MAYO CLINIC

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

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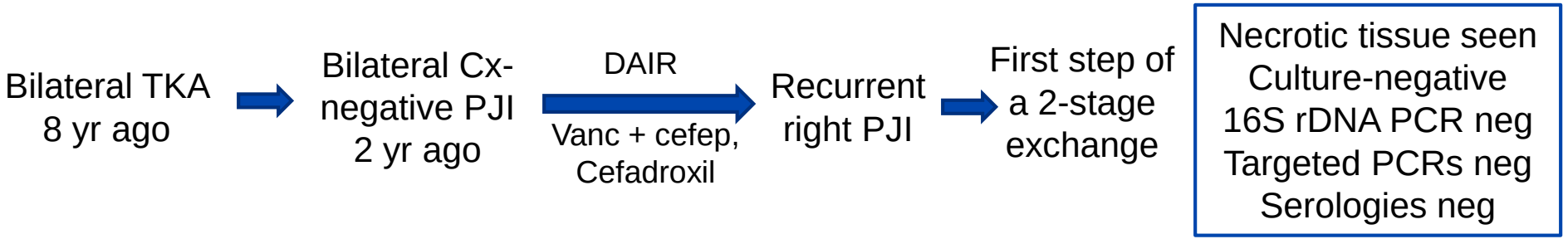
I have no financial disclosures

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



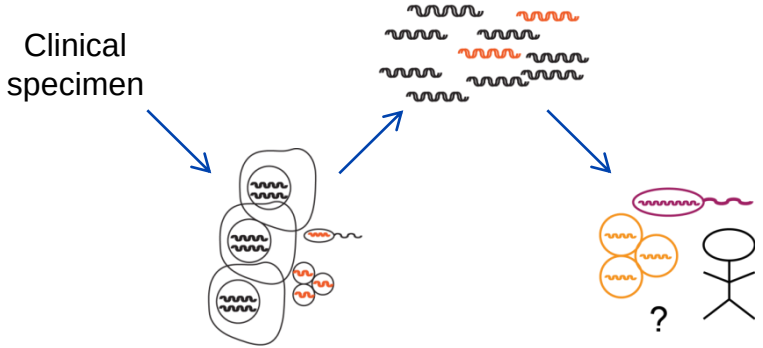
- What do you go from here?

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

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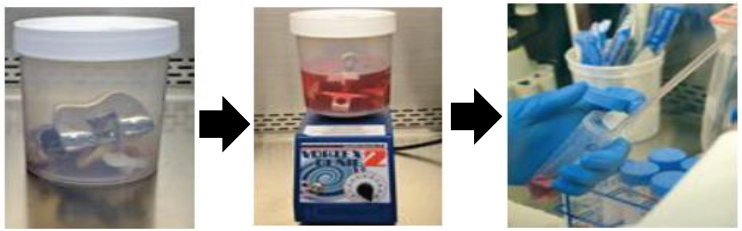
Does metagenomic sequencing work for PJI?

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

Identification of Prosthetic Joint Infection Pathogens Using a Shotgun Metagenomics Approach

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

  - 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,[a] Matthew J. Thoendel,[b] Patricio R. Jeraldo,[c] Kerryl E. Greenwood-Quaintance,[a] Arlen D. Hanssen,[d] Matthew P. Abdel,[d] Nicholas Chia,[c] Janet Z. Yao,[c] Aaron J. Tande,[b] Jayawant N. Mandrekar,[e] Robin Patel[a,b]

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, Finegoldia 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, Finegoldia 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[1*], Teresa L Street[1], Dona Foster[1], Jeremy Swann[1], Bridget L Atkins[3,4], Andrew J Brent[1,3], Martin A McNally[3], Sarah Oakley[4], Adrian Taylor[4], Tim E A Peto[1,2], Derrick W Crook[1,2] and David W Eyre[1,2]

