Evaluation of Real-time PCR for Atypical pneumonia

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Abstract
Atypical pneumonia is caused by Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella species which are fastidious bacteria and are treated by antibiotics different from those causing pneumonia. Genetic detection is preferable to culture and antibody detection. This study aimed to evaluate triplex SYBR green real-time, a method using melting curve analysis and probe-based multiplex real-time PCR. Specificity was determined using 63 pathogenic bacterial strains and detection limit was also carried out. The results showed 100% specificity in both methods. Detection limit for diagnosis of M. pneumoniae, C. pneumoniae, and Legionella species using triplex SYBR green real-time was 0.003, 0.01, and 0.02 CFU/assay, respectively, whereas probe-based multiplex real-time PCR showed 11.96, 36 and 6.27 copies/assay, respectively. Overall comparison, triplex SYBR green real-time is useful for screening and probe-based multiplex real-time PCR is appropriate for confirmation of atypical pneumonia.

Background
Atypical pneumonia is caused by Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella species which are fastidious bacteria. Patients have prior symptoms of fever, chill, myalgia similar those caused by other bacterial pneumonia. However, macrolides treatment of atypical pneumonia is not generally drug of choice for bacterial pneumonia. Rapid diagnosis such as genetic detections are preferable and reduces dramatically mortality of patients with progressive severity. This study evaluated triplex SYBR green real-time, a method using melting curve analysis and probe-based multiplex real-time PCR used to detect atypical pneumonia.

Methods
Triplex SYBR Green real-time PCR: This real-time PCR was done according to Kerdsin et al., 2010.

Probe-based multiplex real-time PCR: This real-time PCR was performed as described by Thurman et. al., 2011

Specificity, Detection limit, and Sensitivity. These parameters were analyzed by flowchart shown below.

Results

Result of Triplex SYBR Green real-time PCR detection of atypical pneumonia pathogens by melting-curve analysis

Result of probe-based multiplex real-time PCR detection of atypical pneumonia pathogens by Ct value analysis

Comparison of specificity, sensitivity, detection limit, and cost between triplex SYBR Green real-time PCR and Probe-based multiplex real-time PCR

<table>
<thead>
<tr>
<th>Method</th>
<th>Organism</th>
<th>Specificity (%)</th>
<th>Sensitivity</th>
<th>Detection Limit</th>
<th>Cost/test (baht)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triplex SYBR green real-time</td>
<td>C. pneumoniae</td>
<td>100</td>
<td>0.002 CFU/assay</td>
<td>0.003 CFU/assay</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>M. pneumoniae</td>
<td>100</td>
<td>0.02 CFU/assay</td>
<td>0.01 CFU/assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. pneumophila</td>
<td>100</td>
<td>0.02 CFU/assay</td>
<td>0.02 CFU/assay</td>
<td></td>
</tr>
<tr>
<td>Probe-based multiplex real-time PCR</td>
<td>C. pneumoniae</td>
<td>100</td>
<td>176 copies</td>
<td>36 copies</td>
<td>1400</td>
</tr>
<tr>
<td></td>
<td>M. pneumoniae</td>
<td>100</td>
<td>232 copies</td>
<td>11.96 copies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. pneumophila</td>
<td>100</td>
<td>3890 copies</td>
<td>6.27 copies</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion
Overall comparison, triplex SYBR green real-time is useful for screening a large numbers of specimen and probe-based multiplex real-time PCR is appropriate for confirmation of infections caused by C. pneumoniae, M. pneumoniae and L. pneumophila. Our study have implications to propose real-time PCR assay as a rapid and effective tool for detection of atypical pneumonia.

References