



# Diagnosis of bone and joint infections: the point of view from the clinical metagenomist

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I have no financial disclosures

## Goals of this presentation

- Review prosthetic joint infections (PJIs) and why they are good cases for metagenomic shotgun sequencing (MSS)
- Discuss metagenomic sequencing for PJIs in the context of ideal qualities of a test from the clinical metagenomist point of view
- Review data supporting why MSS may be useful, but not always necessary, for PJI

# Case

- 68 yo female
- PMH: Crohn's disease: adalimumab then vedolizumab

Bilateral TKA  
8 yr ago



Bilateral Cx-  
negative PJI  
2 yr ago



DAIR  
Vanc + cefep,  
Cefadroxil

Recurrent  
right PJI



First step of  
a 2-stage  
exchange

Necrotic tissue seen  
Culture-negative  
16S rDNA PCR neg  
Targeted PCRs neg  
Serologies neg

- What do you go from here?

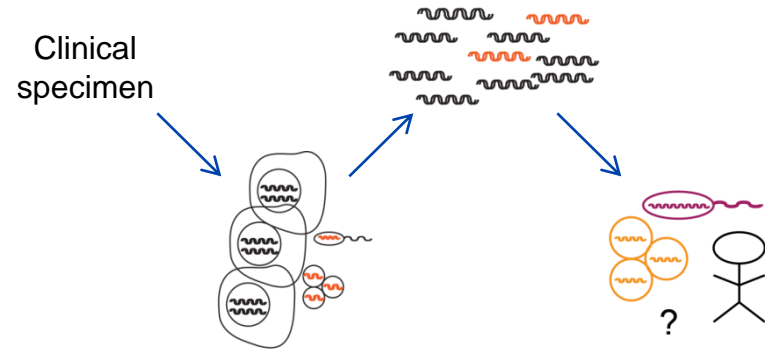
# A little background

## Prosthetic Joint Infection (PJI)



- Primarily bacterial, some fungal
- Acute or chronic
- Treatment is difficult
  - Surgery almost always required
  - Sometimes joint is removed for months (2-stage exchange)
  - Antibiotics from 6 weeks to lifelong

## Metagenomic Shotgun Sequencing



- Nucleic acid (DNA and/or RNA) extracted directly from a clinical specimen
- No targeted amplification (e.g. 16S rRNA)
- Millions of short sequences obtained
- Sequences analyzed to detect microorganisms

# What makes PJI an attractive target for MSS

- Cultures don't always work
- Typically a sterile site
- “Wide” range of pathogens
- The diagnosis CAN wait a few days
- Long-term treatment implications

## Goals of the clinical metagenomist

- To provide an accurate identification of pathogens to aid in the care of patients
  - We want to help patients
    - Tests must be effective, timely, and useful
  - We do not want to harm patients
    - Results must be accurate

## Goals of the clinical metagenomist

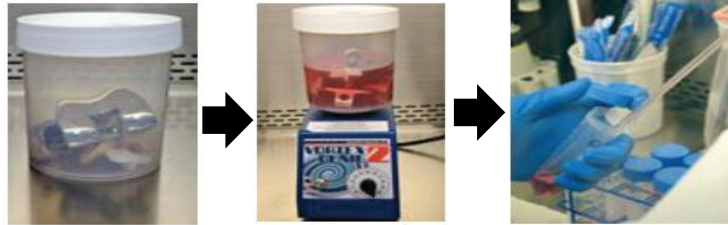
- To provide an accurate identification of pathogens to aid in the care of patients
  - We want to help patients
    - Tests must be **effective**, timely, and useful
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# Does metagenomic sequencing work for PJI?