- 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

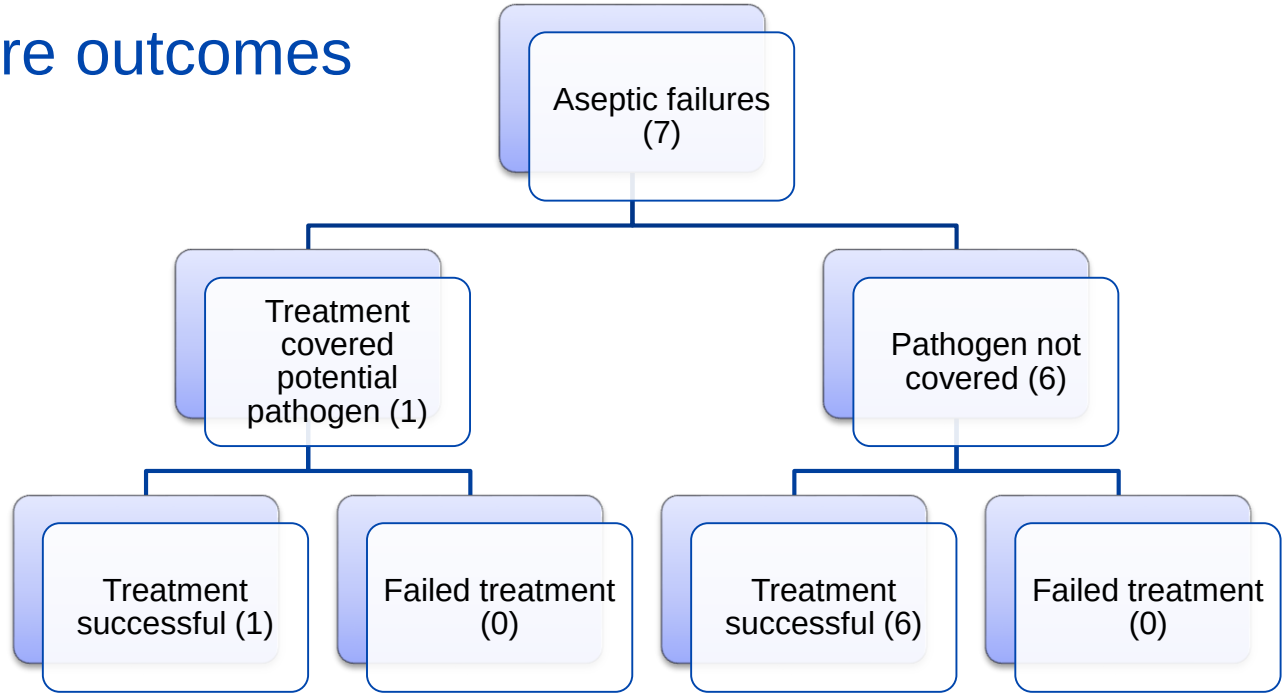
- Looked at treatment and outcomes of individuals where new potential pathogens were discovered by MSS

Study Design

- 39 subjects identified
  - 32 classified as PJI
  - 7 classified as aseptic failure

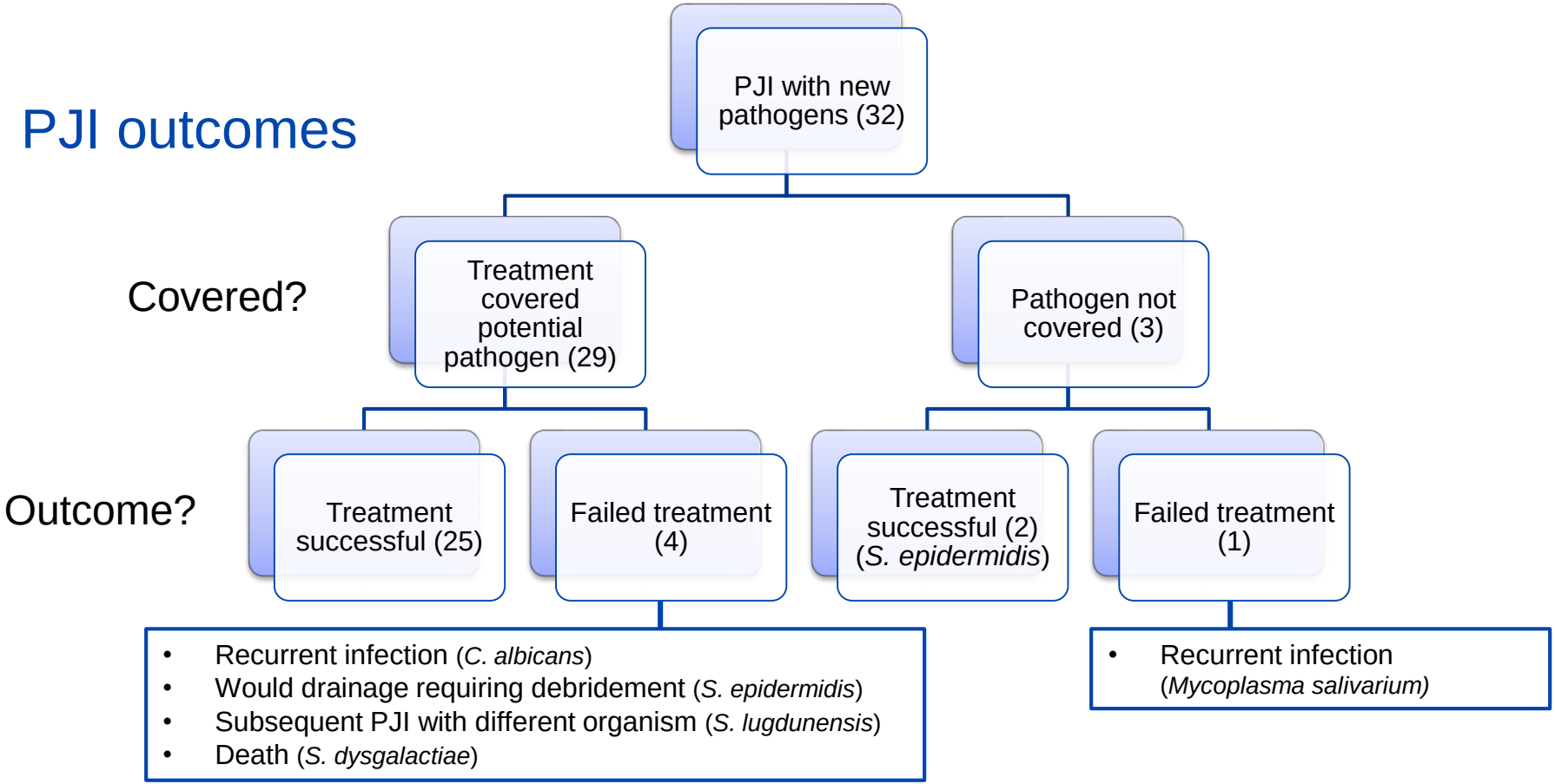
- Determined whether subsequent IV antibiotic therapy covered the identified microorganism
  - Also evaluated the reason the IV therapy was chosen
- Evaluated outcomes after surgery

