Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Performance of the BIOFIRE® Joint Infection Panel in the diagnosis of osteoarticular infections in adults and children in Santiago, Chile

Dona Benadof

donaben@gmail.com

Hospital Roberto del Rio

Mirta Acuña

Hospital Roberto del Rio

Yennybeth Leiva

Centro de investigación clínica avanzada Hospital Roberto del río (CICA)

Karla Yohannessen

Centro de investigación clínica avanzada Hospital Roberto del río (CICA)

Álvaro Martínez

Hospital San José

Andrés Oyarzun

Hospital San José

Jose Tomas Aldunate

Hospital San José

Pascale Clement

Centro de investigación clínica avanzada Hospital Roberto del río (CICA)

Constanza Bohle

Hospital San José

Verónica Vilches

Centro de investigación clínica avanzada Hospital Roberto del río (CICA)

Research Article

Keywords: Osteoarticular infections, multiplex PCR, molecular diagnoses, BIOFIRE Joint Infection Panel

Posted Date: November 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11281631/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Purpose

To evaluate the diagnostic performance and clinical utility of the BIOFIRE® Joint Infection multiplex PCR panel compared to conventional culture and 16S rRNA sequencing for the diagnosis of acute osteoarticular infections (OAI) in a Latin American setting.

Methods

A prospective study was conducted between August 2022 and January 2024 at two tertiary hospitals in Santiago, Chile, involving a dual cohort of 124 adults and 32 children. A total of 156 samples (synovial fluid and osteoarticular tissue) were analyzed. JI panel's performance was compared against a Composite Reference Standard (CRS) consisting of standard-of-care culture and universal 16S rRNA PCR with sequencing.

Results

Staphylococcus aureus was the predominant pathogen identified in both cohorts (49.3% by culture vs. 46.8% by JI). For all on-panel microorganisms, the JI panel demonstrated an overall sensitivity of 90.9% (95% CI: 81.3–96.6%) and a negative predictive value (NPV) of 92.4% (95% CI: 84.2–97.2%). In the pediatric population, the panel achieved a specificity of 95.2% and an NPV of 95.2%. Notably, the JI panel maintained high performance in 20.5% of patients with prior antibiotic exposure and successfully identified polymicrobial infections that were missed or underreported by traditional cultures.

Conclusions

The JI panel is a rapid, highly accurate tool for the microbiological documentation of OAIs in real-world clinical settings in Latin America. Its high NPV and rapid turnaround time (65 minutes) support its integration into diagnostic stewardship algorithms to optimize early and optimal antimicrobial therapy, particularly in pediatric cases and patients with suspected fastidious or culture-negative infections.

INTRODUCTION

Osteoarticular infections are common across all age groups. In adults, they are primarily associated with prosthetic joints, whereas in children, they predominantly affect native bone and joints. *Staphylococcus aureus* is the main etiologic agent in both populations (1).

Standard microbial culture remains the gold standard for diagnosing osteoarticular diseases. However, this method frequently lacks sensitivity, with a reported yield ranging from 35% to 78% (2)(3).

Furthermore, culture is a time-consuming process dependent on bacterial proliferation, which, in osteoarticular samples, often requires more than 72 hours for definitive results. This lack of sensitivity and the resulting diagnostic delay compromise timely, targeted antimicrobial therapy and stewardship. To overcome these limitations, various molecular diagnostic methods have been developed to improve microbial identification in osteoarticular samples, though many remain expensive or have suboptimal turnaround times.

Among these technologies, the BIOFIRE® Joint Infection (JI) panel performs automated nucleic acid extraction and multiplex polymerase chain reaction (PCR) in a closed system with a turnaround time (TAT) of 65 minutes, with a limit of detection for bacteria and yeast ranged from 100 – 10,000 CFU/mL (4). It simultaneously detects 15 Gram-positive pathogens (including 7 anaerobes), 14 Gram-negative pathogens (including 1 anaerobe), yeasts (*Candida spp*. and *Candida albicans*), and 8 antimicrobial resistance genes (Table S1 in Supplementary Material).

Initial validation studies have demonstrated high diagnostic accuracy for this platform. Kokenda et al. reported a positive predictive value (PPV) of 89.7% and a negative predictive value (NPV) of 99.7% across 201 synovial fluid samples (5). Similarly, Schoenmakers et al. published an overall sensitivity of 100%, with an 83% sensitivity specifically in patients with clinical suspicion of native septic arthritis (n = 45) and Esteban et al. showed in 1,544 samples a sensitivity of 90.9% and a positive percent agreement of 100% for antimicrobial resistance genes, a specificity of 98.5% and a negative percent agreement of 95.7% for antimicrobial resistance genes (6)(7). Furthermore, Gaillard et al. showed a PPV of 100% and an NPV of 82.6% in 307 specimens (8).

Recent literature on the JI panel, including a comprehensive meta-analysis of multiplex PCR (mPCR) platforms in joint infections, reported a high pooled specificity of 96.2% and a diagnostic odds ratio of 42.1, although the overall pooled sensitivity was moderate at 62.2%(9)(10). However, when assessing the specific performance of the JI panel for its targeted (on-panel) microorganisms, studies have demonstrated an excellent diagnostic yield (10)(11). In synovial fluid samples, the panel exhibits clinical sensitivities of 90.9% to 91% and specificities of 98.5% to 100% for on-panel pathogens (10)(11). Despite these strong diagnostic metrics, the assay's overall clinical sensitivity can be compromised by the absence of frequent off-panel pathogens, such as *Staphylococcus epidermidis* and *Cutibacterium acnes* in adults (1)(8)(9)(11)(12)(7)(13), and *Neisseria meningitidis* in children (14)(15), which must be considered during clinical interpretation.

