Clinical Metagenomics in Bone and Joint Infections

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**Bone and Joint Infections: Proof of Concept Study**

(≥1 pg/μL bacterial DNA, <99% human DNA)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Samples</th>
<th>Age</th>
<th>Gender</th>
<th>Post-operative infection (type of surgery)</th>
<th>Delay between surgery and infection</th>
<th>Body site</th>
<th>Material involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>51</td>
<td>M</td>
<td>Yes (material)</td>
<td>&lt;1 month</td>
<td>Ankle</td>
<td>Osteosynthesis</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>50</td>
<td>F</td>
<td>No</td>
<td></td>
<td>Clavicle</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>54</td>
<td>M</td>
<td>Yes (material)</td>
<td>&lt;1 month</td>
<td>Toe</td>
<td>Osteosynthesis</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>66</td>
<td>M</td>
<td>Yes (material)</td>
<td>1 and 3 months</td>
<td>Tibia</td>
<td>Osteosynthesis</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>61</td>
<td>F</td>
<td>Yes (material)</td>
<td>&lt;1 month</td>
<td>Knee</td>
<td>Total knee prothesis</td>
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<tr>
<td>F</td>
<td>1</td>
<td>63</td>
<td>M</td>
<td>Yes (material)</td>
<td>&lt;1 month</td>
<td>Mandible</td>
<td>Osteosynthesis</td>
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<tr>
<td>G</td>
<td>3</td>
<td>69</td>
<td>M</td>
<td>Yes (bone resection)</td>
<td></td>
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<tr>
<td>H</td>
<td>2</td>
<td>64</td>
<td>F</td>
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<td>I</td>
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<td>86</td>
<td>F</td>
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<td>1 and 3 months</td>
<td>Knee</td>
<td>Total knee prothesis</td>
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<tr>
<td>J</td>
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<td>50</td>
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<td>K</td>
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<td>86</td>
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<td>No</td>
<td>&gt;3 months</td>
<td>Knee</td>
<td>Osteosynthesis</td>
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<td>L</td>
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<td>51</td>
<td>M</td>
<td>Yes (material)</td>
<td>&gt;3 months</td>
<td>Tibia</td>
<td>Osteosynthesis</td>
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<tr>
<td>M</td>
<td>1</td>
<td>87</td>
<td>F</td>
<td>Yes (material)</td>
<td>&lt;1 month</td>
<td>Knee</td>
<td>Total knee prothesis</td>
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<tr>
<td>N</td>
<td>1</td>
<td>60</td>
<td>M</td>
<td>No</td>
<td></td>
<td>Greater trochanter and ischium</td>
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</table>

Proportions of Species Recovered In Culture and From Reads (Metaphlan2)

Monomicrobial samples by culture (n = 8)
Presence of pathogen confirmed by metagenomics

Polymicrobial samples by culture (n = 16)
32/55 (58%) found species level, 41/55 (75%) genus level
#Species Found By Culture and Metagenomic Sequencing

Additional 273 bacteria found
182 possible pathogens
91 contaminants

<table>
<thead>
<tr>
<th>Prosthetic Joint Infection Microbiology</th>
<th>Hip and Knee</th>
<th>Hip</th>
<th>Knee</th>
<th>Shoulder</th>
<th>Elbow</th>
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<tr>
<td>All time periods</td>
<td>2435</td>
<td>637</td>
<td>1979</td>
<td>1427</td>
<td>199</td>
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<tr>
<td>Number of joints</td>
<td></td>
<td></td>
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<tr>
<td>Staphylococcus aureus</td>
<td>27</td>
<td>38</td>
<td>13</td>
<td>23</td>
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<td>Coagulase negative staphylococci</td>
<td>27</td>
<td>22</td>
<td>30</td>
<td>23</td>
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<tr>
<td>Streptococcus species</td>
<td>8</td>
<td>4</td>
<td>6</td>
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<td>4</td>
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<td>Enterococcus species</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Aerobic gram negative bacilli</td>
<td>9</td>
<td>24</td>
<td>7</td>
<td>5</td>
<td>10</td>
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<td>Anaerobic bacteria</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>5</td>
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<tr>
<td>Cutibacterium acnes</td>
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<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Other anaerobes</td>
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<td></td>
<td></td>
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<td>3</td>
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<tr>
<td>Culture negative</td>
<td>14</td>
<td>10</td>
<td>7</td>
<td>11</td>
<td>15</td>
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<td>Polymicrobial</td>
<td>15</td>
<td>31</td>
<td>14</td>
<td>12</td>
<td>16</td>
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<tr>
<td>Other</td>
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</tr>
</tbody>
</table>

