

Concordance Between 16S-to-23S Interspace Profiling and 16S rRNA Sequencing in Culture-Negative Bone and Joint Infections



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INTRODUCTION

- Bone and joint infections (BJI) remain difficult to diagnose, particularly when conventional microbial cultures are **negative**.
- Molecular tests such as **16S rRNA gene sequencing** may improve pathogen detection but are limited by cost, turnaround time, and interference from host DNA.
- Interspace profiling (IS-pro)** identifies bacteria by length polymorphisms in the 16S–23S rRNA intergenic spacer region, enabling rapid, simultaneous detection of multiple taxa.
- Purpose:** evaluate the diagnostic performance of IS-pro in suspected BJI with negative cultures and assess its concordance with 16S rRNA sequencing.

METHODS

- Retrospective **single-center** study of consecutive patients with suspected BJI and **negative cultures** (Nov 2024 – Aug 2025).
- Synovial fluid** samples were analyzed; all cultures were incubated for **9 days**.
- Following DNA extraction, each sample was tested with **both IS-pro and 16S rRNA sequencing**.
- Overall **concordance** between the two methods was calculated.
- Positive and negative percent agreement (PPA / NPA)** and **Cohen’s κ** assessed agreement beyond chance.

RESULTS

- 20 patients** (mean age 54.5 ± 24.0 years; 45% female); **13 native** and **7 prosthetic** joints.
- Overall concordance between IS-pro and 16S rRNA sequencing was **14/20 (70%)**.
- PPA 25%, NPA 100%**; fair agreement beyond chance (**κ = 0.29**).
- 2 samples** uninterpretable by 16S (strong human DNA) yielded identifiable organisms with **IS-pro** — proteobacteria and *S. bovis* group + *S. intermedius*.
- 2 detections of *C. acnes* on initial 16S were not confirmed on repeat testing, suggesting probable **contamination**.

CONCLUSION

- IS-pro showed **moderate concordance** with 16S rRNA sequencing in suspected culture-negative BJI.
- IS-pro **identified organisms** in cases where sequencing was uninterpretable due to high human DNA background.
- May serve as a **rapid adjunctive molecular diagnostic tool** when cultures are negative.
- Larger prospective cohorts** are needed to define its role within the BJI diagnostic algorithm.

Concordant 14/20 (70%)

Discordant 6/20 (30%)

25%

Positive percent agreement (PPA)

100%

Negative percent agreement (NPA)

κ = 0.29

Agreement beyond chance (fair)

IS-pro identified organisms in 2 samples that were uninterpretable by 16S rRNA sequencing.

Table 1. Clinical characteristics of the cohort

Characteristic	Value (N = 20)
Age, mean ± SD (years)	54.5 ± 24.0
Female sex	9 (45%)
Native joint	13 (65%)
Prosthetic joint	7 (35%)
Joint — knee / shoulder / hip / elbow / wrist / foot	8 / 4 / 3 / 3 / 1 / 1
Inflammatory arthritis	2 (10%)
Immunocompromised	1 (5%)
Antibiotics ≤2 weeks prior to sampling	8 (40%)
Negative cultures	20 (100%)

Joint distribution and comorbidities summarized across all 20 patients.

Table 2. Microbiological data

	Joint	Type	Abx ≤2 wk	Culture	16S rRNA	Repeat 16S	IS-pro
1	Knee	Native	Yes	Neg	<i>C. acnes</i>	Neg	Neg
2	Elbow	Native	Yes	Neg	Neg	—	Neg
3	Elbow	Prosthetic	No	Neg	Neg	—	Neg
4	Knee	Native	No	Neg	Neg	—	Neg
5	Foot	Native	Yes	Neg	Unknown*	—	Unknown proteobacteria (1.00, high)
6	Wrist	Native	Yes	Neg	Neg	—	Neg
7	Shoulder	Native	Yes	Neg	Unknown*	—	<i>S. bovis</i> grp + <i>S. intermedius</i> (0.70, med)
8	Knee	Native	No	Neg	Unknown*	—	Neg
9	Shoulder	Native	Yes	Neg	Unknown*	—	Neg
10	Knee	Native	No	Neg	Neg	—	Neg
11	Knee	Native	No	Neg	Unknown*	—	Neg
12	Shoulder	Native	Yes	Neg	Neg	—	Neg
13	Hip	Prosthetic	No	Neg	Neg	—	Neg
14	Hip	Prosthetic	No	Neg	Neg	—	Neg
15	Knee	Prosthetic	No	Neg	<i>C. acnes</i>	Neg	Neg
16	Knee	Prosthetic	No	Neg	Unknown*	—	Neg
17	Knee	Prosthetic	Yes	Neg	Neg	—	Neg
18	Elbow	Prosthetic	No	Neg	Neg	—	Neg
19	Shoulder	Native	No	Neg	Neg	—	Neg
20	Hip	Native	No	Neg	Neg	—	Neg

All samples were synovial fluid; all cultures were negative. *Microbial band detected but uninterpretable due to a strong human band. IS-pro detections show (confidence score, abundance). — not performed.