- To date, three larger studies
  - Two studies focused on sonicate fluid as a sample

Sonicate fluid:



## Molecular Diagnosis of Orthopedic-Device-Related Infection Directly from Sonication Fluid by Metagenomic Sequencing

Teresa L. Street,<sup>a</sup> Nicholas D. Sanderson,<sup>a</sup> Bridget L. Atkins,<sup>b,c</sup> Andrew J. Brent,<sup>a,b</sup> Kevin Cole,<sup>d,e</sup> Dona Foster,<sup>a</sup> Martin A. McNally,<sup>b</sup> Sarah Oakley,<sup>c</sup> Leon Peto,<sup>a</sup> Adrian Taylor,<sup>b</sup> Tim E. A. Peto,<sup>a,f</sup> Derrick W. Crook,<sup>a,f</sup> David W. Eyre<sup>a,f</sup>

## Identification of Prosthetic Joint Infection Pathogens Using a Shotgun Metagenomics Approach

Matthew J. Thoendel,<sup>1</sup> Patricio R. Jeraldo,<sup>2</sup> Kerryl E. Greenwood-Quaintance,<sup>3</sup> Janet Z. Yao,<sup>4</sup> Nicholas Chia,<sup>5</sup> Arlen D. Hanssen,<sup>4</sup> Matthew P. Abdel,<sup>1</sup> and Robin Patel<sup>1,5</sup>

- One studied synovial fluid prior to surgery

## Direct Detection and Identification of Prosthetic Joint Infection Pathogens in Synovial Fluid by Metagenomic Shotgun Sequencing

Morgan I. Ivy,<sup>a</sup> Matthew J. Thoendel,<sup>b</sup> Patricio R. Jeraldo,<sup>c</sup> Kerryl E. Greenwood-Quaintance,<sup>a</sup> Arlen D. Hanssen,<sup>d</sup> Matthew P. Abdel,<sup>d</sup> Nicholas Chia,<sup>e</sup> Janet Z. Yao,<sup>e</sup> Aaron J. Tande,<sup>b</sup> Jayawant N. Mandrekar,<sup>a</sup> Robin Patel<sup>a,b</sup>

# Sonicate fluid MSS results

- Street, *et al.* results
  - 97 samples: 62 culture-positive, 35 culture-negative

	Sensitivity	Specificity	New Identifications
Versus sonicate fluid culture	88% (Genus level=93%)	88%	9 probable pathogens
Vs. sonicate fluid and PPT culture	68%	88%	6 probable pathogens

New Identifications:

*Fusobacterium nucleatum*, *Veillonella parvula*, *Fingoldia magna*, *Parvimonas micra*, *Staphylococcus aureus*, and *Streptococcus dysgalactiae*

## Sonicate fluid MSS results

- Thoendel *et al.* results
  - 408 samples: 115 Culture-pos, 98 Cx-neg PJI, 195 aseptic failure

	Sensitivity	Specificity	New Identifications
Versus sonicate fluid culture	94.8% (115 Cx-pos PJI)	96.4% (Aseptic failures)	11 from Cx-pos PJI (9.6%) 43 from Cx-Neg PJI (43.9%)
Vs. sonicate fluid and PPT culture	90.5% (137 Cx-pos PJI)		12 from Cx-pos PJI (8.8%) 27 form Cx-neg PJI (35.5%)
Vs. sonicate fluid, PPT, and synovial fluid culture	89% (146 Cx-pos PJI)		12 from Cx-pos PJI (8.2%) 21 from Cx-neg PJI (31.3%)

# Synovial Fluid MSS results

- Ivy, *et al.*
  - 168 samples: 82 Cx-pos PJI, 25 Cx-neg PJI, 61 aseptic failure

	Sensitivity	Specificity	New Identifications
Vs. synovial fluid culture	82.9%	93.4% (Aseptic failures)	3 from Cx-pos PJI (3.7%) 4 from Cx-neg PJI (16%)

New Identifications:

*S. aureus*, *Salpingoeca rosetta*, *Afipia broomeae*, *Bradyrhizobium japonicum*,  
*Enterococcus faecalis*, *Fingoldia magna*, *Anaerococcus vaginalis*

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# Nanopore-based sequencing for PJIs

Sanderson et al. *BMC Genomics* (2018) 19:714  
<https://doi.org/10.1186/s12864-018-5094-y>


