Rapid detection of carbapenemase-producing Enterobacteriaceae directly from positive blood cultures by a new immunochromatographic assay

Axel Hamprecht a,b, Harald Seifert a,b and Ahmad Saleh a,b

aInstitute for Medical Microbiology, Immunology and Hygiene, University Hospital of Cologne, Cologne, Germany; bDZIF (German Centre for Infection Research), partner site Cologne-Bonn; presenting author

Background
- Bloodstream infections caused by carbapenemase-producing Enterobacteriaceae (CPE) associated with treatment failure and increased mortality
- Detection of CPE from blood cultures (BC) by standard methods takes 20-96 hours

Objectives
- to develop and evaluate a protocol for the rapid detection of carbapenemases directly from positive BC using a new multiplex immunochromatographic test (ICT)

Methods
- Blood cultures spiked with Enterobacteriaceae and incubated in a BD Bactec FX system until positive
- 170 molecularly characterized clinical isolates
  - K. pneumoniae (N=84), E. coli (N=53), F. tularensis (N=15), E. aerogenes (N=6), C. freundii (N=5), other species (N=7)
  - 126 CPE
    - 79 OXA-48-like, 18 KPC, 29 NDM
  - 44 carbapenemase negative isolates
- Blood from positive BC bottles was hemolyzed, bacteria concentrated by centrifugation and lysed
- Lysate was transferred to the RESIST-3 O.K.N. ICT® (Coris BioConcept, Gembloux, Belgium), which detects OXA-48-like, KPC and NDM (Fig. 1)

Results
- Without pretreatment, results cannot be easily determined because of red background
- After pretreatment with SDS, all carbapenemases can be easily identified (Fig. 2)
- Sensitivity and specificity 100% (Table 1)
- Easy and rapid protocol, time to result 20-30 min

Figure 1. Workflow for the preparation of ICT directly from positive blood cultures; SDS: sodium dodecyl sulfate; PBS: phosphate buffered saline

Figure 2. Result of the ICT from blood cultures

<table>
<thead>
<tr>
<th>Carbapenemase</th>
<th>positive tests/total isolates</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-48-like</td>
<td>79/79</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>OXA-48</td>
<td>54/54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-181</td>
<td>8/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-232</td>
<td>7/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-162</td>
<td>7/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-48-like/NDM</td>
<td>3/3</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity and specificity of the ICT from blood cultures

Conclusion
- OXA-48-like, KPC and NDM carbapenemases can be reliably detected directly from positive BC bottles with the new protocol
- More rapid than other currently available assays
- Can be performed in any routine microbiology laboratory
- Can help to rapidly identify patients with CPE BSI and early optimize treatment

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Contact Information
Axel Hamprecht, Institute for Medical Microbiology, Immunology and Hygiene, University Hospital Cologne, Baldiehstrasse 19-21, D-50935 Cologne, Germany; Tel. (+49) 221-478 32160; e-mail: axel.hamprecht@uk-koeln.de