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Staphylococcus epidermidis Biofilm on Polycarbonate Coupons
Scanning Electron Microscopy
Current Orthopedic Implant Processing - Mayo Clinic

400 ml Ringer’s Solution Added

Prosthesis Placed in Container (Operating Room)

Plating

Aspiration

Centrifuge 5 min

Vortex 30 sec

Sonicate 5 min

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# Comparison of Sonicate Fluid and Tissue Culture Diagnosis of Orthopedic Foreign Body Infection

<table>
<thead>
<tr>
<th>Implant type</th>
<th>Sensitivity</th>
<th>Periimplant tissue</th>
<th>p value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip/knee arthroplasties</td>
<td>Sensitivity</td>
<td>79%</td>
<td>61%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>99%</td>
<td>99%</td>
<td>Trampuz et al, NEJM 2007 Vol 357:654</td>
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<tr>
<td>Shoulder arthroplasties</td>
<td>Sensitivity</td>
<td>67%</td>
<td>55%</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>98%</td>
<td>95%</td>
<td>Piper et al, JCM 2009 Vol 47:1878</td>
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<tr>
<td>Spine implants</td>
<td>Sensitivity</td>
<td>91%</td>
<td>73%</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>97%</td>
<td>93%</td>
<td>Sampedro et al, Spine 2010 Vol 25:1218</td>
</tr>
<tr>
<td>Elbow arthroplasties</td>
<td>Sensitivity</td>
<td>89%</td>
<td>55%</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>100%</td>
<td>93%</td>
<td>Vergidis et al, JSES 2011 Vol 20;1275</td>
</tr>
<tr>
<td>Hip/knee/shoulder arthroplasties, mega-prostheses, osteosyntheses, spine implants</td>
<td>Sensitivity</td>
<td>83%</td>
<td>61%</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>95%</td>
<td>95%</td>
<td>Holinka et al. J Orthop Res 2011 Vol 29:617</td>
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<tr>
<td>Hip arthroplasties</td>
<td>Sensitivity</td>
<td>75%</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>97%</td>
<td>100%</td>
<td>Bogut et al. Polish J Microbiol 2014:63:299</td>
</tr>
<tr>
<td>Hip/knee arthroplastises, internal device</td>
<td>Sensitivity</td>
<td>77%</td>
<td>34%</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>97%</td>
<td>100%</td>
<td>Scorzolini et al. New Microbiol 2014:37:321</td>
</tr>
<tr>
<td>Fracture fixation implant (plate, screws, spine implant, intramedullary nail)</td>
<td>Sensitivity</td>
<td>90%</td>
<td>57%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>91%</td>
<td>96%</td>
<td>Yano et al. J Clin Microbiol 2014;52:4176</td>
</tr>
<tr>
<td>Hip/knee arthroplastises, tibial inserts, acetabular components, fixation devices, spinal devices, etc.</td>
<td>Sensitivity</td>
<td>90%</td>
<td>67%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>99%</td>
<td>99.5%</td>
<td>Puig-Verdie et al. Bone Joint J 2013;95-B(2):244-9</td>
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<tr>
<td>Hip/knee/shoulder/elbow arthroplasties</td>
<td>Sensitivity</td>
<td>81%</td>
<td>61%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>99%</td>
<td>100%</td>
<td>Portillo et al. J Infect 2014:69:35e41</td>
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<tr>
<td>Hip/knee arthroplastises</td>
<td>Sensitivity</td>
<td>97%</td>
<td>70%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>90%</td>
<td>97%</td>
<td>Rothenberg et al. Clin Orthop Relat Res 2017;475:1827</td>
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<tr>
<td>Modular megaprostheses</td>
<td>Sensitivity</td>
<td>91%</td>
<td>52%</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
<td>Puchner et al. J Orthop Res 2016</td>
</tr>
<tr>
<td>Test</td>
<td>Sensitivity 135 PJI</td>
<td>Specificity 231 Aseptic Failure</td>
<td>Accuracy</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------</td>
<td>--------------------------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% (95% Confidence Interval)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue culture</td>
<td>70.4 (64.5-76.3)</td>
<td>98.7 (97.2-100)</td>
<td>88.3 (84.2-92.4)</td>
<td></td>
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<tr>
<td>Sonicate fluid culture</td>
<td>72.6 (66.8-78.4)</td>
<td>98.3 (96.6-100)</td>
<td>88.8 (84.7-92.9)</td>
<td></td>
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<tr>
<td>Sonicate fluid broad-range PCR</td>
<td>70.4 (64.5-76.3)</td>
<td>97.8 (95.9-99.7)</td>
<td>87.7 (83.5-91.9)</td>
<td></td>
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<tr>
<td>Combination of two tests above</td>
<td>83.0 (78.2-87.8)</td>
<td>95.7 (93.1-98.3)</td>
<td>91.0 (87.3-94.7)</td>
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<tr>
<td>Sonicate fluid culture plus PCR</td>
<td>78.5 (73.2-83.8)</td>
<td>97.0 (94.8-99.2)</td>
<td>90.2 (86.4-94.0)</td>
<td></td>
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<tr>
<td>Synovial fluid culture</td>
<td>64.7 (56.5-72.9)</td>
<td>96.9 (93.9-99.9)</td>
<td>84.1 (77.8-90.4)</td>
<td></td>
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<tr>
<td>Sonicate fluid PCR - lower cutoff (CP &lt;27.59 cycles)</td>
<td>80.0 (74.8-85.2)</td>
<td>90.9 (87.2-94.6)</td>
<td>86.8 (82.5-91.3)</td>
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</table>
## Sonication Studies – PJI PCR Panel