BMC Genomics

METHODOLOGY ARTICLE

Open Access

## Real-time analysis of nanopore-based metagenomic sequencing from infected orthopaedic devices



Nicholas D Sanderson<sup>1\*</sup> , Teresa L Street<sup>1</sup>, Dona Foster<sup>1</sup>, Jeremy Swann<sup>1</sup>, Bridget L Atkins<sup>3,4</sup>, Andrew J Brent<sup>1,3</sup>, Martin A McNally<sup>3</sup>, Sarah Oakley<sup>4</sup>, Adrian Taylor<sup>4</sup>, Tim E A Peto<sup>1,2</sup>, Derrick W Crook<sup>1,2</sup> and David W Eyre<sup>1,2</sup>

- Successful in 7 out of 7 culture-positive PJIs

## Goals of the clinical metagenomist

- To provide an accurate identification of pathogens to aid in the care of patients
  - We want to help patients
    - Tests must be effective, timely, and **useful**
  - We do not want to harm patients
    - Results must be accurate

## How much difference could metagenomics make?

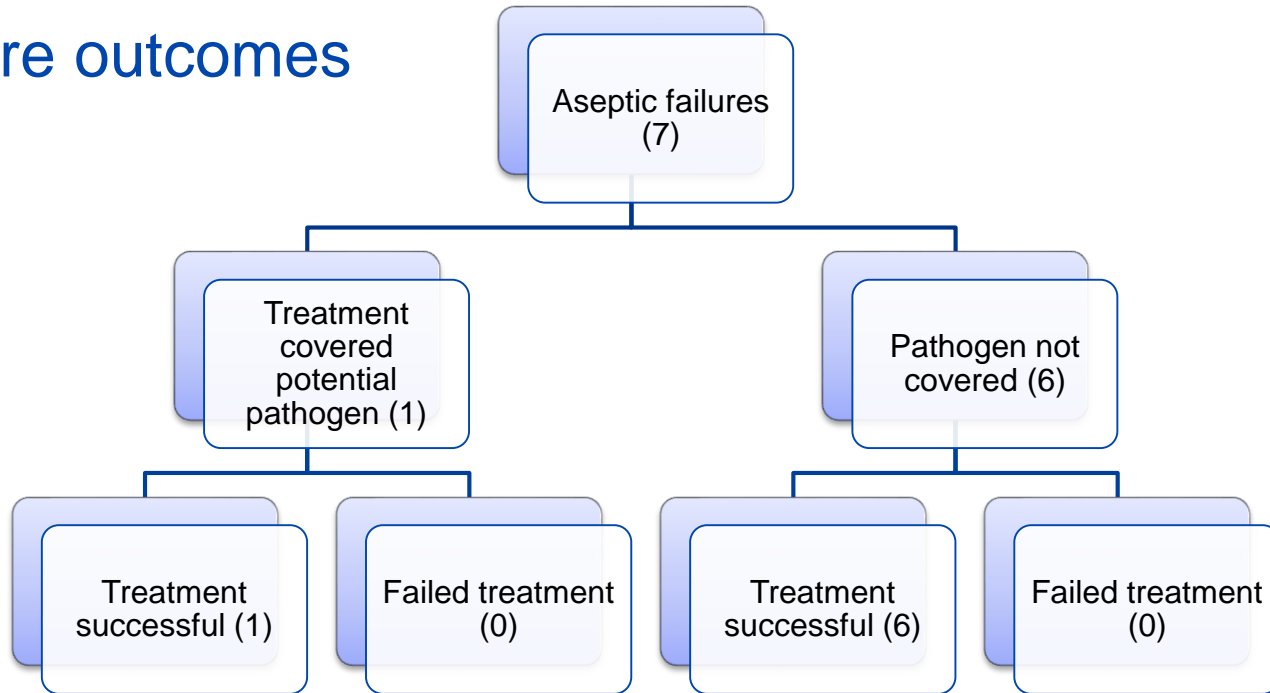
- Looked back at our study of 408 subjects
  - Now 2 to 8 years outcome data available
- Looked at treatment and outcomes of individuals where new potential pathogens were discovered by MSS



