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PRECISION DIAGNOSIS OF BONE AND JOINT INFECTIONS: INTEGRATING PATHOLOGY, CLINICAL MICROBIOLOGY, MOLECULAR DIAGNOSTICS, AND SURGICAL MANAGEMENT- A SYSTEMATIC REVIEW

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ABSTRACT

Background: Bone and joint infections (BJIs), including native osteomyelitis, septic arthritis, vertebral osteomyelitis, fracture-related infection (FRI), and periprosthetic joint infection (PJI), remain diagnostically challenging because previous antimicrobial exposure, biofilm formation, low microbial burden, sampling error, and overlap with sterile inflammatory disorders reduce the reliability of any single test.

Objective: To systematically review contemporary evidence on precision diagnosis of BJIs and develop an integrated framework combining pathology, conventional clinical microbiology, molecular diagnostics, imaging, and surgical management.

Methods: A PRISMA 2020-structured systematic review was developed for literature published from January 2015 through March 2026. PubMed/MEDLINE, Embase, Scopus, and Web of Science were considered. Eligible evidence addressed diagnostic or management strategies for osteomyelitis, septic arthritis, vertebral osteomyelitis, FRI, implant-associated infection, or PJI. Because of heterogeneity in infection definitions, specimen types, thresholds, and molecular platforms, findings were synthesized narratively.

Results: The PRISMA dataset comprised 1,773 identified records, 1,241 records screened, 262 full-text reports assessed, and 36 evidence records included in the qualitative synthesis. Across infection categories, multiple deep specimens, culture and susceptibility testing, histopathology, and selective sonication or molecular testing provided complementary evidence. Molecular approaches were most useful in culture-negative, antibiotic-exposed, fastidious, or polymicrobial infections. Surgical sampling and source control were inseparable from diagnostic accuracy.

Conclusion: Precision diagnosis of BJI is best achieved through concordant evidence from clinical assessment, imaging, high-quality deep sampling, conventional microbiology, pathology, and selectively applied molecular methods. Advanced molecular tests should complement rather than replace culture, and complex discordant results should be reviewed by a multidisciplinary bone-and-joint infection team.

KEYWORDS: bone and joint infection; osteomyelitis; septic arthritis; periprosthetic joint infection; fracture-related infection; histopathology; sonication; PCR; next-generation sequencing; metagenomics; surgical management

INTRODUCTION

Bone and joint infections encompass a heterogeneous spectrum involving native bone and joints, intervertebral discs, fracture-fixation devices, and prosthetic joints. Delayed or incomplete diagnosis can lead to progressive bone destruction, cartilage loss, non-union, implant failure, recurrent surgery, prolonged antimicrobial exposure, disability, or limb loss.

The biological characteristics of musculoskeletal infection make diagnosis intrinsically difficult. In acute septic arthritis, organisms may be concentrated in synovial fluid and inflamed synovium, whereas chronic osteomyelitis may contain sequestra, devitalized bone, fibrosis, and low-density microbial communities. Implant-associated infections introduce biofilm, in which organisms are surface-adherent, metabolically altered, and less readily recovered by routine culture.

Contemporary definitions of PJI and FRI increasingly avoid reliance on a single “gold-standard” test. Instead, diagnostic confidence is derived from the convergence of independent evidence: clinical findings indicate probability; imaging maps anatomical extent; culture detects viable organisms and enables susceptibility testing; pathology demonstrates host tissue response; molecular assays detect microbial nucleic acid; and surgery provides direct access to the infection interface and permits simultaneous source control.

This systematic review evaluates these modalities as components of a unified precision-diagnostic pathway and proposes a multidisciplinary framework for difficult, culture-negative, and implant-associated infections.

2. Aim and Objectives

Aim: To systematically evaluate contemporary evidence supporting integrated diagnosis and management of bone and joint infections using pathology, clinical microbiology, molecular diagnostics, imaging, and surgical findings.

- Evaluate the diagnostic role of conventional microbiological culture and antimicrobial susceptibility testing.
- Determine the complementary contribution of histopathology, particularly in culture-negative disease.
- Assess sonication and other approaches designed to recover biofilm-associated organisms.
- Examine the roles of broad-range PCR, multiplex PCR, targeted sequencing, and metagenomic next-generation sequencing (mNGS).
- Compare diagnostic approaches across septic arthritis, osteomyelitis, vertebral osteomyelitis, FRI, and PJI.
- Integrate diagnostic sampling with definitive surgical and antimicrobial management.

