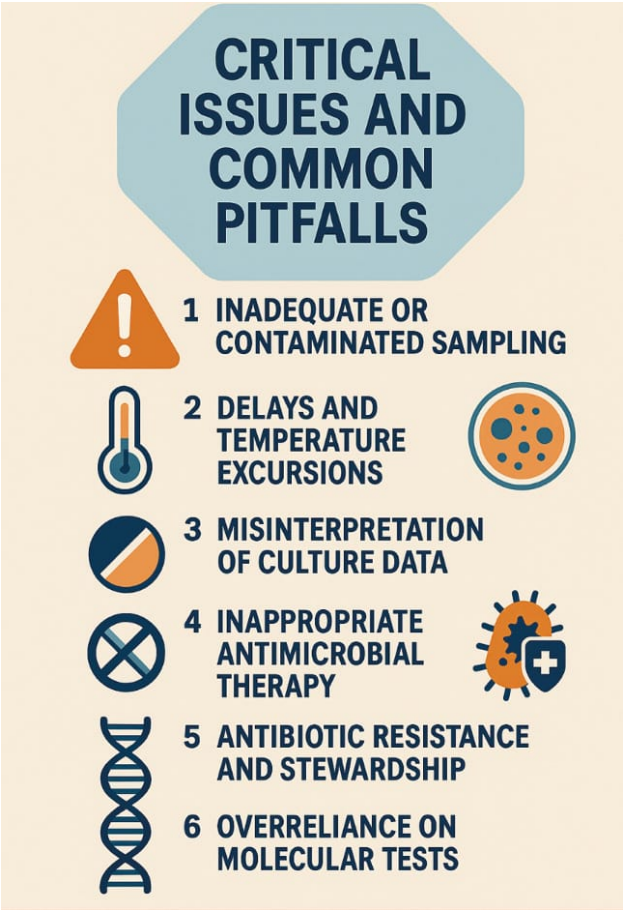

Figure 5. Critical Issues and Common Pitfalls in IRI Diagnosis Illustration of six frequently encountered challenges that can compromise microbiological diagnosis of implant-related infections: (1) inadequate or contaminated sampling leading to false negatives/positives; (2) delays and temperature excursions causing loss of viable pathogens; (3) misinterpretation of low-level growth without quantitative thresholds; (4) inappropriate antimicrobial therapy fueling patient harm and resistance; (5) antibiotic resistance undermining stewardship efforts; and (6) overreliance on molecular tests without clinical correlation.

Illustration of six frequently encountered challenges that can compromise microbiological diagnosis of implant-related infections:

1. inadequate or contaminated sampling leading to false negatives/positives;
2. delays and temperature excursions causing loss of viable pathogens;
3. misinterpretation of low-level growth without quantitative thresholds;
4. inappropriate antimicrobial therapy fueling patient harm and resistance;
5. antibiotic resistance undermining stewardship efforts; and
6. overreliance on molecular tests without clinical correlation.

CONCLUSIONS

Successful diagnosis of implant-related infections hinges on rigorous attention to each step of the sample's journey—from precise, aseptic intra-operative collection through rapid, cold-chain transport, to targeted biofilm disruption and extended culture protocols. When surgeons, microbiologists, and infectious-disease specialists collaborate closely—adhering to consensus-driven guidelines, using sonication or DTT (including closed-system devices), and reserving molecular assays for truly culture-negative cases—they can sharply reduce both false negatives and false positives. This integrated, multidisciplinary approach not only ensures accurate pathogen identification and tailored antimicrobial therapy but also supports antimicrobial stewardship, minimizes patient morbidity, and optimizes clinical outcomes in the management of implant-related infections.

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