Driven by the limitations in sensitivity and the prolonged turnaround time of conventional culture, the FDA approved the JI panel for clinical use in May 2022. To date, however, there are few published data evaluating its clinical utility in Latin America (16). Therefore, the primary objective of this study was to evaluate the diagnostic performance of the JI panel joint infections in comparison with conventional culture results and 16S rRNA followed by sequencing. Additionally, the TAT of the JI panel was compared to that of culture in both pediatric and adult populations across two tertiary hospitals in Santiago, Chile.

MATERIALS AND METHOD

This prospective study was conducted from August 29th, 2022, to January 1st, 2024, at two tertiary healthcare centers in the northern metropolitan area of Santiago, Chile: the Hospital de Niños Roberto del Río (HRR), a specialized pediatric center, and the Hospital San José (HSJ), an adult general hospital. Both institutions are categorized by the Chilean Ministry of Health as high-complexity and high-resolution tertiary-care hospitals.

At the time of the study, HRR was a 200-bed public pediatric facility that served as a national referral center for children with complex medical conditions. HSJ is the sole public general hospital for adults in this metropolitan zone, operating with 590 beds and serving an assigned catchment population of approximately 600,000 adults.

Based on the hypothesis that the JI panel provides superior diagnostic yield compared to conventional culture, and utilizing the performance metrics described by Kokenda et al. (5), a target sample size of 150 specimens was estimated. Inclusion criteria comprised: (i) clinical suspicion of acute osteoarticular infection; (ii) consultation at a participating center; (iii) collection of synovial fluid or osteoarticular tissue for microbiological analysis; and (iv) written informed consent provided by the patients or their legal guardians. Specimens with insufficient sample volume for complete processing were excluded. The samples were collected in the operating room based on the clinical judgment of the attending orthopedist

Tissue samples were homogenized in 2 mL of sterile 0.9% saline solution before testing. Of note, while the JI panel is not regulatory-approved for tissue specimens, this off-label evaluation was conducted to explore its diagnostic utility in this specific sample matrix. Additionally, an aliquot was sent to TAAG Genetics (Santiago, Chile) for microbial identification. Briefly, the workflow consisted of enriching the samples for eukaryotic DNA, followed by amplification using a multiplex PCR kit targeting short microbial DNA fragments. This kit utilizes primers directed against conserved regions of both the bacterial 16S rRNA and rpoB genes. The resulting amplicons were sequenced on an Oxford Nanopore platform. Finally, taxonomic assignment and data analysis were performed using a proprietary bioinformatics platform based on sequence similarity against curated reference databases.

To evaluate the diagnostic performance of the JI panel, a composite reference standard (CRS) was defined, which included conventional bacterial culture and universal 16S rRNA PCR followed by sequencing. The JI result was recorded as positive or negative for microorganism identification, consistent with the CRS classification. Patient recruitment commenced upon receipt of synovial fluid or osteoarticular tissue samples at the participating centers' laboratories. These samples were processed according to local laboratory protocols using standard-of-care microbiology based on culture. The remaining aliquot was utilized for JI testing after obtaining informed consent. Additionally, an aliquot was sent to TAAG Genetics labs (Santiago, Chile) for microbial identification. Briefly, the workflow consisted of enriching the samples for eukaryotic DNA, followed by amplification using a multiplex PCR kit targeting short microbial DNA fragments. This kit utilizes primers directed against conserved regions

of both the bacterial 16S rRNA and rpoB genes. The resulting amplicons were sequenced on an Oxford Nanopore platform. Finally, taxonomic assignment and data analysis were performed using a proprietary bioinformatics platform based on sequence similarity against curated reference databases.

Descriptive statistics were used to report the baseline demographic and clinical characteristics of the patients, the microbiological results, and the overall diagnostic performance of the study. Continuous variables were expressed as medians and interquartile ranges (IQRs) owing to their non-normal distribution. Absolute and relative frequencies were calculated for categorical variables. To evaluate the diagnostic performance of the JI panel relative to the reference standard (culture combined with 16S rRNA sequencing), 2×2 contingency tables were constructed. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated along with their corresponding 95% confidence intervals (CIs). Statistical analyses were performed using Stata version 17 software (StataCorp, College Park, TX, USA).

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the hospital administrations and the Scientific Ethics Committee of the Northern Metropolitan Health Service (Approval No. 020/2022). Written informed consent was obtained from all participants or their parents/legal guardians prior to participation.

RESULTS

During the study period, a total of 156 osteoarticular samples were obtained across both participating hospitals, comprising 124 (79.5%) specimens from adult patients at Hospital San José (HSJ) and 32 (20.5%) from pediatric patients at Hospital de Niños Roberto del Río (HRR). A predominance of male patients was observed in both cohorts, accounting for 68.5% (85/124) of adults and 56.3% (18/32) of children, which represented 66.0% (103/156) of the overall study population. The median age was 47.2 years (range: 16.3–88.1) in the adult group and 5.8 years (range: 0.2–16.2) in the pediatric group. Regarding patient nationality, the majority of the overall population was Chilean (78.2), followed by foreign nationals from other Latin American and Caribbean countries (8.3% total; including 4.5% Venezuelan, 1.9% Peruvian, 1.3% Colombian, and 0.6% Haitian), while sociodemographic data were missing for 13.4% of the cases. Notably, baseline comorbidities were substantially more frequent in the adult population than in the pediatric cohort, being present in 51.6% (64/124) and 25.0% (8/32) of patients, respectively (Table 1).