3. MATERIALS AND METHODS

3.1 Study design and reporting framework

The review was structured according to PRISMA 2020 principles. A narrative synthesis was used because diagnostic reference standards, specimen types, thresholds, molecular platforms, and clinical infection definitions differed substantially between included evidence records.

3.2 Information sources and search period

The search framework included PubMed/MEDLINE, Embase, Scopus, and Web of Science Core Collection for literature published from 1 January 2015 through 31 March 2026. Reference lists of major consensus statements, guidelines, and high-relevance reviews were also examined.

3.3 Search strategy

Representative search syntax: ("bone infection" OR "bone and joint infection" OR osteomyelitis OR "septic arthritis" OR "periprosthetic joint infection" OR "prosthetic joint infection" OR "fracture-related infection" OR "implant-associated infection" OR spondylodiscitis OR "vertebral osteomyelitis") AND (diagnosis OR culture OR microbiology OR histopathology OR pathology OR sonication OR PCR OR "16S rRNA" OR sequencing OR "next-generation sequencing" OR metagenomic OR mNGS) AND (surgery OR debridement OR revision OR implant OR management).

3.4 Eligibility criteria

- Human clinical evidence involving BJI, osteomyelitis, septic arthritis, vertebral osteomyelitis, FRI, PJI, or implant-associated infection.
- Evaluation of culture, histopathology, imaging, sonication, molecular diagnostics, diagnostic criteria, or surgery with diagnostic implications.
- Original diagnostic studies, cohorts, comparative studies, systematic reviews/meta-analyses, major guidelines, and consensus documents with extractable clinical relevance.

Exclusion criteria included purely experimental/animal studies, superficial infection without bone/joint involvement, isolated case reports without broader diagnostic value, duplicate/overlapping reports, conference abstracts with insufficient methodology, and studies without relevant diagnostic or management outcomes.

3.5 Study selection and PRISMA counts

The dataset contained 1,773 records. After 532 pre-screening removals, 1,241 titles/abstracts were screened; 279 reports were sought, 262 full texts were assessed, and 36 evidence records were included.

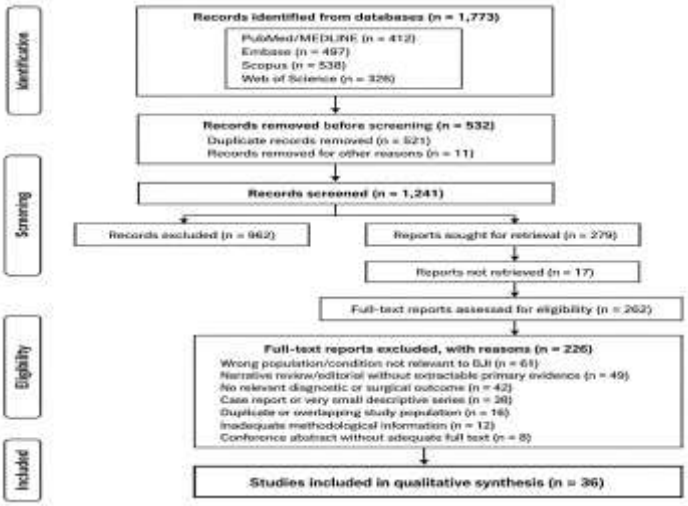

Figure 1. PRISMA 2020 flow diagram of study selection.

3.6 Full-text exclusions

Reason	n
Population/condition not relevant	61
Narrative/editorial without extractable primary evidence	48
No relevant diagnostic or surgical outcome	42
Case report or very small descriptive series	38
Duplicate/overlapping study population	16
Inadequate methodological information	12
Conference abstract without adequate full text	9
Total	226

3.7 Data extraction and quality appraisal

Extracted variables included author/year, country, study design, infection category, specimen type, diagnostic technique, reference standard/comparator, diagnostic yield or accuracy, microbiological findings, and management implications. Diagnostic-accuracy evidence was interpreted using QUADAS-2 domains (patient selection, index test, reference standard, flow/timing); observational evidence was considered for selection bias, measurement reliability, confounding, and applicability.