### Hip/Knee Prostheses

<table>
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<th>Test</th>
<th>Aseptic failure (290)</th>
<th>PJI (144)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tr>
<td></td>
<td>No. of patients with positive specimens</td>
<td>% (95% confidence interval)</td>
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<td>Synovial-fluid culture</td>
<td></td>
<td>5/161</td>
<td>59/89</td>
<td>66.3 (55.5-76.0)</td>
<td>96.9 (92.9-99.0)</td>
<td>92.2 (82.7-97.4)</td>
</tr>
<tr>
<td>Tissue culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any growth</td>
<td></td>
<td>45</td>
<td>119</td>
<td>82.6 (75.4-88.4)</td>
<td>84.5 (79.8-88.5)</td>
<td>72.6 (65.1-79.2)</td>
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<tr>
<td>≥2 positive tissues (same organism)</td>
<td></td>
<td>6</td>
<td>101</td>
<td>70.1 (62.0-77.5)</td>
<td>97.9 (95.6-99.2)</td>
<td>94.4 (88.2-97.9)</td>
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<tr>
<td>Sonicate fluid culture</td>
<td></td>
<td>5</td>
<td>105</td>
<td>72.9 (64.9-80.0)</td>
<td>98.3 (96.0-99.4)</td>
<td>95.5 (89.7-98.5)</td>
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<tr>
<td>Sonicate fluid PCR (10 assay panel)</td>
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<tr>
<td>Any positive result</td>
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<td>6</td>
<td>111</td>
<td>77.1 (69.3-83.7)</td>
<td>97.9 (95.6-99.2)</td>
<td>94.9 (89.2-98.1)</td>
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<td><em>Staphylococcus</em> sp</td>
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<td>2</td>
<td>75</td>
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<td><em>S. aureus</em></td>
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<td>0</td>
<td>28</td>
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<td>Coagulase-negative staphylococci</td>
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<td>47</td>
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<td><em>Streptococcus</em> sp</td>
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<tr>
<td><em>Enterococcus/Granulicatella/Abiotrophia</em> sp</td>
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<td>0</td>
<td>11</td>
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<td>Enterobacteriaceae</td>
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<td>Gram-positive anaerobic cocci</td>
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<td>0</td>
<td>8</td>
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<tr>
<td><em>Cutibacterium</em> sp</td>
<td></td>
<td>0</td>
<td>8</td>
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<td><em>P. aeruginosa</em></td>
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<td>0</td>
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<td><em>Corynebacterium</em> sp</td>
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<td><em>C. jeikium/urealyticum</em></td>
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<tr>
<td>Non-jeikeium sp</td>
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<td>0</td>
<td>4</td>
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<td><em>Proteus</em> sp</td>
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<td><em>B. fragilis</em> group</td>
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<td>0</td>
<td>0</td>
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</table>
Metagenomic Shotgun Whole Genome Sequencing