Table 1
Sociodemographic characteristics of patients.

Sex, n (%)	**HSJ (n = 124)**	**HRR (n = 32)**	**Overall (n = 156)**
Male	85 (68.5)	18 (56.3)	103 (66.0)
Female	25 (20.2)	14 (43.7)	39 (25.0)
Missing data	14 (11.3)	-	14 (9.0)
Age (years), P_{50} (min-max)	**47.2 (16.3–88.1)**	**5.8 (0.2–16.2)**	**40.3 (0.2–88.1)**
Patient nationality, n (%)			
Chilean	94 (75.8)	28 (87.5)	122 (78.2)
Venezuelan	5 (4.0)	2 (6.3)	7 (4.5)
Peruvian	3 (2.4)	-	3 (1.9)
Colombian	1 (0.8)	1 (3.1)	2 (1.3)
Haitian	-	1 (3.1)	1 (0.6)
Missing data	21 (16.9)	-	21 (13.4)
Parental nationality, n (%)			
Both Chilean	1 (0.8)	24 (74.0)	25 (16.0)
Both foreign	-	7 (21.9)	7 (4.5)
One foreign	-	1 (3.1)	3 (1.9)
Missing data	123 (99.2)	-	123 (78.8)
Comorbidities, n (%)			
Yes	64 (51.6)	8 (25.0)	72 (46.2)
No	32 (25.8)	24 (75.0)	56 (35.9)
Unknown	28 (22.6)	-	28 (17.9)

SSMN: Servicio de Salud Metropolitano Norte

Regarding the clinical presentation upon admission, pain, localized swelling, and functional impairment were the predominant symptoms across both study cohorts, with pediatric patients generally displaying a higher symptom burden than adults (Fig. 1; individual data points are detailed in Table S2 of the Supplementary Material). A striking difference was observed in the prevalence of fever: 62.5% of

pediatric patients versus 9.7% of adult patients. Localized pain was present in 96.9% of children and 68.6% of adults. In contrast, localized swelling was recorded slightly more often in adults (58.1%) than in children (50.0%). Functional impairment was highly prevalent in both groups, affecting 71.9% of pediatric cases and 56.5% of adult cases. Furthermore, a history of trauma was reported by 37.9% of adults and 28.1% of children, while pus at the infection site was clinically evident in 24.2% of the adult cohort versus 9.4% of the pediatric cohort. Finally, joint effusion was notably rare in adults, observed in only 0.8% (1/124) of cases, compared with 12.5% (4/32) in the pediatric population (Fig. 1).

Table 2 details the clinical reasons for consultation and variables associated with the hospitalization course. In the adult cohort, the primary reasons for consultation were pain or discomfort at the anatomical site (29.0%), postoperative complications (20.9%), and accidents (19.4%). These were followed by a suspicion of osteoarthritis (OA) (12.9%) and the presence of a symptomatic prosthesis (8.1%). Conversely, among pediatric patients, the most frequent reasons for seeking medical care were pain or discomfort at the anatomical site (37.5%), followed equally by a suspicion of OA (21.9%) and general symptoms such as fever (21.9%), while accidents accounted for 18.7% of the cases. Hospitalization was required for most of the study population (80.8%, 126/156), with a significantly higher rate in children (100.0%) than in adults (75.8%). For hospitalized patients, the overall median length of hospital stay was 15 days (range: 1–99). Notably, adult patients experienced substantially longer hospitalizations, with a median of 17.5 days (range: 1–94), compared with 6.5 days (range: 2–99) in the pediatric group. Admission to the intensive care unit (ICU) was infrequent, occurring in only 7.1% (11/156) of the total sample (6.5% of adults and 9.4% of children). Among those requiring critical care, the median ICU length of stay was 12 days (range: 1–99), with 15 days (range: 1–99) for adults and 11 days (range: 6–23) for children. Finally, clinical complications during the course of care were reported in 7.1% (11/156) of the overall population, all of which were strictly restricted to adult patients (8.9%) (Table 2).

Table 2
Reasons for consultation and variables related to hospitalization.

Reason for consultation, n (%)	**HSJ (n = 124)**	**HRR (n = 32)**	**Overall (n = 156)**
Pain or discomfort at anatomical site	36 (29.0)	12 (37.5)	48 (30.7)
Accident	24 (19.4)	6 (18.7)	30 (19.2)
Postoperative complication	26 (20.9)	-	26 (16.7)
Suspicion of OM	16 (12.9)	7 (21.9)	23 (14.7)
General symptoms (e.g., fever)	8 (6.5)	7 (21.9)	15 (9.6)
Symptomatic prosthesis	10 (8.1)	-	10 (6.4)
Elective surgery	2 (1.6)	-	2 (1.3)
Follow-up or consultation	2 (1.6)	-	2 (1.3)
Previous antibiotic use, n (%)	26 (20.9)	6 (18.7)	32 (20.5)
Hospitalization, n (%)			
Yes	94 (75.8)	32 (100.0)	126 (80.8)
No	6 (4.8)	-	6 (3.8)
Missing data	24 (19.4)	-	24 (15.4)
Hospitalization days, P_{50} (min-max)[1]	17.5 (1–94)	6.5 (2–99)	15 (1–99)
ICU, n (%)			
Yes	8 (6.5)	3 (9.4)	11 (7.1)
No	86 (69.4)	29 (90.6)	115 (73.7)
Missing data	30 (24.1)	-	30 (10.2)
ICU days, P_{50} (min-max)[2]	15 (1–99)	11 (6–23)	12 (1–99)
Complications, n (%)			
Yes	11 (8.9)	-	11 (7.1)
No	86 (69.3)	32 (100.0)	118 (75.6)
Missing data	27 (21.8)	-	27 (17.3)