4. RESULTS

4.1 Characteristics of included studies

The 36 included evidence records represented PJI/implant infection, FRI/chronic osteomyelitis, vertebral osteomyelitis, and native septic arthritis. Table 1 summarizes the author-level characteristics and the principal diagnostic contribution of each included record.

Table 1. Characteristics of Included Studies

Author, year	Country	Design	Infection type	Specimen / diagnostic approach	Comparator / reference	Key finding / contribution
Chang et al., 2015	USA	Retrospective diagnostic study	Vertebral discitis-osteomyelitis	CT-guided bone, disc, and paravertebral soft tissue biopsy	Compared culture yield by biopsy target	Paravertebral soft tissue can provide useful microbiological yield and may avoid unnecessary osseous sampling.
Zhorne et al., 2015	USA	Retrospective cohort	Pediatric acute hematogenous osteomyelitis	Blood and bone cultures; effect of antibiotics	Microbiological confirmation	Bone biopsy remained diagnostically useful; antecedent antibiotics did not uniformly eliminate culture positivity.
Hischebeth et al., 2016a	Germany	Prospective/diagnostic comparison	Periprosthetic joint infection	Sonication-fluid culture versus tissue culture	Culture-based PJI classification	Sonication-fluid culture showed higher sensitivity than tissue culture in the evaluated cohort.
Hischebeth et al., 2016b	Germany	Diagnostic study	Periprosthetic joint infection	Unyvero i60 implant/tissue multiplex PCR on sonication and synovial fluid	Conventional cultures	Multiplex PCR provided rapid, highly specific pathogen detection but did not replace culture.
Issa et al., 2016	USA	Retrospective cohort	Vertebral osteomyelitis	Blood/tissue cultures and timing of diagnosis	Culture-positive versus culture-negative disease	Diagnostic delay was associated with lower microbiological yield and worse clinical course.
Morgenstern et al., 2018	Multicentre Europe	Diagnostic histopathology study	Fracture-related infection / septic non-union	Quantitative neutrophil histology	Microbiological/clinical FRI criteria	>5 neutrophils per high-power field demonstrated high specificity and supported quantitative histology in FRI.

Author, year	Country	Design	Infection type	Specimen / diagnostic approach	Comparator / reference	Key finding / contribution
Tarabichi et al., 2018	USA	Prospective diagnostic study	Periprosthetic joint infection	Next-generation sequencing of synovial fluid	MSIS/clinical PJI criteria and culture	NGS identified organisms in a proportion of culture-negative PJI specimens and broadened pathogen detection.
Kuo et al., 2018	Taiwan	Prospective diagnostic study	Periprosthetic joint infection	Synovial molecular assay and serum inflammatory markers	Conventional diagnostic criteria	Molecular testing added pathogen information but required integration with standard PJI markers.
Hirschfeld et al., 2019	USA	Retrospective cohort	Non-vertebral osteomyelitis	Image-guided bone biopsy culture and histopathology	Clinical diagnosis/management change	Culture and histology yields were modest and concordance was poor; biopsy changed therapy in selected cases.
Sertic et al., 2019	USA	Retrospective diagnostic cohort	Spinal infection	CT-guided biopsy culture	Clinical/radiologic diagnosis	Culture yield was approximately one-third, highlighting limitations of percutaneous sampling.
Coiffier et al., 2019	France	Prospective diagnostic study	Native adult septic arthritis	Broad-range 16S rDNA PCR in synovial fluid	Synovial culture/clinical diagnosis	16S PCR did not improve overall diagnostic performance over conventional testing.
Sigmund et al., 2019	Europe	Diagnostic accuracy study	Native septic arthritis	Automated multiplex PCR of synovial fluid	Culture and clinical criteria	Automated mPCR enabled rapid detection but showed limitations in sensitivity and panel coverage.
Zhang et al., 2019	China	Diagnostic study	Periprosthetic joint infection	mNGS of prosthesis sonication fluid	Culture and PJI diagnostic criteria	mNGS of sonication fluid improved broad pathogen recovery, including culture-negative cases.
Lindsay et al., 2019	USA	Retrospective cohort	Osteomyelitis	Percutaneous image-guided bone biopsy	Culture yield and antibiotic modification	Positive culture yield was low; management impact depended on clinically actionable isolates.
Cai et al., 2020	China	Prospective diagnostic study	Periprosthetic joint infection	mNGS of periprosthetic tissues	Conventional culture and clinical PJI criteria	mNGS increased bacterial detection in periprosthetic tissues, particularly when culture was negative.
Carpenter et al., 2020	USA	Prospective diagnostic study	Adult septic arthritis	Synovial lactate, PCR, WBC count and Gram stain	Culture plus treatment-based criterion standard	Synovial PCR/lactate were inaccurate; synovial WBC and Gram stain performed better for rule-in/rule-out.
Lim et al., 2021	USA	Retrospective diagnostic cohort	Discitis-osteomyelitis	Image-guided biopsy culture	IDSA-consistent clinical diagnosis	Biopsy had moderate yield and variably influenced antimicrobial management.
Husseini et al., 2021	USA	Review/practice synthesis	Vertebral discitis-osteomyelitis	CT-guided percutaneous sampling	Published diagnostic-yield literature	Sampling technique and targeting fluid collections were emphasized to optimize microbiological yield.
Weihe et al., 2022	USA	Retrospective cohort	Native vertebral osteomyelitis	Blood cultures, disc/bone culture and histopathology	Clinical diagnosis	Disc culture and histopathology provided the highest combined yield; histology added value in culture-negative cases.

