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

RNA-seq

1. Total RNA extraction (RiboPure® Bacteria Kit)
2. Human RNA removal (MICROB Express® Human RNA Removal Kit)
3. Bacterial rRNA removal (ACTQuick® rRNA Removal Kit)
4. Sequencing library preparation (TruSeq® RNA Sample Preparation Kit)
5. Illumina paired-end sequencing
6. Bioinformatics analysis

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><em>S. aureus</em> reads</td>
<td>270,000 (1.17%)</td>
<td>113,000 (0.98%)</td>
</tr>
<tr>
<td><em>S. aureus</em> mRNA reads</td>
<td>17,000 (0.074%)</td>
<td>7,000 (0.061%)</td>
</tr>
</tbody>
</table>

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