[1] based on hospitalized patients; [2] based on patients admitted to the ICU; OM: Osteomyelitis

Regarding overall diagnostic yield, no statistically significant difference was observed between conventional culture and the JI panel (Fig. 2; detailed statistical comparisons and comprehensive species-level breakdowns are available in Supplementary Tables S3-S5). More than half of the specimens yielded negative results by both methods (54.5% for culture and 50.6% for JI), while a statistically similar proportion of positive results was detected (45.5% vs. 49.4%, respectively; p = 0.490). When evaluating the distribution of specific microbial groups among the positive isolates, the pathogen profiles remained highly consistent across both diagnostic modalities, with no significant differences in any category (Table S3). *Staphylococcus aureus* was the predominant pathogen identified by both culture and the JI panel (49.3% vs. 46.8%, respectively; p = 0.869), followed by other Gram-positive cocci (OGPC; 23.9% vs. 26.0%; p = 0.850), and enterobacterial species (16.9% vs. 11.7%; p = 0.480). Anaerobes and other less frequent pathogens accounted for the remaining isolates, with a statistically uniform distribution across both testing methods (Fig. 2). In the pediatric cohort none *Kingella kingae* identification.

The diagnostic performance metrics of the JI panel compared with the composite reference standard are summarized in Table 3. For the detection of all on-panel microorganisms, the assay demonstrated an overall sensitivity of 90.9% (95% CI: 81.3–96.6%) and a specificity of 81.1% (95% CI: 71.5–88.6%). The overall positive predictive value (PPV) and negative predictive value (NPV) were 77.9% (95% CI: 67.0–86.6%) and 92.4% (95% CI: 84.2–97.2%), respectively. When stratified by patient population, the panel displayed a highly consistent sensitivity of 90.9% across both cohorts. In the pediatric group (HRR), the panel achieved a specificity of 95.2% (95% CI: 76.2–99.9%), a PPV of 90.9% (95% CI: 58.7–99.8%), and an NPV of 95.2% (95% CI: 76.2–99.9%). For the adult cohort (HSJ), the specificity was 76.8% (95% CI: 65.1–86.1%), with a PPV of 75.8% (95% CI: 63.6–85.5%) and an NPV of 91.4% (95% CI: 81.0–97.1%). For *Staphylococcus aureus*, the most frequently isolated pathogen in this study, the JI panel demonstrated an overall sensitivity and specificity of 81.6% (95% CI: 65.7–92.3% for both metrics), coupled with a high PPV of 96.9% (95% CI: 83.8–99.9%) and an NPV of 79.4% (95% CI: 62.1–91.3%). In the adult group (HSJ), the performance for *S. aureus* remained robust, yielding a sensitivity of 80.6% (95% CI: 62.5–92.5%), a specificity of 95.8% (95% CI: 78.9–99.9%), and a PPV of 96.2% (95% CI: 80.4–99.9%). In the pediatric population (HRR), the panel reached a sensitivity of 85.7% (95% CI: 42.1–99.6%), while both specificity and PPV achieved 100.0%. Diagnostic performance metrics for less frequent targeted pathogens are detailed in Supplementary Table S5. Notably, the panel demonstrated flawless diagnostic accuracy for *Escherichia coli* and *Enterococcus faecalis*, achieving 100.0% sensitivity and 100.0% specificity for both pathogens. For *Streptococcus* spp., the overall clinical sensitivity was 83.3% (95% CI: 35.9–99.6%) and the specificity was 91.7% (95% CI: 81.6–97.2%). The unidentified streptococcal species by JI were *Streptococcus constellatus, Streptococcus anginosus, Streptococcus pneumoniae, Streptococcus dysgalactiae*, and *Streptococcus gallolyticus*. (Table S4*)*. In Table 3, it shows the performance of JI versus the Gold Standard.

Table 3
Diagnostic performance of BFJI versus the Gold Standard.

	n	TP/FN	TN/FP	Se (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
All BFJI microorganisms							
Overall	156	60/6	73/17	90.9 (81.3–96.6)	81.1 (71.5–88.6)	77.9 (67.0-86.6)	92.4 (84.2–97.2)
HSJ	124	50/5	53/16	90.9 (80.0–97.0)	76.8 (65.1–86.1)	75.8 (63.6–85.5)	91.4 (81.0-97.1)
HRR	32	10/1	20/1	90.9 (58.7–99.8)	95.2 (76.2–99.9)	90.9 (58.7–99.8)	95.2 (76.2–99.9)
Staphylococcus aureus							
Overall	66	31/7	27/1	81.6 (65.7–92.3)	81.6 (65.7–92.3)	96.9 (83.8–99.9)	79.4 (62.1–91.3)
HSJ	55	25/6	23/1	80.6 (62.5–92.5)	95.8 (78.9–99.9)	96.2 (80.4–99.9)	79.3 (60.3–92.0)
HRR	11	6/1	4/0	85.7 (42.1–99.6)	100.0 (39.8–100.0)	100.0 (54.1–100.0)	80.0 (28.4–99.5)