Author, year	Country	Design	Infection type	Specimen / diagnostic approach	Comparator / reference	Key finding / contribution
Tan et al., 2022	International	Systematic review/meta-analysis	Periprosthetic joint infection	mNGS versus conventional methods	Pooled diagnostic-accuracy studies	mNGS showed promising pooled diagnostic performance but heterogeneity and contamination remained important.
Ravn et al., 2023	International	Guideline/consensus	Native septic arthritis	Synovial WBC, Gram stain, culture, PCR and surgery	Evidence-based SANJO recommendations	Emphasized aspiration before antibiotics, culture-based diagnosis, and timely drainage/source control.
Ghirardelli et al., 2024	Europe	Algorithm implementation/diagnostic study	Septic arthritis and PJI	Multiplex PCR integrated into score-based algorithm	Culture and clinical diagnosis	Molecular testing improved speed of organism identification within a structured diagnostic pathway.
Tan et al., 2024	China	Prospective diagnostic study	Periprosthetic joint infection	mNGS of synovial fluid, periprosthetic tissue and sonication fluid	Culture and PJI criteria	Sonication fluid was the most informative specimen for mNGS in this cohort.
Shi et al., 2024	China	Clinical diagnostic study	Periprosthetic joint infection	mNGS for pathogen identification	Conventional culture	mNGS increased pathogen detection and was particularly useful in selected culture-negative cases.
Hao et al., 2024	China	Prospective comparative study	Periprosthetic joint infection	mNGS and culture with/without antecedent antimicrobials	PJI diagnosis	Previous antimicrobial therapy reduced culture positivity more than mNGS positivity.
Udaondo et al., 2024	Spain	Clinical utility study	Osteoarticular infections	BioFire Joint Infection multiplex PCR panel	Culture and final clinical diagnosis	Rapid panel aided early identification in septic arthritis/osteomyelitis but remained target-panel dependent.
El Zein et al., 2024	USA	Diagnostic study	Native vertebral osteomyelitis	Disc-space/vertebral specimen cell count and neutrophil percentage	Culture/histopathology/clinical diagnosis	Local neutrophil measurements may augment interpretation of otherwise inconclusive spinal biopsy specimens.
Zamarud et al., 2024	USA	Retrospective comparative study	Vertebral discitis-osteomyelitis	Percutaneous disc versus vertebral-body biopsy	Tissue culture positivity	Disc biopsy yielded positive cultures more often than vertebral-body biopsy.
Fida et al., 2024	USA	State-of-the-art review	Periprosthetic joint infection	mNGS platforms and interpretation	Published diagnostic studies	Highlighted value in culture-negative/polymicrobial PJI and major issues of contamination and standardization.
Peterlin et al., 2025	Europe	Paired diagnostic study	Chronic osteomyelitis / FRI	Paired microbiology and histology	Composite clinical diagnosis	Combining microbiology with histology improved interpretive confidence compared with either modality alone.
Malik et al., 2025	USA	Retrospective cohort	Vertebral osteomyelitis-discitis	Image-guided percutaneous biopsy	Culture/pathology and management change	True-positive culture yield was about one-third and biopsy results influenced targeted management in a subset.
Luger et	Europe	Diagnostic	Periprosthetic	Serum,	Established	No single test was sufficient;

