Evaluation of a new multiplex immunochromatographic assay OKN K-SeT for the rapid detection of OXA-48, KPC and NDM carbapenemases from cultured bacteria

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Introduction

• Accurate and timely detection of CPE is essential for patient management and for rapid implementation of appropriate infection control measures.
• Recently, lateral flow assays based on monoclonal antibodies generated by immunization in mice have been developed for easy and rapid detection of OXA-48-like and KPC carbapenemases.

This technology was first shown by our group2 and subsequently confirmed by several other investigators in different countries as a powerful means (100% sensitivity and 100% specificity) to identify OXA-48 and KPC producers within 15 minutes directly from bacterial colonies but as single confirmatory test.

The present study aimed to evaluate the performance of a new triplex assay (OKN K-SeT) which incorporates specific antibodies against OXA-48, NDM and KPC to detect these three carbapenemases in a single test from culture colonies.

Methods

• Retrospective study: 200 collection isolates of Enterobacteriaceae with characterized β-lactamases collected at the French associated NRC (period 2008-2014) including 60 non CPE (mostly ESBLs or AmpC-/- impermeability) and 140 CPE producers (see Figure 1a and Table 1). All isolates were verified before testing for the presence of carbapenemases) by an updated version of the Carba NP test; PCR/sequencing of β-lactam genes was used as reference gold standard.

• Prospective analysis (2 months in 2016): 183 non-duplicate clinical isolate with decreased susceptibility or resistance to carbapenems (meropenem and/or imipenem) referred to the Belgian NRC as putative CPE (Fig. 1b, Table 2).

• All isolates were verified for the presence of carbapenemase by BVG Carba v2.0.

• Two in house multiplex PCR assays (ISO15189 certified) targeting the major carbapenemases were performed on all isolates. Amplicons were sequenced using external sequencing services for allele identification (Macrogen Inc., South Korea).

• The OKN K-SeT assay (Coris BioConcept, Gentilhouse, Belgium) was used according to the recommendations of the manufacturer. All strains tested were grown on trypticase soy agar supplemented with 5% sheep blood (bioMérieux, Marcy l’Etoile, France) for 16-24 h at 37°C.

Table 1. Description of the retrospective collection panel

<table>
<thead>
<tr>
<th>β-lactamase content Species</th>
<th>N° of isolates</th>
<th>OKN K-SeT assay</th>
<th>Carba NP assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-48, KPC, NDM</td>
<td>200</td>
<td>200</td>
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<tr>
<td>OXA-48, KPC</td>
<td>200</td>
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Table 2. Results of the OKN K-SeT assay in the prospective study phase

Results I

Table 1. Results of the OKN K-SeT assay for the detection of OXA-48 like, KPC and NDM carbapenemases

Prospective study part:

• 100% sensitivity and specificity for the detection of OXA-48 like, KPC and NDM carbapenemases

• 100% positive predictive value and 100% negative predictive value for the detection of these three carbapenemase families

• Rapid detection of 97 carbapenemases out of 109 (98%) in 107 CPE isolates

• Additional confirmatory tests would have been required only for the detection of 12 CPE isolates (Table 2)

• The multiplex OKN assay allows rapid, direct identification from culture colonies of 90% of the CPE in countries such as Belgium and France

Conclusions

• Globally, the performance of the OKN K-SeT assay reached 100% sensitivity and 100% specificity for the detection of OXA-48 like, KPC and NDM carbapenemases

• The OKN K-SeT assay did correctly detect isolates that contained a combination of different carbapenemases.

• In the prospective study, the OKN assay allowed the detection of 97 of the 109 carbapenemases (98%), and yielded a positive and a negative predictive value of 100% for the three targeted carbapenemases.

• The assay is efficient and very easy to implement in the workflow of a clinical microbiology laboratory for the confirmation of OXA-48, NDM and KPC carbapenemases.

• The OKN K-SeT assay test represents a powerful diagnostic tool as it enables the rapid detection (<15 minutes) from culture colonies of three among the most important carbapenemase families without the need of more costly and less frequently available molecular assays.

References


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