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; Se: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value

DISCUSSION

To our knowledge, this study represents the first clinical evaluation and the largest series to date of the JI panel in Latin America, uniquely assessing its real-world performance across a dual cohort of adult and pediatric patients with acute osteoarticular infections. The overall performance of the JI panel was 90.9% clinical sensitivity and 92.4% NPV when evaluated against the composite reference standard. Interestingly, the pooled sensitivity of mPCR platforms for joint infections has been traditionally reported as moderate; a recent milestone meta-analysis by Olearo *et al.* reported a pooled mPCR sensitivity of only 62.2% (9), which closely aligns with the overall sensitivities of 56% to 58% observed by Azad et al.

and Miamidian et al. in large periprosthetic joint infection (PJI) testing cohorts (11)(17). The significantly higher sensitivity achieved in our series (90.9%) can be explained clinically by the epidemiological characteristics of our sample. While standard PJI cohorts are heavily compromised by off-panel pathogens such as *Staphylococcus epidermidis* or *Cutibacterium acnes*, our population included a substantial proportion of native acute infections and pediatric patients in whom *Staphylococcus aureus* and other targeted on-panel streptococci were predominant. When targeted pathogens are present, the analytical performance of the JI system is robust, matching the on-panel sensitivities of 90.5% to 100% documented in recent international literature (12)(7)(17)(18)(19).

A major strength of our findings lies in the stratification by age groups, which is often missing in molecular diagnostic trials for bone and joint infections. In the pediatric cohort, the panel achieved robust sensitivity of 90.9%, along with high specificity (95.2%) and NPV (95.2%). This mirrors the clinical observations of Schoenmakers et al., who demonstrated that the clinical benefit of the JI panel is maximized in native septic arthritis—yielding a sensitivity of 83.3%—whereas its utility drops drastically to 30.0% in early post-operative infections due to the predominance of skin contaminants not covered by the syndromic menu (6). In pediatric acute hematogenous osteomyelitis and septic arthritis, where sample volumes are often highly restricted and fastidious pathogens like *Kingella kingae* are common, a sample volume of just 200 µL yielding an accurate microbiological diagnosis within approximately one hour represents a paradigm shift in pediatrics. Surprisingly, unlike other studies (16)(20)(21)(13)(7)(8)(2), no *K. kingae* was identified in ours. However, the exclusion of *Neisseria meningitidis* remains an important consideration, so a negative panel result cannot rule out this pathogen without adjunctive conventional microbiology.

In the adult cohort, the panel maintained an identical sensitivity of 90.9%, although the specificity (76.8%) and positive predictive value (PPV: 75.8%) were lower than those observed in children. This phenomenon does not necessarily reflect suboptimal assay design but rather highlights a well-known limitation of culture-based comparisons. As noted by Berinson et al. and Salar-Vidal et al., conventional standard-of-care cultures frequently produce false-negative results due to bacterial sequestration within dense biofilms or unviable growth states (18)(10). Consequently, when a highly sensitive mPCR assay detects bacterial DNA in a culture-negative sample from a patient with clear clinical signs of infection, the standard culture reference misclassifies these episodes as "false positive" artificially penalizing the assay's specificity. This structural limitation underscores the imperative to embrace composite reference standards that combine phenotypic and molecular methods to accurately assess advanced diagnostic platforms.

In real-world clinical practice, the diagnostic sensitivity of conventional culture methods declines substantially when systemic antibiotics are administered before sample collection. In our study, previous antibiotic use was documented in 20.5% (32/156) of the overall cohort, affecting 20.9% of adult patients and 18.7% of pediatric cases. This rate reflects the common clinical reality of tertiary healthcare settings. Despite this active antimicrobial pressure within a fifth of our study population, the JI panel maintained its strong performance metrics. Because mPCR targets and amplifies highly conserved

bacterial DNA sequences rather than relying on cell viability, the platform successfully circumvents the limitations of proliferation-dependent standard cultures in pre-treated individuals

Another major analytical advantage highlighted in our series was the unmasking of polymicrobial infections that were underreported or missed by traditional standard-of-care cultures. This clinical phenomenon is well supported by Salar-Vidal et al., who observed a significant increase in polymicrobial detections with the JI panel compared to cultures, demonstrating that traditional methodologies often overlook mixed infections (10). When a highly virulent or rapidly proliferating organism—such as *Staphylococcus aureus*—is present alongside slower-growing or fastidious microbes, it aggressively dominates the agar plate. Consequently, standard laboratory protocols often sweep only the dominant strain, leaving co-pathogens undetected. Camacho-Moreno et al. also documented multiple instances in a Latin American pediatric cohort where the syndromic panel successfully identified pyogenic coinfections that traditional cultures reported as strictly monomicrobial (16).

In a prospective study, Berinson et al. established that the JI panel delivers definitive results 21 hours faster than conventional culture methods, leading directly to early treatment optimization or de-escalation in 28.9% of active cases (18). Lund-Andersen et al. similarly found that the rapid one-hour processing window of the mPCR assay maximizes antibiotic stewardship, allowing for targeted therapeutic modifications within hours of admission rather than days (19).