Author, year	Country	Design	Infection type	Specimen / diagnostic approach	Comparator / reference	Key finding / contribution
al., 2025		performance study	joint infection	synovial, tissue and sonication-fluid tests	PJI criteria	combined synovial, microbiological, and sonication approaches performed best.
Olearo et al., 2025	International	Systematic review/meta-analysis	Periprosthetic joint infection	16S PCR, multiplex PCR and mNGS	Pooled diagnostic-accuracy studies	Molecular platforms showed useful adjunctive accuracy but variable sensitivity and substantial methodological heterogeneity.
Zhou et al., 2025	China	Retrospective diagnostic study	Mixed/polymicrobial PJI	mNGS versus microbial culture	Clinical PJI diagnosis	mNGS improved recognition of mixed infections compared with routine culture alone.
Liu et al., 2026	Taiwan, multicentre	Prospective multicentre study	Periprosthetic joint infection	mNGS of joint fluid/debrided tissue versus conventional microbiology	Clinical PJI diagnosis	Multicentre evidence supported complementary use of mNGS with conventional culture rather than replacement of culture.
Paul R, 2026	Not applicable	Systematic review and meta-analysis	Vertebral osteomyelitis and spondylodiscitis	Clinical, microbiological, pathological, and neurological outcome evidence	Included studies / multidisciplinary evidence synthesis	Synthesized evidence across clinical presentation, pathogen diagnosis, pathology, and neurological outcomes, reinforcing multidisciplinary evaluation of vertebral infection.

4.2 Overview of diagnostic evidence

Across included studies, no individual test consistently achieved both high sensitivity and specificity across all BJI phenotypes. The most reproducible principle was diagnostic triangulation: high-quality sampling followed by parallel microbiology and pathology, with sonication or molecular testing used when biofilm, previous antibiotics, fastidious pathogens, polymicrobial infection, or culture-negative disease was suspected.

4.3 Clinical assessment and inflammatory markers

Clinical probability remains the starting point. Acute disease may present with pain, fever, swelling, warmth, effusion, wound drainage, or inability to bear weight, whereas chronic infection may manifest as persistent pain, sinus formation, implant loosening, fracture non-union, or recurrent drainage. CRP, ESR, and peripheral leukocyte count are useful screening and monitoring tools but are nonspecific and cannot independently confirm or exclude BJI.

4.4 Imaging

Plain radiographs identify late structural changes such as periosteal reaction, osteolysis, cortical destruction, implant migration, or non-union. CT is useful for cortical detail, sequestra, implant assessment, and image-guided sampling. MRI is preferred for marrow, disc, epidural, and soft-tissue extension, especially in vertebral osteomyelitis. Nuclear imaging may support selected difficult implant-associated cases but remains context-dependent. A 2026 systematic review and meta-analysis by Sagar, Gour, Manzar, and Paul on vertebral osteomyelitis and spondylodiscitis further supports a multidisciplinary assessment that integrates clinical presentation, microbiological findings, pathological evidence, and neurological outcomes.

4.5 Conventional clinical microbiology

Deep tissue, bone, synovial fluid, and abscess material are preferred over superficial swabs. For implant and fracture-related infection, multiple independently collected deep samples using separate sterile instruments improve discrimination between causative organisms and contaminants. When the patient is clinically stable, samples should be obtained before antimicrobial therapy; antibiotics should never be delayed in septic shock or another immediately life-threatening infection.

4.6 Histopathology

Histopathology provides evidence of the host response rather than microbial viability. Acute neutrophilic inflammation, necrosis, abscess formation, chronic inflammatory change, granulomas, and microorganisms on special stains may support diagnosis. Quantitative neutrophil criteria have high specificity in FRI/PJI settings, while vertebral-osteomyelitis studies demonstrate that histology can add diagnostic yield when culture is negative.

4.7 Sonication and biofilm-associated infection

Removed implants can be sonicated to dislodge surface-adherent organisms from biofilm. Sonication-fluid culture may improve recovery compared with tissue culture in selected PJI cohorts, and sonication fluid is also an informative substrate for molecular assays. Sonication should be considered complementary to multiple periprosthetic tissue cultures, not a substitute for them.