## Study Design

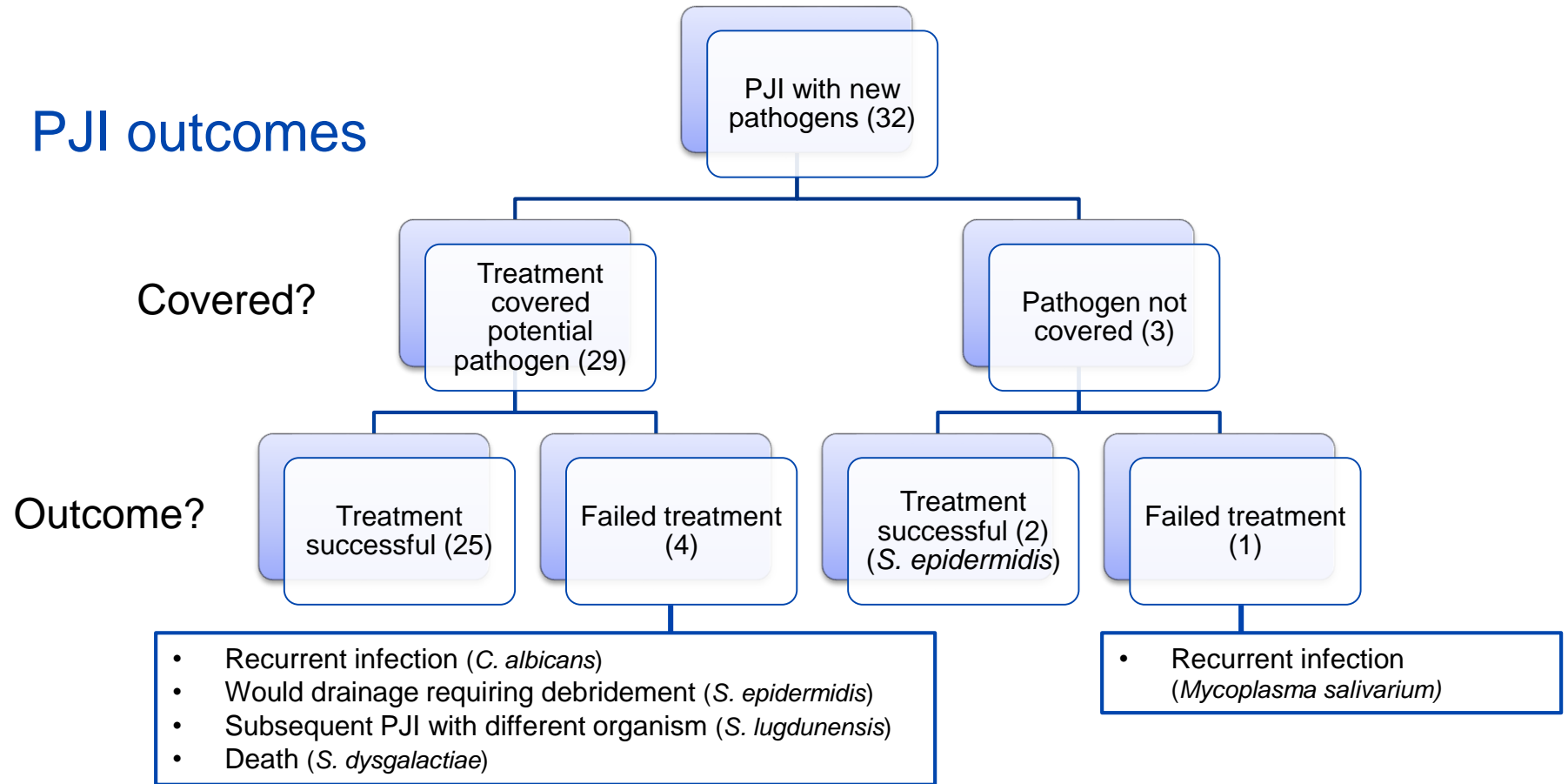
- 39 subjects identified
  - 32 classified as PJI
  - 7 classified as aseptic failure
- Determined whether subsequent IV antibiotic therapy covered the identified microorganism
  - Also evaluated the reason the IV therapy was chosen
- Evaluated outcomes after surgery