Sequence ALL DNA present using short 150-300 bp reads
Evaluation of Arthroplasty Failure Using Metagenomic Whole Genome Shotgun Sequencing

• Goals:
  • **Improve sensitivity**
    • Difficult to culture organisms
    • Fastidious organisms
    • Recent antibiotic exposure
  • Polymicrobial infections
    • Identify subpopulations within species
    • Identify important genes
    • Antibiotic resistance markers
    • Virulence factors

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Comparison of Microbial Enrichment Methods
Metagenomic Analysis of Arthroplasty Failure

DNA Extraction

NEBNext Microbiome DNA Enrichment

DNA Extraction

DNA for Next Generation Sequencing

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Comparison of Microbial Enrichment Methods
Metagenomic Analysis of Arthroplasty Failure

• DNA extraction: Mobio BIOstic Bacteremia DNA isolation kit
• Whole genome amplification: Illustra GenomiPhhi V2 kit
• Amplified DNA purification: Agencourt AMPure XP beads
• Paired-end library prep: NEBNext Ultra DNA Library Prep Kit
• Sequencing: Illumina HiSeq 2500 in rapid run mode with 2 x 250 bp reads (multiplexed 6 up to samples/lane)
• Adapter sequence removal: Trimmomatic (v0.35)
• Human & PhiX sequence removal: BioBloom tools (v2.0.12)
• Data analysis: Livermore Metagenomics Analysis Toolkit (LMAT) with kML + Human.v4.14.20.g10.db database
## Comparison of Microbial Enrichment Methods
### Metagenomic Analysis of Arthroplasty Failure

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No Enrichment</th>
<th>NEBNext Microbiome DNA Enrichment</th>
<th>MolYsis Enrichment</th>
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<tbody>
<tr>
<td><strong>S. aureus PJI</strong></td>
<td></td>
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<tr>
<td>% of reads</td>
<td>0.02%</td>
<td>0.2%</td>
<td>7.7%</td>
</tr>
<tr>
<td>(4,158 of 25,609,460)</td>
<td>(350,625 of 169,981,133)</td>
<td>(2,286,890 of 29,530,730)</td>
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<tr>
<td>Enrichment factor</td>
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<td>13X</td>
<td>481X</td>
</tr>
<tr>
<td><strong>S. epidermidis PJI</strong></td>
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<td></td>
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</tr>
<tr>
<td>% of reads</td>
<td>0.007%</td>
<td>0.2%</td>
<td>7.0%</td>
</tr>
<tr>
<td>(1,682 of 23,606,476)</td>
<td>(133,680 of 74,544,475)</td>
<td>(2,268,087 of 32,184,381)</td>
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</tr>
<tr>
<td>Enrichment factor</td>
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<td>25X</td>
<td>986X</td>
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<tr>
<td><strong>E. faecalis PJI</strong></td>
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</tr>
<tr>
<td>% of reads</td>
<td>0.006%</td>
<td>0.5%</td>
<td>59.4%</td>
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**S. aureus PJI**

