RNA-seq profiling of pathogens in prosthetic joint infection

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**Introduction**
Prosthetic joint infections (PJIs) are serious complications of joint alloplasties. These device-associated infections are typically caused by bacteria growing in biofilms, which protect them from antimicrobial agents and host immune responses in poorly vascularized joints. Our current knowledge of how pathogens cope with the complex conditions within the host is based on *in vitro* studies and animal models, which can differ substantially from their behavior in the human host. Studying gene expression of pathogens during infection is a way to understand pathogenesis. RNA sequencing (RNA-seq) is capable of characterizing the entire transcriptome, both quantitatively and qualitatively, of an organism.

**Aim**
To study the gene expression of pathogens directly in PJI in a human host using RNA-seq.

**Methods**

**Identification**

- Synovial fluid
- 16S rRNA gene cloning
- 16S rRNA gene amplicon sequencing
- S. aureus N315, 2.84 Mb, 2694 genes, 2613 proteins

**RNA-seq**

- Total RNA extraction RiboPure® Bacteria Kit
- Human RNA removal MICROB Express Kit
- Bacterial rRNA removal RiboPure® Bacterial rRNA Removal Kit
- Sequencing library preparation TruSeq® RNA Sample Preparation
- Illumina paired-end sequencing
- Bioinformatics analysis

**Results**

**Identification**
Monomicrobial infection: *Staphylococcus aureus* was identified by 3 independent methods (culture, 16S rRNA gene cloning, and 16S rRNA gene amplicon sequencing).

**Mapping statistics**

<table>
<thead>
<tr>
<th></th>
<th>Number of reads</th>
<th>Number of paired reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total reads</td>
<td>23,000,000</td>
<td>11,500,000</td>
</tr>
<tr>
<td>S. aureus reads</td>
<td>270,000 (1.17%)</td>
<td>113,000 (0.98%)</td>
</tr>
<tr>
<td>S. aureus mRNA reads</td>
<td>17,000 (0.074%)</td>
<td>7,000 (0.061%)</td>
</tr>
</tbody>
</table>

*Reference genome: S. aureus N315, 2.84 Mb, 2694 genes, 2613 proteins*

**Top 100 genes (ordered by RPKM)**
- 52 Essential genes
- 22 Hypothetical proteins
- 8 Infection related genes

**Virulence factors:**
- *hlgA, hlgB* and *hlgC*: form pores in immune cells
- *Sbi*: interferes with the host complement system

**Virulence regulation genes:**
- *saeS* and *saeR*

**Vancomycin resistance sensor/regulator:**
- *vraS* and *vraR*

**Acknowledgement**
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www.joint-prosthesis-infection-pain.dk