# Aseptic failure outcomes



New organisms: *S. aureus* (3), *C. acnes* (2), *Streptococcus sanguinis* (2)

# PJI outcomes



# How were we able to cover new pathogens in 29 of 32 cases?



## Mainly Yes....

*Corynebacterium pseudogenitalium*  
*Cutibacterium acnes* (4)  
*Staphylococcus aureus* (10)  
*Staphylococcus epidermidis* (7)  
*Staphylococcus haemolyticus*  
*Streptococcus agalactiae* (3)  
*Streptococcus dysgalactiae* (2)  
*Streptococcus sanguinis*  
*Enterococcus faecalis*

## but also No...

*Aerococcus urinae*  
*Candida albicans*  
*Clostridium perfringens*  
*Clostridium species*  
*Facklamia languida*  
*Finegoldia magna*  
*Peptoniphilus harei*  
*Peptoniphilus lacrimalis*

## Reasons for choosing correct coverage in 29 cases

- 9 cases were empiric coverage
  - Daptomycin + ertapenem (2)
  - Vancomycin + ertapenem (2)
  - Vancomycin + cefepime
  - Ceftriaxone (2)
  - Cefepime
  - Cefazolin + rifampin
- 9 cases: past prior infection with detected pathogen
  - All underwent DAIR and were on suppression
- 8 cases: therapy directed at other culture-positive organisms
- 3 cases: Other positive cultures from acute episode

Conclusion: Metagenomics can help, but a good ID physician can go a long ways

## Goals of the clinical metagenomist

- To provide an accurate identification of pathogens to aid in the care of patients
  - We want to help patients
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    - Results must be **accurate**

## Why is accuracy difficult?

- How do you define prosthetic joint infection?
- Pathogen versus background?
  - Culture-negative PJI: often low burden of disease
  - Significant overlap between common reagent contaminants and reported PJI pathogens
  - Background varies
- New pathogens to discover?

# Do analysis tools affect accuracy?

**+ PATHOGENOMIX**

**PHASE**  
GENOMICS

**FASTERIS**  
member of the sonic healthcare network

**taxonmer** BETA

**Kraken**  
Taxonomic Sequence Classification System

**COSMOSID**

**KARIUS**  
clarity at speed™

**QIIME**  
Quantitative Insights Into Microbial Ecology

**ARC**  
BIO

**DeepMicrobes**

DeepMicrobes: taxonomic classification for metagenomics with deep learning

**Metaxa2**

**IDbyDNA**

**PathoQuest**

**MetaPhlAn v2.0**

**MOCAT2**  
by the Bork Group @ EMBL

**MG-RAST**  
metagenomics analysis server  
version 4.0.3  
cite us

**ONE CODEX**



# Does analysis tool choice matter?

- Evaluated three commercial analysis tools
- Hand-selected 24 “challenging” samples from PJI study
  - Uncommon pathogens, polymicrobial, culture-negative, etc.
- Submitted identical sequencing files to each company for analysis
- Determined whether there were differences in final interpretations based on the tool used
  - Culture-positive species detected?
  - New identifications?
    - If so, were the “corroborated” by other tools?

# Does analysis tool choice matter?

**LMAT**

**CosmosID**

**One Codex**

**IDbyDNA**

## Why is accuracy important?

- An accurate diagnosis can ideally lead to narrower and more effective therapy with better outcomes and fewer adverse effects
- An inaccurate diagnosis can lead to harm
  - Overtreatment if additional non-pathogens reported
  - Possible loss of treatment if only non-pathogen(s) reported
- A negative test will not create harm
- You cannot rely on physicians to sort out real versus not real

## Summary

- Metagenomics has a role for PJI pathogen detection
- At this time metagenomic sequencing should be reserved for when conventional testing fails
- Accurate results will be key for clinical integration
- For PJI, specificity should trump sensitivity

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## Pitfalls? How...?