To balance high analytical accuracy with institutional cost constraints in Latin America, we propose a structured diagnostic stewardship algorithm. Rather than using costly molecular panels as an unselective up-front screening method for every routine sample, which lowers pre-test probability, the JI panel should be strategically reserved for pediatric populations where fastidious organisms are clinically suspected, critically ill or unstable patients requiring immediate targeted data, and culture-negative cases displaying highly objective joint inflammation biomarkers. When the specialized mPCR panel is negative, a broad range 16S rRNA PCR with sequencing should be utilized as an unbiased secondary line (9). Ultimately, molecular diagnostics must be implemented as a complementary strategy alongside conventional cultures, which remain irreplaceable for isolating live pathogens, testing off-panel species, and determining true phenotypic susceptibilities.

Several limitations of this study must be acknowledged. First, the sample size (N = 156), while comparable to or larger than several single-region molecular validation trials, limits our ability to extrapolate performance to extremely rare or specialized bone and joint pathogens. Second, a fraction of the analyzed specimens underwent tissue homogenization; while testing tissues on the JI panel is an off-label sample type according to the manufacturer's instructions for use, our composite tissue results demonstrated excellent consistency and higher diagnostic yield compared to composite tissue cultures, matching the experiences described by Salar-Vidal et al. and Lazic et al. (10)(22). Third, because our university medical centers routinely manage high-complexity cases, a selection bias toward more severe clinical presentations and higher infection prevalence might be present. Finally, the low prevalence of multidrug-resistant organisms in our specific cohort prevented a robust evaluation of the panel's

resistance gene detection capabilities in a real-world clinical setting. This differs from previous studies (7)(16).

In conclusion, the JI panel proved to be a highly accurate, reliable, and rapid diagnostic tool in a real-life Latin American clinical setting, demonstrating excellent sensitivity and NPV across both adult and pediatric populations. The assay proved particularly valuable for identifying pathogens in polymicrobial environments and unmasking etiologies, including patients with a history of prior antibiotic exposure and it is a considerable faster than traditional cultures. While its syndromic architecture cannot replace traditional cultures, its integration into structured diagnostic stewardship programs has the clear potential to accelerate targeted antimicrobial selection, improve patient care, and optimize surgical decision-making in bone and joint infections.

Declarations

ACKNOWLEDGEMENT

Acknowledgments to the following laboratory professionals: Juan Pablo Perez, Pamela Morales, Judith Aguirre, Abigail Veas, and Agustin Zamorano.

CONFLICT OF INTEREST STATEMENT

The authors declare financial support from the bioMérieux Center of Excellence for the provision of laboratory consumables and reagents used in the analysis of study samples. The funder had no influence on the study design, data interpretation, manuscript preparation, or publication decisions.

FUNDING

This study was supported by a grant awarded to Hospital Roberto del Río through the bioMérieux Center of Excellence (COE) program, which provided funding for BIOFIRE Joint Infection Panel reagents and consumables required to conduct the study. No payments, salaries, or other financial compensation were provided to the study authors or healthcare professionals from the participating institutions through this grant.

Author Contribution

D.B. and M.A. conceptualized the study, coordinated the project, and wrote the main manuscript text. Y.L. and K.Y. performed the statistical analyses, contributed to data interpretation, and prepared the figures. A.M., A.O., and J.T.A. participated in patient enrollment, clinical assessment, data collection, and revision of the initial manuscript draft. P.C., C.B., and V.V. carried out sample processing, laboratory analyses,

quality control procedures, and review of the study tables and results. All authors contributed to manuscript revision, approved the final manuscript, and accepted responsibility for the content of the work.

Acknowledgement

Acknowledgments to the following laboratory professionals: Juan Pablo Perez, Pamela Morales, Judith Aguirre, Abigail Veas, and Agustin Zamorano.

References

1. Lemaignen A, Bernard L, Marmor S, Ferry T, Grammatico-Guillon L, Astagneau P et al. Epidemiology of complex bone and joint infections in France using a national registry: The CRIOAc network. J Infect [Internet]. 2021;82(2):199–206. Available from: https://doi.org/10.1016/j.jinf.2020.12.010
2. Carter K, Doern C, Abmm D, Jo C, Copley LAB. The Clinical Usefulness of Polymerase Chain Reaction as a Supplemental Diagnostic Tool in the Evaluation and the Treatment of Children With Septic Arthritis. J Pediatr Orthop. 2016;36(2):167–72.
3. Gouliouris T, Aliyu SH, Brown NM. Spondylodiscitis: update on diagnosis and management. J Antmicrobial Chemother. 2010;65(January 1980):11–24.
4. Francis N, Barbier L, Dubost C, Billet E, Manwaring J, Southwick J, et al. 640. Analytical Validation of the BioFire® Bone and Joint Infection (BJI) Panel for the Identification of Bacteria, Yeast, and Antimicrobial Resistance Genes from Synovial Fluid. Open Forum Infect Dis. 2020;7(Suppl 1):S380.
5. Kokenda C, Legendre T, Abad L, Graue C, Jay C, Ferry T et al. Evaluation of an Automated Multiplex PCR Joint Infection Panel for the Detection/Identification of Pathogens in 201 Synovial Fluid Specimens in a Monocentric Study. Orthop Proc. 2021;103-B(SUPP:23.
6. Schoenmakers JA, de Boer R, Gard L, Kampinga GA, van Oosten M, van Dijl JM, et al. acute joint infections. J Bone Jt Infect. 2023;8(ii):45–50.
7. Esteban J, Salar-vidal L, Schmitt BH, Waggoner A, Laurent F, Abad L, et al. Multicenter evaluation of the BIOFIRE Joint Infection Panel for the detection of bacteria, yeast, and AMR genes in synovial fluid samples. J Clin Microbiol. 2023;61(11):1–13.
8. Gaillard T, Dupieux-Chabert C, Roux A-L, Tessier E, Boutet-Dubois A, Courboulès C et al. A prospective multicentre evaluation of BioFire ® Joint Infection Panel for the rapid microbiological documentation of acute arthritis. Clin Microbiol Infect [Internet]. 2024;30:905–10. Available from: https://doi.org/10.1016/j.cmi.2024.03.022
9. Olearo F, Zein S, El, Portillo ME, Zapf A, Rohde H. Diagnostic accuracy of 16S rDNA PCR, multiplex PCR and metagenomic next-generation sequencing in periprosthetic joint infections : a systematic review and meta-analysis. 2025;31:1115–25.

