RNA-seq profiling of pathogens in prosthetic joint infection

Xu, Yijuan; Nielsen, Kåre Lehmann; Nielsen, Per Halkjær; Nielsen, Jeppe Lund; Thomsen, Trine Rolighed

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Xu, Yijuan; Nielsen, Kåre L.; Nielsen, Per H.; Nielsen, Jeppe L.; Thomsen, Trine R.
1: Center for Microbial Communities, Aalborg University, Denmark
2: Danish Technological Institute, Denmark

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
- Mono/microbial 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>
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<tbody>
<tr>
<td>Total reads</td>
<td>23,000,000</td>
<td>11,500,000</td>
</tr>
<tr>
<td>S. aureus reads¹</td>
<td>270,000 (1.17%)</td>
<td>113,000 (0.98%)</td>
</tr>
<tr>
<td>S. aureus mrNA reads</td>
<td>17,000 (0.74%)</td>
<td>7,000 (0.061%)</td>
</tr>
</tbody>
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¹Reference 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
- Agr quorum sensing signal

Vancomycin resistance sensor/regulator:
- vraS and vraR

Acknowledgement
The study is part of the Danish “Prosthesis-related Infection and Pain” innovation Project, supported by a grant from the Danish Agency of Science and Technology (no. 09-052174).
www.joint-prosthesis-infection-pain.dk

Conclusions
This study indicates that RNA-seq is a challenging but powerful tool to profile the gene expression of pathogens in vivo.