- Interpreting the data is hard
- Tools were not designed to answer whether a pathogen is present
- As pathogen loads go down, reagent contaminant signals go up

Sample #1 (with read #'s)	Sample #2	Sample #3
Staphylococcus 334,354 Malassezia 8 Corynebacterium 2	Acinetobacter 4,915 Streptococcus 873 Prevotella 288 Bradyrhizobium 326 Oribacterium 193	Staphylococcus 666 Cutibacterium 161 Streptococcus 141 Acinetobacter 133 Malassezia 52

*S. aureus* PJI

Aseptic failure

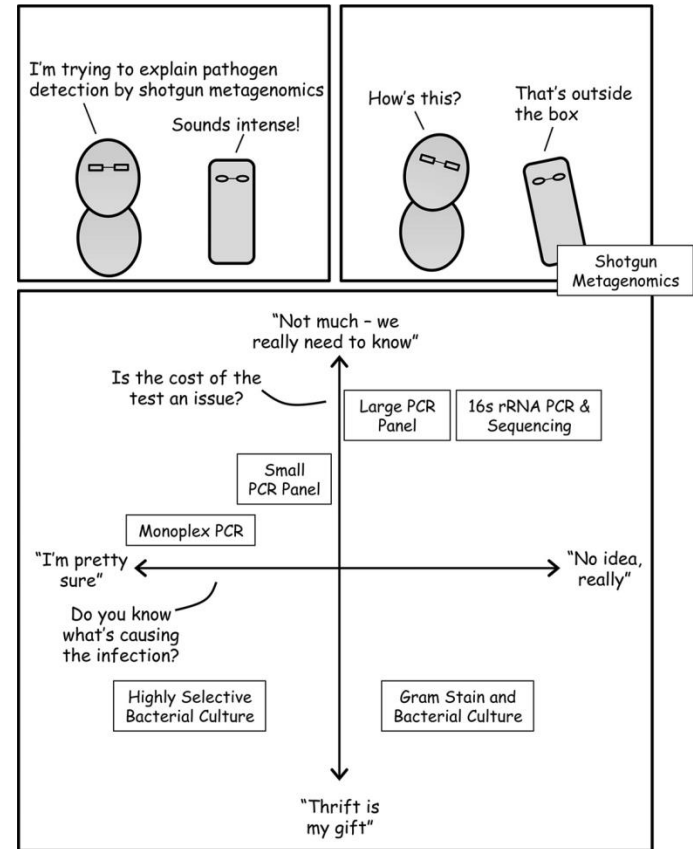
*S. epidermidis* PJI

## Lessons learned from MSS analysis

- Simple read count or percentage cutoffs aren't sufficient
  - Host DNA content and multiplexing influences these too much
- Subtracting negative control reads isn't easy
  - Contains potential pathogens, changes over time
- Different tools can give different results
- A combination of metrics will likely be optimal
  - Signal strength, genome coverage, signal vs. internal controls
- A false positive result is much more dangerous than a negative result

# Proposed role for MSS

- Currently: When all else fails
  - Cultures
  - Directed PCRs
  - 16S rRNA gene PCR
  - Serologic tests
- Best way to preserve samples?
- Future needs:
  - Faster and cheaper
  - Avoiding false positives



Alexander McAdam. J Clin Micro 2018, 56(8)

# Study Methods Used

Study	Samples	Microbial Enrichment	Extraction	Library + Sequencer	Analysis Tools	Comparison
Street, et al. J Clin Micro, 2017	Sonicate fluid (n=97)	5 µm filter	Pathogen lysis tubes + EtOH precipitation	Nextera XT and MiSeq	Kraken	Sonicate fluid culture ± PPT
Thoendel, et al. CID, 2018	Sonicate fluid (n=408)	MoYsis	MoBio Bacteremia DNA kit	NEBNext Ultra and HiSeq 2500	LMAT + Metaphlan2	Sonicate fluid culture ± PPT ± synovial fluid culture
Ivy, et al. J Clin Micro, 2018	Synovial fluid (n=168)	MoYsis	MoBio Bacteremia DNA kit	NEBNext Ultra and HiSeq 2500	LMAT + Metaphlan2	Synovial fluid

LMAT = Livermore Metagenomics Analysis Toolkit  
PPT = Periprosthetic tissue (intraoperative)