10. Salar-Vidal L, Esteban J, Laurent F, Abad L, Putnam A, Graue C et al. Clinical comparison of the BIOFIRE Joint Infection Panel using synovial fluid and tissue biopsy samples. 2026;14(4):1–10.
11. Azad MA, Wolf MJ, Strasburg AP, Daniels ML, Starkey JC, Donadio AD, et al. Comparison of the BioFire Joint Infection Panel to 16S Ribosomal RNA Gene-Based Targeted Metagenomic Sequencing for Testing Synovial Fluid from Patients with Knee Arthroplasty Failure. J Clin Microbiol. 2022;60(12):1–8.
12. Berinson B, Spenke L, Krivec L, Tanida K, Both A, Keller J, et al. Performance and Hypothetical Impact on Joint Infection Management of the BioFire Joint Infection Panel: a Retrospective Analysis. J Clin Microbiol. 2023;61(8):1–9.
13. Fox-lewis S, Douglass M, Roberts S, Fox-lewis S, Douglass M, Roberts S. Performance and potential utility of the BioFire Joint Infection Panel with synovial fluid and joint tissue specimens. J Clin Microbiol. 2025;63(10):1–11.
14. Hänel A, Rodríguez P, Silva A, Meza G, Piñera C. Infecciones osteoarticulares en niños: experiencia de cinco años Osteoarticular infections in children: five-year experience. Rev Chil Infectología. 2020;37(6):742–9.
15. De Tezanos-Pinto A, Acuña M, Benadof D, Yohannessen K. Meningococcal Disease in a Pediatric Hospital in Chile: An 8 Year Review. J Pediatr Child Heal Care. 2018;3(1):1–6.
16. Camacho-Moreno G, Vergara-Amador E, Martínez-Villegas T, Aragón-Joya J, Romero-Cardozo L, Lores-Garcia F et al. Correlation between the results of cultures and the molecular BIOFIRE® joint infection panel in a cohort of pediatric patients with bone and joint infections in Bogotá, Colombia. Front Pediatr. 2024;(April):1–8.
17. Miamidian JL, Toler K, Somasundaram S, Mclaren A, Cholewa J, Deirmengian C. Evaluating the Diagnostic Performance of a Multiplex Polymerase Chain Reaction Panel for the Detection of Synovial Fluid Pathogens in the Setting of Periprosthetic Joint Infection. Cureus. 2025;17(8):e91300.
18. Berinson B, Tanida K, Spenke L, Krivec L, Keller J, Rolvien T et al. Prospective evaluation of real-world performance and clinical impact of the Biofire FilmArray joint infection panel. 2025;13(4):1–12.
19. Lund–Andersen J, Petersen MLH, Kostadinov K, Friis–Hansen L, Calum H, Overgaard S. Clinical evaluation of a multiplex PCR – based test for joint infection: a prospective diagnostic accuracy study of forty – nine patients. Eur J Orthop Surg Traumatol [Internet]. 2024;34(8):4105–11. Available from: https://doi.org/10.1007/s00590-024-04114-2
20. Pascual S, Noble B, Ahmad-saeed N, Aldridge C, Ambretti S, Amit S et al. real-world Evid study. 2024;87–97.
21. Saeed K, Ahmad N, Rachel S, Gavin A, Barrett L, Boyd SE. A multicentre evaluation and expert recommendations of use of the newly developed BioFire Joint Infection polymerase chain reaction panel. Eur J Clin Microbiol Infect Dis [Internet]. 2022;(0123456789). Available from: https://doi.org/10.1007/s10096-022-04538-w
22. Lazic I, Feihl S, Prodinger PM, Banke IJ, Trampuz A, Rothe RVE et al. Diagnostic accuracy of multiplex polymerase chain reaction on tissue biopsies in periprosthetic joint infections. Sci Rep

[Internet]. 2021;11:1–6. Available from: https://doi.org/10.1038/s41598-021-99076-4