**No Enrichment**

**NEBNext Enrichment**

**Molyysis Enrichment**

**Read Score** | **# of Reads** | **TaxID** | **Taxonomy group** | **Organism** | **Rank**
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Host DNA Depletion Depletes DNA Quantities for Sequencing

Vortex + Sonication

MolYsis Human Cell Lysis

DNA Extraction

Data Analysis
- Identify pathogens
- Antibiotic resistance genes
- Subpopulations

Next Generation Sequencing (HiSeq 2500, 2 x 250bp)

Whole Genome Amplification

© 2017 Mayo Foundation for Medical Education and Research
Impact of Contaminating DNA in Whole Genome Amplification Kits on PJI Diagnosis

- 8 sonicate fluids, including culture-positive and –negative PJI, and aseptic failure
- Microbial DNA enrichment: MolYsis Basic5 kit
- DNA extraction: MoBio Bacteremia DNA isolation kit
- Whole genome amplification:
  1. Qiagen REPLI-g Single Cell kit (Qiagen, Hilden Germany)
  2. Illustra V2 Genomiphi kit (GE Healthcare Bio-Sciences, Pittsburgh PA)
  3. Illustra Single Cell Genomiphi kit
- Amplified DNA purification: Agencourt AMPure XP beads
- Paired-end library preparation: NEBNext Ultra DNA Library Prep Kit
- Sequencing: Illumina HiSeq 2500 rapid run mode, 2x250 bp reads (multiplexed 6 samples/lane)
- Controls (no WGA) - library preparation with NEBnext Ultra II DNA library Prep Kit (4 samples/lane)
- Illumina adapters removal: Trimmomatic
- Human and PhiX sequences prefiltration: BioBloom tools
- Taxonomic assignment of reads: LMAT with kML + Human.v4.14.20.g10.db database
- Trimming of low-quality reads: Trimmomatic
- Analysis of trimmed reads: MetaPhlAn2

## Sonicate Fluid Samples Tested and Relative Read Counts (LMAT)

<table>
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<tr>
<th>Sample</th>
<th>Site</th>
<th>Microbiology</th>
<th>Qiagen REPLI-g</th>
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### Culture-positive PJI

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#### 980 H
- **Group C Streptococcus**<20 CFUs
  - 1 of 3
  - Total reads: 27,244,012
  - Pathogen reads: 2,694,906
  - Non-pathogen reads: 103
  - Total reads: 36,576,986
  - Pathogen reads: 286,577
  - Non-pathogen reads: 556
  - Total reads: 25,754,214
  - Pathogen reads: 3,075,181
  - Non-pathogen reads: 566

#### 982 K
- **S. epidermidis**>100 CFUs
  - 3 of 4
  - Total reads: 28,320,234
  - Pathogen reads: 3,272,895
  - Non-pathogen reads: 591
  - Total reads: 30,284,116
  - Pathogen reads: 102,934
  - Non-pathogen reads: 203
  - Total reads: 26,508,366
  - Pathogen reads: 9,539,147
  - Non-pathogen reads: 17,625

#### 986 K
- **S. aureus**<20 CFUs
  - 1 of 5
  - Total reads: 26,835,306
  - Pathogen reads: 26,397
  - Non-pathogen reads: 77,581
  - Total reads: 28,991,289
  - Pathogen reads: 424
  - Non-pathogen reads: 308
  - Total reads: 168,046
  - Pathogen reads: 27,391,148
  - Non-pathogen reads: 240,703

#### 996 H
- **B. fragilis**, >100 CFUs
  - 3 of 3
  - Total reads: 28,388,841
  - Pathogen reads: 155,761
  - Non-pathogen reads: 4,853
  - Total reads: 28,745,844
  - Pathogen reads: 11,134
  - Non-pathogen reads: 1,346
  - Total reads: 96,154
  - Pathogen reads: 8,140
  - Non-pathogen reads: 8,140

