Detection of carbapenemase-producing Enterobacteriaceae with a commercial DNA microarray

James Cohen Stuart, Guido Voets, Jelle Scharringa, Ad C. Fluit and Maurine A. Leverstein-Van Hall

The Check-MDR CT102 DNA microarray enables detection of the most prevalent carbapenemases (NDM, VIM, KPC, OXA-48 and IMP) and extended-spectrum β-lactamase (ESBL) gene families (SHV, TEM and CTX-M). The test performance of this microarray was evaluated with 95 Enterobacteriaceae isolates suspected of being carbapenemase producers, i.e. with meropenem MICs ≥0.5 mg l⁻¹. The collection of isolates contained 70 carbapenemase-producing isolates, including 37 blaKPC-, 20 blaVIM-, five blaOXA-48-, four blaKPC/blaVIM- and four blaNDM-positive isolates; and 25 carbapenemase-gene-negative isolates. ESBLs were produced by 51 of the isolates. PCR and sequencing of β-lactamase genes was used as reference test. For detection of carbapenemases, the sensitivity of the microarray was 97 % (68/70), with 100 % specificity. The two negative isolates tested positive when the microarray test was repeated; these isolates were an OXA-48- and a KPC-producing isolate. For ESBL detection, the sensitivity was 100 % (51/51) and the specificity was 98 % (43/44), although 20 % of the SHV-12 ESBLs were categorized as SHV-2-like ESBLs. In conclusion, the CDT102 microarray is a rapid and accurate tool for the detection of carbapenemase and ESBL genes, although the array seems less suitable for epidemiology of ESBL genes.

INTRODUCTION

Carbapenemase-producing Enterobacteriaceae are an emerging problem worldwide. Rapid and accurate detection of carbapenemase-producing strains is pivotal for adequate antibiotic therapy and infection control, especially in an outbreak setting.

Phenotypic detection of carbapenemases in Enterobacteriaceae has several disadvantages. First, the two most frequently used confirmation tests, the modified Hodge test and the carbapenemase inhibition test, require overnight incubation and do not provide information on the carbapenemase gene. Second, the modified Hodge test is difficult to interpret and has limited specificity because extended-spectrum β-lactamase (ESBL)- and/or AmpC-producing isolates with decreased permeability may give false positive results (Pasteran et al., 2009, 2010), and a low sensitivity has been reported for detection of NDM-producing isolates (Girlich et al., 2012). Third, although the carbapenemase inhibition tests with boronic acid derivatives/cloxacillin and DPA/EDTA are sensitive and specific for detection of Ambler class A and B carbapenemases (Giske et al., 2011; Tsakris et al., 2010), respectively, the available data are mainly from VIM- and KPC-producing isolates. Fourth, the carbapenemase inhibition tests cannot differentiate between ESBL- and AmpC-producing isolates with decreased permeability and OXA-48 carbapenemase, which is encoded by an emerging carbapenemase gene and has recently been implicated in numerous outbreaks in several regions in the world (Cuzon et al., 2011; Pitart et al., 2011; Goren et al., 2011; O’Brien et al., 2011).

Because of the limitations of the phenotypic carbapenemase confirmation tests, genotypic detection of carbapenemase genes is the gold standard, although this only detects a prespecified set of known carbapenemase genes. The Check-MDR CT102 microarray (Check points Health BV) has been designed for detection of genes encoding NDM, KPC, VIM, IMP and OXA-48 (Naas et al., 2011; Woodford et al., 2011), which are currently the most prevalent carbapenemases (Cohen Stuart & Leverstein-Van Hall, 2010). In addition, this microarray enables detection of the most clinically relevant ESBL gene families, i.e. blaCTX-M, blaSHV and blaTEM. The main
goal of this study was to determine the test characteristics of this microarray for the detection of carbapenemase genes.

METHODS

The ability of the Check-MDR CT102 microarray to detect carbapenemase genes was evaluated in 95 well-characterized non-duplicate Enterobacteriaceae isolates, which had a meropenem MIC of $\geq 0.5$ mg l$^{-1}$, and, as such, were suspected of being carbapenemase producers (Cohen Stuart & Leverstein-Van Hall, 2010). Of these isolates, 45 were from a beta-lactamase reference centre (University Medical Centre, Utrecht, The Netherlands), collected in 2010 for detection of carbapenemase genes, 24 were from Greece, 20 were from New York, and six were ATCC or NTCC reference strains. The collection contained 66 Klebsiella pneumoniae, nine Escherichia coli and two Proteus mirabilis isolates as well as 19 Enterobacter isolates (15 E. cloacae, four E. aerogenes and one E. cancerogenus). Of the 95 isolates, 70 were carbapenemase-positive (37 KPC-2 or KPC-3, 20 VIM-1, five OXA-48, four NDM-1, four VIM-1 plus KPC-2) and 25 were carbapenemase-negative (17 ESBL and eight AmpC, one of which produced plasmid-borne CMY-2 and seven of which were chromosomal AmpC-hyperproducing E. cloacae). Of all the isolates (carbapenemase-positive and -negative), 51 (54%) harboured an ESBL gene (19 $bla_{CTX-M}$, 30 $bla_{SHV}$ and two $bla_{CTX-M}$ plus $bla_{SHV}$).

As reference test for the presence of beta-lactamase genes, PCR and sequencing were performed as described previously (Voets et al., 2011), using the Ultraclean Microbial DNA Isolation kit (Mo Bio Laboratories).