Figures

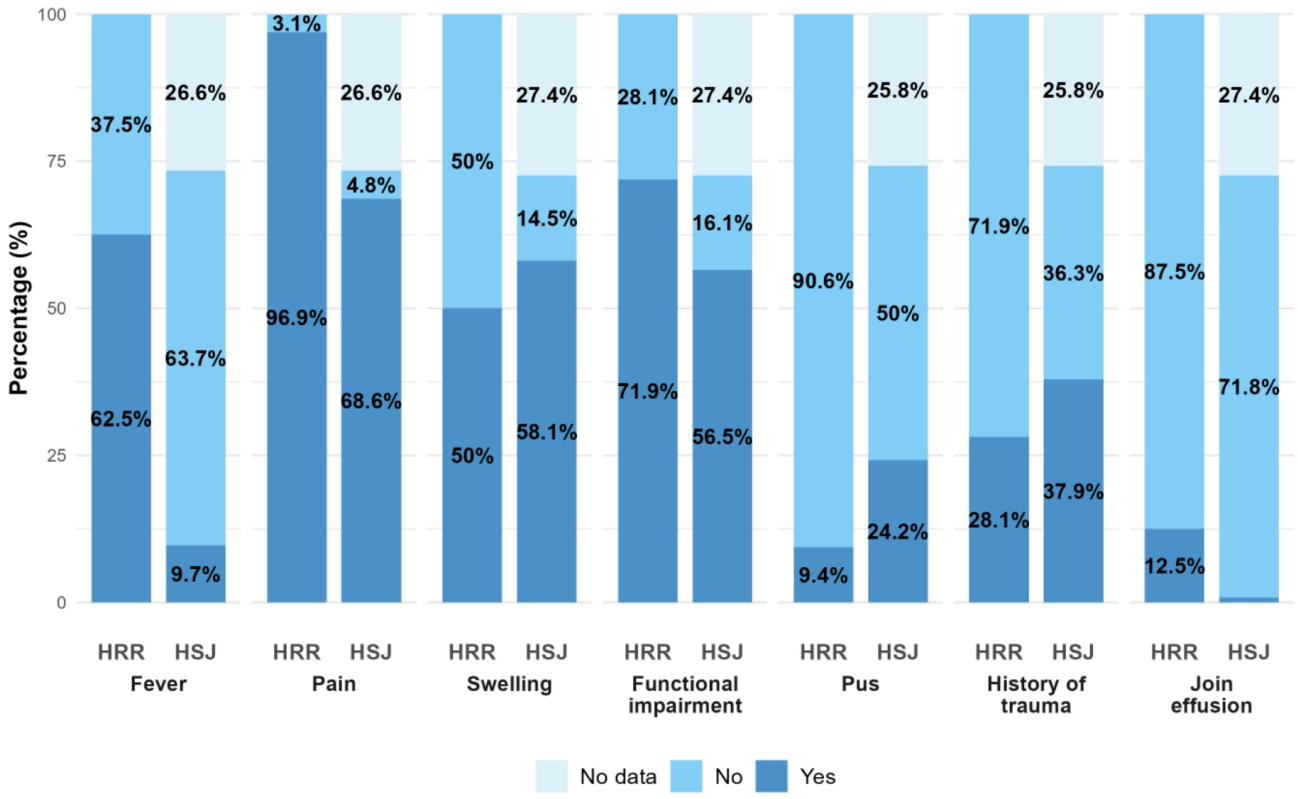


Figure 1

Signs and symptoms on admission.

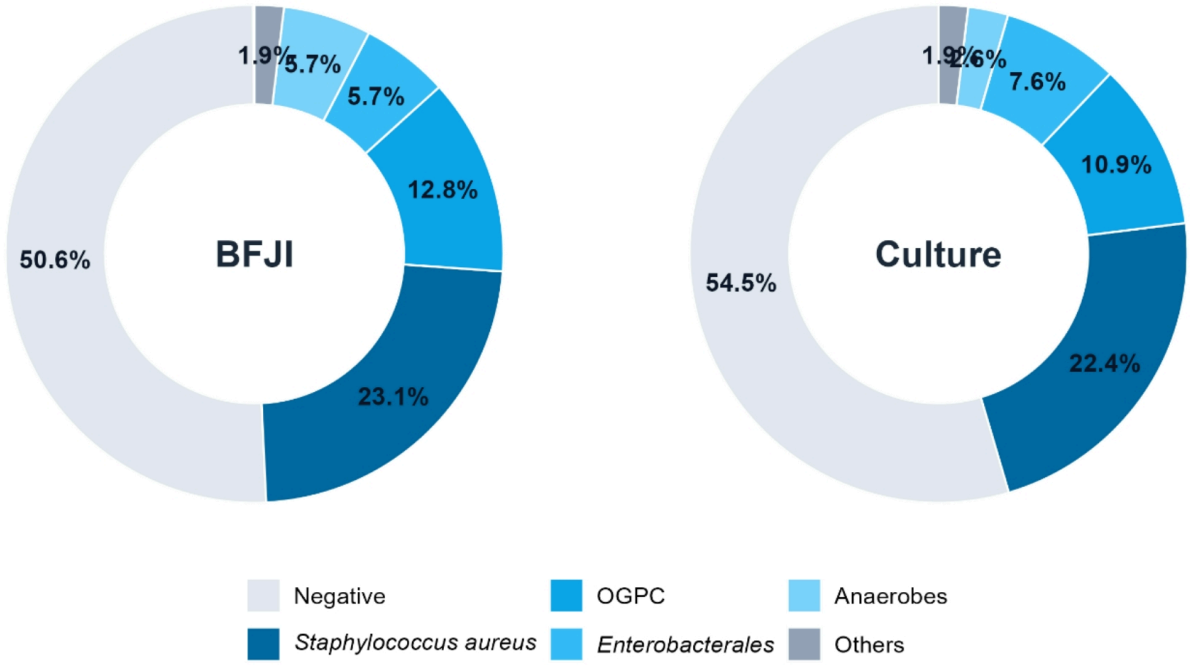


Figure 2

Comparison of culture and BFJI results.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial20260830.docx