#### 1002 K
- **C. striatum**, 51-100 CFUs
  - 3 of 3
  - Total reads: 32,165,206
  - Pathogen reads: 32,165,206
  - Non-pathogen reads: 27,925,551
  - Total reads: 32,338,186
  - Pathogen reads: 536,155
  - Non-pathogen reads: 2,594
  - Total reads: 1,000,810
  - Pathogen reads: 53,019
  - Non-pathogen reads: 53,019

### Culture-negative PJI

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#### 984 K
- **Culture negative**
  - 0 of 4
  - Total reads: 31,616,449
  - Pathogen reads: 31,616,449
  - Non-pathogen reads: 26,240,307
  - Total reads: 36,808,230
  - Pathogen reads: 305
  - Non-pathogen reads: 16,974
# Sonicate Fluid Samples Tested and Relative Read Count (LMAT)

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## MetaPhlAn2 Identification of Species

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β-Diversity of gene content of the samples was determined using QIIME and plotted on a principal coordinate analysis plot. Samples are colored according to the WGA kit used for amplification (A) or by template sample source (B). NT no template.
53 yo Man – Right Knee PJI

A. R knee arthroplasty
Knee pain, stiffness, swelling, and sinus tract development
Multiple courses of oral antibiotics
Referral to Mayo Clinic for evaluation
6 weeks IV antibiotics
R knee arthroplasty reimplantation
Sinus tract development

Nov 2012
April 2013
2014
July 2015
Feb 2016
April 2016
July 2016
April 2017

Synovial fluid: 28 756 cells/mm³ (93% PMNs), cultures negative, ESR: 53, CRP: 71.6
Synovial fluid: 2288 cells/mm³ (80% PMNs), cultures negative. PCR for M. hominis negative, ESR: 40, CRP: 93.5
ESR: 44, CRP: 51.3
Metagenomic analysis of Feb 2016 sonicate fluid positive for M. salivarium
Synovial fluid: 11 596 cells/mm³ (94% PMNs). 16S rRNA gene PCR/sequencing positive for M. salivarium

B. All Reads (27 984 652)
Microbial Reads (1906)
Bacterial Reads (1881)

- Human (27 049 593)
- Low LMAP Score (812 657)
- Microbial (1906)
- No Database Hits (16 796)
- Chimeras (420)
- Cellular Organism (15 412)

- Bacteria (1881)
- Protozoa (14)
- Fungi (11)
- Viruses (0)
- Mycoplasma (1796)
- Acinetobacter (58)
- Propionibacterium (19)
- Rubrivivax (1)
- Paenibacillus (4)

C. All Reads (234 088 137)
Microbial Reads (21 081)
Bacterial Reads (20 914)

- Human (228 541 886)
- Read Too Short (395 776)
- Low LMAP Score (2 763 397)
- Microbial (21 081)
- No Database Hits (883 550)
- Chimeras (38 803)
- Cellular Organism (24 395)
- PhiX (1 419 249)

- Bacteria (20 914)
- Protozoa (144)
- Fungi (20)
- Viruses (3)
- Mycoplasma (19 642)
- Propionibacterium (371)
- Pseudomonas (247)
- Corynebacterium (86)
- Arthrobacter (73)
- Staphylococcus (36)
- Streptococcus (35)
- Burkholderia (33)
- Ralstonia (35)
- Other Bacteria (356)
Antibiotic Resistance Prediction

Macrolide resistance-associated mutations in 23S rRNA gene of *M. pneumoniae*

Alignment of case to reference *M. salivarium* and *M. pneumoniae* 23S rRNA genes


Correct susceptibility inferred
94% monomicrobial
77% polymicrobial samples
Conclusions

• Metagenomic analysis of bone and joint infection promising
  – Culture-negative cases
  – Specimen type matters
  – Need for specific reagents/protocols/bioinformatics tools for bone and joint infection, especially prosthetic joint infection
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