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

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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?

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

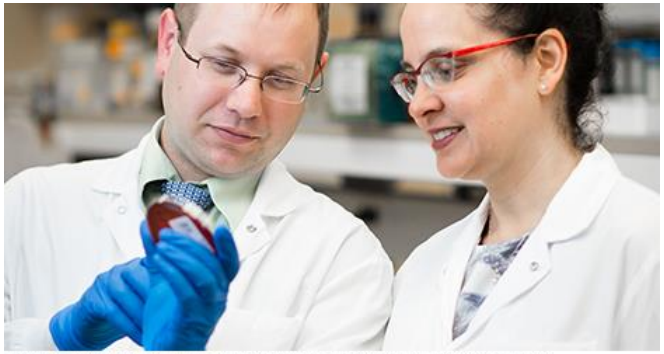
- An inaccurate diagnosis can lead to harm
  - Overtreatment if additional non-pathogens reported
  - Possible loss of treatment if only non-pathogen(s) reported
- A negative test will not create harm

- 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

Acknowledgements



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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

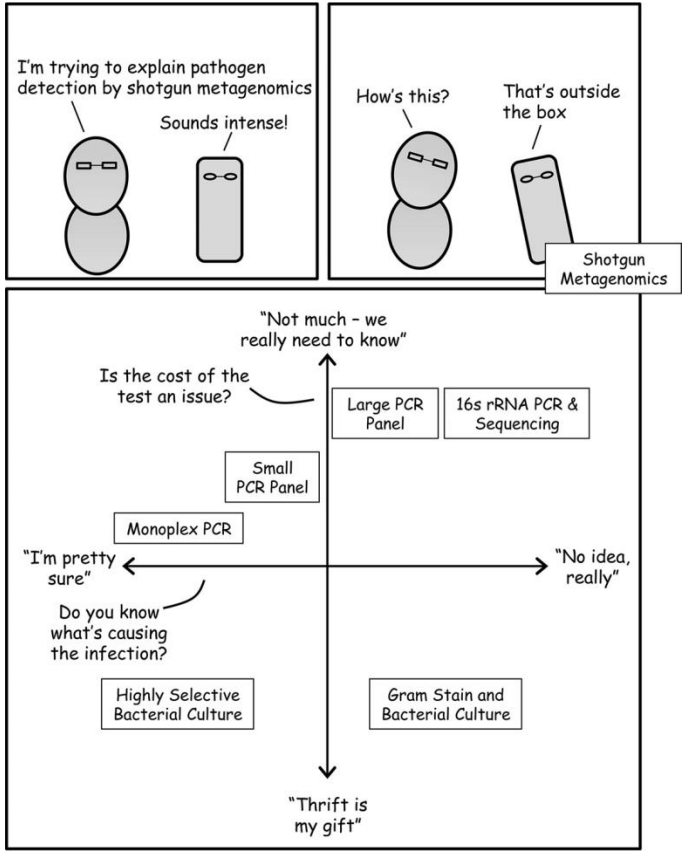
Lessons learned from MSS analysis

- 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)	MolYsis	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)	MolYsis	MoBio Bacteremia DNA kit	NEBNext Ultra and HiSeq 2500	LMAT + Metaphlan2	Synovial fluid

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