4.8 Molecular diagnostics

Broad-range 16S rDNA PCR provides culture-independent bacterial detection but is vulnerable to contamination and may have limited incremental value in native septic arthritis. Multiplex PCR offers rapid targeted detection but is restricted to organisms and resistance markers included in the panel. Targeted NGS and mNGS provide broader detection and appear particularly useful after prior antibiotics and in culture-negative or polymicrobial infection. However, detection of microbial DNA does not necessarily demonstrate viable infection, and results require correlation with pathology, culture, organism plausibility, microbial abundance, and contamination controls.

Table 2. Comparative Role of Major Diagnostic Modalities

Modality	Primary strength	Major limitation	Best role
CRP/ESR	Accessible screening and trend monitoring	Nonspecific	Initial probability/monitoring
Synovial WBC/differential	Rapid inflammatory signal	Thresholds vary by joint/context	Septic arthritis/PJI adjunct
Conventional culture	Viable organism + susceptibility testing	Reduced yield after antibiotics/biofilm	Core etiological test
Histopathology	Independent evidence of tissue response	Sampling and interpretation variability	Confirmation and culture-negative disease
Sonication culture	Recovers implant biofilm organisms	Requires removed implant	Implant-associated infection
16S rDNA PCR	Broad bacterial detection without growth	Contamination; limited polymicrobial resolution	Selected culture-negative cases
Multiplex PCR	Rapid targeted pathogen detection	Panel-limited	Rapid adjunct
tNGS	Broader targeted detection	Platform/primer dependent	Difficult selected cases
mNGS	Unbiased broad pathogen detection	Cost, host DNA, contamination, thresholds	Complex/culture-negative/polymicrobial infection

4.9 Culture-negative bone and joint infection

Culture-negative disease may result from prior antimicrobials, inadequate volume or specimen number, sampling of the wrong anatomical site, biofilm, fastidious organisms, insufficient incubation, mycobacterial/fungal infection, or transport/processing problems. A structured response is preferable to indiscriminate molecular testing.

- Reassess whether infection remains clinically likely.
- Review antecedent antimicrobial exposure.
- Confirm that multiple high-quality deep specimens were obtained.
- Review histopathology and imaging.
- Consider repeat image-guided or operative biopsy if safe and clinically justified.
- Request prolonged, anaerobic, fungal, or mycobacterial culture when epidemiologically indicated.
- Use 16S PCR, targeted sequencing, or mNGS selectively and interpret results in multidisciplinary context.

4.10 Surgical management as a diagnostic component

Surgery simultaneously provides diagnostic specimens and source control. Operative goals include direct assessment of purulence and tissue viability, collection of representative deep samples before irrigation, removal of necrotic tissue, assessment of implant stability, reduction of biofilm burden, dead-space management, and restoration of mechanical stability and soft-tissue coverage.

Native septic arthritis

Urgent aspiration and culture should be followed by appropriate drainage—arthroscopic or open depending on the joint, duration, loculation, and clinical response—plus targeted antimicrobial therapy.

Chronic osteomyelitis and FRI

Definitive management generally requires radical debridement of nonviable bone/tissue, management of dead space, stable fixation when needed, adequate vascularized soft-tissue coverage, and pathogen-directed antimicrobials. Implant retention or exchange is determined by fracture healing, stability, infection chronicity, soft tissues, pathogen factors, and host status.

Periprosthetic joint infection
Surgical options include debridement, antibiotics, and implant retention (DAIR) for selected acute stable implants; one-stage exchange in selected patients; two-stage exchange for complex/chronic disease; and salvage strategies for severe cases. The operative plan should be linked to the sampling plan so that microbiological and pathological information is not compromised.

