Comparison of broad-range 16S rDNA PCR with blood culture in diagnosis of bloodstream infections

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Introduction: Rapid identification of causative agents of bloodstream infection (BSI) is one of crucial needs of today’s microbiology. A delay in the start of targeted antimicrobial therapy is associated with a decrease in survival of 7.6% with each hour after the start of BSI-associated hypotension1. As blood cultures are found to be positive in only 30-40% of patients with serious BSI, alternative tests with a higher sensitivity are required to ensure timely targeted treatment and/or de-escalation of empiric antibiotic therapy. For nucleic acid amplification techniques (NAAT), the greatest obstacles in BSI diagnostics seem to be low starting volume of blood and an excess of interfering human DNA.

Objectives of the study: To compare the performance of conventional blood culture (BC) and commercially available broad-range 16S rDNA PCR followed by sequencing (16S PCR).

Material and Methods:
482 blood samples (330 pts) with suspected BSI hospitalized at:
• Department of Internal Medicine (IM) – 257 samples (207 pts)
• Department of Paediatric Haematology and Oncology (MOD) – 225 samples (123 pts)

BC (BACTEC™ FX, Beckton Dickinson, USA) at least one pair aerobic/anaerobic
BC bottles per episode

Clinical significance of positive broad-range PCR findings results was evaluated based on:
• Agreement with blood culture result
• Patient’s medical history
• Additional patient’s microbiological findings (pre- and post-study testing, when available)
• Opinion of clinician taking care of the patient

Table 1: Comparison of 16S PCR and Blood culture results

<table>
<thead>
<tr>
<th>Sample</th>
<th>16S PCR (UMD SelectNA, Molzym, Germany)</th>
</tr>
</thead>
<tbody>
<tr>
<td>482 blood samples</td>
<td>330 pts with suspected BSI</td>
</tr>
<tr>
<td>BC</td>
<td>at least one pair aerobic/anaerobic BC bottles per episode</td>
</tr>
</tbody>
</table>

Table 2: Analytical parameters of 16S PCR

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.781</td>
<td>0.835</td>
</tr>
<tr>
<td>0.558</td>
<td>0.934</td>
</tr>
</tbody>
</table>

Results:

Conclusions: The NAAT-based approach was applied to 5-10 ml of blood samples after depletion of human DNA which was expected to improve the diagnostic value of the method. The 16S PCR and the culture provided concordant results in 347 of 482 samples. In addition, the 16S PCR detected a new or additional relevant organism in 36 (7.5%) samples, while in 23 (4.8%) more samples it failed and was regarded to be false negative. Therefore, the NAAT-based method seems to be worth considering to complete the BSI diagnostics, but negative PCR results are requested to be carefully checked by blood culture.

Acknowledgements: Supported by Ministry of Health of the Czech Republic, grant no. AZV15-28157A. All rights reserved.

References:

Table 3: Analysis of discordant results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added value of 16S PCR+ results (%)</th>
<th>16S PCR failureb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOD Children</td>
<td>61 (6.7)</td>
<td>6 (2.7)</td>
</tr>
<tr>
<td>IM</td>
<td>74 (21 (8.2)</td>
<td>17 (6.6)</td>
</tr>
<tr>
<td>Total</td>
<td>135 (36 (7.5)</td>
<td>23 (4.8)</td>
</tr>
</tbody>
</table>

a Antibiological agent of BSI detected only by 16S PCR
b False negative 16S PCR results

Czechia

Concordant results

Concordant results

Correctly

Positive

Antibiological agent of BSI detected only by 16S PCR

False negative 16S PCR results

In 10 of 23 samples presence of multiple organisms

Sample DNA extraction PCR amplification

16S panbacterial PCR (18S panfungal PCR)

Gram – Gram + (Fungi)

Database search

Sanger/SyberGreen based qPCR followed by HRM analysis

Positive result

Human cell lysis and depletion of human and free DNA

Detection of panbacterial and panfungal 16S rDNA

Sample of DNA is depleted of human DNA

Antibacterial agent of BSI detected only by 16S PCR

False negative 16S PCR results

In 10 of 23 samples presence of multiple organisms

16S PCR+ results

16S PCR+ results

Concordant results

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16S PCR+ results

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