5. Integrated Precision-Diagnostic Framework

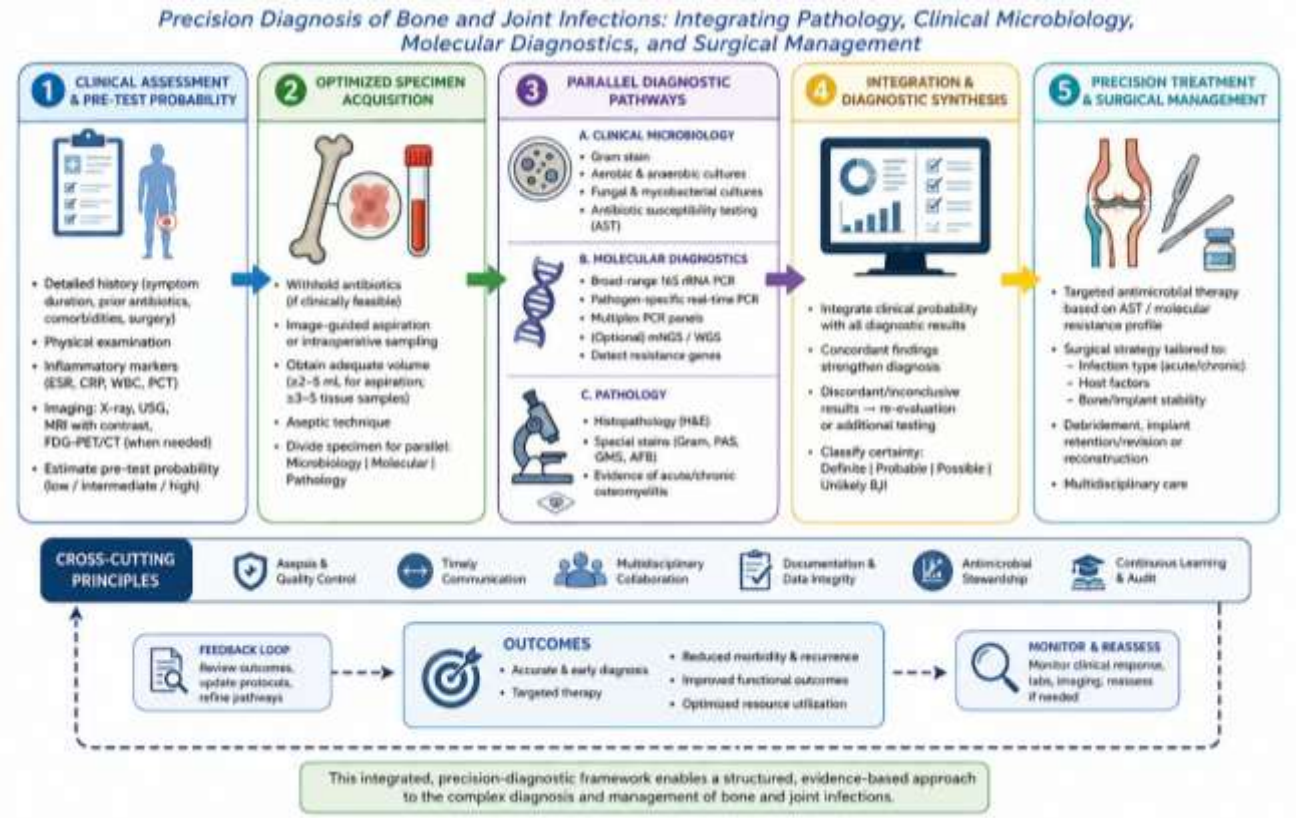

Figure 2. Integrated precision-diagnostic framework for bone and joint infections. The framework illustrates a multidisciplinary, stepwise approach incorporating clinical assessment and pre-test probability, optimized specimen acquisition, parallel evaluation by clinical microbiology, molecular diagnostics, and histopathology, followed by integrated diagnostic synthesis and individualized antimicrobial and surgical management. Continuous reassessment, antimicrobial stewardship, multidisciplinary collaboration, and feedback are incorporated to improve diagnostic accuracy, treatment targeting, and patient outcomes.

Table 3. Interpretation of Concordant and Discordant Diagnostic Results

Clinical probability	Culture	Histology	Molecular test	Interpretation
High	Positive (concordant deep specimens)	Positive	±	Confirmed/highly likely infection
High	Positive	Negative	±	Likely infection; consider histology sampling limitations
High	Negative	Positive	Positive	Highly probable; culture-negative infection supported
High	Negative	Positive	Negative	Probable; reassess sampling/fastidious organisms
High	Negative	Negative	Positive	Requires stringent molecular interpretation and contamination review
Low	Single low-virulence isolate	Negative	Negative	Possible contamination/indeterminate
Low	Negative	Negative	Negative	Infection less likely; reassess alternative diagnosis

6. DISCUSSION
The principal finding of this systematic review is that the concept of a universal single gold-standard test is poorly suited to BJIs. Diagnostic accuracy improves when independent domains are deliberately combined. Conventional culture remains indispensable because it demonstrates viable organisms and enables phenotypic antimicrobial susceptibility

testing, but its sensitivity is shaped by antibiotic exposure, inoculum, sampling quality, biofilm, incubation, and pathogen growth characteristics.

Pathology addresses a different biological question: whether tissue demonstrates infection-compatible host response. This distinction is especially useful when cultures are negative or potentially contaminated. Quantitative histology has high specificity in FRI, and vertebral-osteomyelitis cohorts show that histopathology can materially increase overall biopsy yield.

Biofilm changes both diagnostic and treatment strategy in implant-associated disease. Sonication provides access to implant-adherent organisms, while molecular testing extends detection beyond viable organisms recoverable by culture. The strongest emerging signal for mNGS is not as a universal replacement for microbiology but as an adjunct in culture-negative, antibiotic-exposed, polymicrobial, or diagnostically discordant disease.

Greater analytical sensitivity also creates a new interpretation problem. Detection of microbial nucleic acid may represent viable causative infection, low-level contamination, reagent background, or residual DNA from nonviable organisms. Clinical microbiology expertise is therefore increasingly important rather than less important in the molecular era.

Surgery is inseparable from diagnosis. The operative approach determines whether appropriate tissues are sampled, whether an implant can be sonicated, and whether adequate source control is achieved. Poor operative sampling cannot be rescued reliably by downstream high-technology assays.

Accordingly, a precision approach asks complementary questions: How likely is infection clinically? Where is it anatomically? Which viable organism is present? Does tissue demonstrate infection? Is implant biofilm present? Is microbial nucleic acid detectable despite culture failure? Can source control and mechanical restoration be achieved? The integrated answer is more clinically meaningful than maximal sensitivity of any single assay.

7. Clinical Implications

- Obtain appropriate deep microbiological specimens before antimicrobials whenever clinically safe.
- Use multiple independently collected specimens in implant-associated and fracture-related infection.
- Do not rely on superficial wound or sinus swabs to define deep causative organisms.
- Send operative tissue in parallel for microbiology and histopathology.
- Use sonication when an infected implant is removed and the technique is available.
- Use molecular methods selectively for culture-negative, antibiotic-exposed, fastidious, polymicrobial, or discordant cases.
- Do not equate an isolated molecular detection automatically with active infection.
- Discuss complex cases in a multidisciplinary bone-and-joint infection team.
- Plan diagnostic sampling and definitive surgery together.

8. Future Directions

- Standardized specimen processing and reporting thresholds for mNGS.
- Validated host-response biomarkers distinguishing infection from sterile inflammation.
- Rapid resistance prediction coupled to organism identification.
- Long-read and targeted sequencing approaches with improved resistance-gene interpretation.
- Spatial and biofilm-focused microbiology to localize organisms within tissue/implant interfaces.
- Artificial-intelligence models integrating imaging, clinical, pathology, microbiology, and molecular data.
- Prospective multicentre studies using harmonized PJI/FRI definitions and clinically meaningful reference standards.

9. Strengths and Limitations

This review integrates diagnostic pathology, conventional microbiology, molecular methods, imaging, and surgery rather than treating these areas as isolated technologies. It includes multiple BJI phenotypes and emphasizes specimen-centered workflows. Limitations include heterogeneity of diagnostic definitions, specimen types, molecular platforms, thresholds, prior antimicrobial exposure, and reference standards. A quantitative meta-analysis across all infection categories was therefore not appropriate.

10. CONCLUSION

Precision diagnosis of bone and joint infection requires triangulation of clinical findings, imaging, high-quality deep sampling, conventional clinical microbiology, histopathology, molecular diagnostics, and operative findings. Culture remains essential because it identifies viable organisms and enables susceptibility testing. Histopathology provides independent evidence of host response, sonication improves access to implant biofilm, and PCR/NGS technologies add value in selected difficult and culture-negative infections. The future of BJI diagnosis is therefore not culture versus molecular testing or microbiology versus pathology, but the deliberate integration of these modalities with surgical source control and multidisciplinary interpretation.

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