The principles of the microarray system and interpretation software have been described previously (Cohen Stuart et al., 2010). Concisely, the system combines ligation-mediated amplification with detection of amplified products on a microarray to detect the various carbapenemase genes ($bla_{OXA-48}$, $bla_{NDM}$, $bla_{AmpC}$, $bla_{VIM}$ and $bla_{KPC}$), $bla_{CTX-M}$, $bla_{SHV}$ and $bla_{KPC}$, $bla_{CTX-M}$ groups (1, 2 and 9, or combined 8/25), and the most prevalent ESBL-associated single nucleotide polymorphisms (SNPs) in $bla_{TEM}$ and $bla_{SHV}$ variants. The assay cannot provide a sequence or Lahey number (www.lahey.org/studies/) of the $bla_{TEM}$ and $bla_{SHV}$ genes (e.g. $bla_{TEM-6}$ or $bla_{SHV-2}$), but reports which group they belong to (Cohen Stuart et al., 2010). The microarray is designed to analyse three isolates at a time, which makes it costly to analyse a single isolate. The microarray assays were performed according to the manufacturer’s instructions using software version 20110215T170816R29 and including the use of two separate rooms (one room for DNA isolation and ligation, and one for amplification, hybridization and detection). The time to result of the microarray system was 8 h (3 h for DNA isolation and 5 h for amplification, hybridization and detection). To mimic a routine clinical setting, the test characteristics were based on the first test result. When there were discrepancies between the microarray result of the carbapenemase gene detection and the PCR and sequencing result, the array was repeated.

RESULTS

In all except two of the 70 carbapenemase-producing isolates, the microarray detected the correct carbapenemase gene, corresponding to a sensitivity of (68/70) 97% (Table 1). One OXA-48 producer and one KPC producer were interpreted as carbapenemase-negative by the microarray interpretation software, although visual inspection of the microarray showed a weak signal on the OXA-48 and KPC spot, respectively. The two false-negative isolates tested positive when the microarray was repeated. There were no carbapenemase false-positive results (specificity 100%). The micro-array detected all ESBL genes (sensitivity of 100%). However, one ESBL-negative (OXA-48-producing) K. pneumoniae isolate was reported as $bla_{CTX-M-1}$-positive, corresponding with a specificity and ESBL detection rate of

### Table 1. Comparison between PCR/sequencing results and microarray results

<table>
<thead>
<tr>
<th>Carbapenemase (70)</th>
<th>Number of concordant microarray results (%)</th>
<th>Number of discordant microarray results (%)</th>
<th>Microarray results of discrepant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC (37)</td>
<td>36/37 (100%)*</td>
<td>1/37 (3%)*</td>
<td>One isolate $bla_{KPC}$-negative*</td>
</tr>
<tr>
<td>NDM (4)</td>
<td>4/4 (100%)</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>VIM (20)</td>
<td>20/20 (100%)</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>OXA-48 (5)</td>
<td>4/5 (100%)*</td>
<td>1/5 (20%)*</td>
<td>One isolate $bla_{OXA-48}$-negative*</td>
</tr>
<tr>
<td>VIM plus KPC (4)</td>
<td>4/4 (100%)</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>Carbapenemase-negative (25)</td>
<td>31/31 (100%)</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>ESBLs (51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-M (19)</td>
<td>19/19 (100%)</td>
<td>0%</td>
<td>NA</td>
</tr>
<tr>
<td>SHV (30)</td>
<td>24/30 (80%)</td>
<td>6/30 (20%)</td>
<td>Six isolates SHV-2 group (G238S) instead of SHV-12 (SHV-4 group G238S, E240K)</td>
</tr>
<tr>
<td>CTX-M plus SHV (2)</td>
<td>2/2 (100%)</td>
<td>0%</td>
<td>One isolate CTX-M1 group</td>
</tr>
<tr>
<td>ESBL-negative (44)</td>
<td>43/44 (98%)</td>
<td>1/44 (2%)</td>
<td></td>
</tr>
</tbody>
</table>

*The microarray detected both carbapenemase genes in the repeated microarray test.*
43/44 (98%). In addition, 6 of the 30 SHV-12-producing isolates (20%) were reported as ESBL bla<sub>SHV-2</sub>-positive instead of belonging to the bla<sub>SHV-4</sub> group, because only the G238S substitution of the enzyme was detected in the bla<sub>SHV</sub> gene by the microarray, and not the E240K substitution. All microarray results were obtained within one working day.

**DISCUSSION**

This study shows the Check-MDR CT102 microarray has a high sensitivity and specificity for detection of carbapenemases. This is in line with two previous reports evaluating this microarray. One study, involving 144 carbapenemase-producing isolates, found sensitivity and specificity values of 100% for detection of bla<sub>VIM</sub>, bla<sub>IMP</sub>, bla<sub>NDM</sub> and bla<sub>OXA-48</sub> carbapenemase genes, whereas values of 85% and 100%, respectively, were found for bla<sub>KPC</sub> (Naas et al., 2011). Another evaluation of the microarray with 41 carbapenemase producers, reported sensitivity and specificity values of 100% (Woodford et al., 2011). However, the present study is the first to exclusively use isolates suspected of carbapenemase production because of a meropenem MIC of ≥0.5 mg l<sup>-1</sup>, i.e. a set of isolates that would require confirmation of carbapenemase production in the clinical setting.

The sensitivity and specificity values for ESBL detection were also high. However, the microarray did not detect the SHV E240K substitution in 20% of SHV-12-producing isolates, making this assay less suitable for epidemiological purposes involving SHV ESBLs. Although the latter finding was not mentioned in the two previous evaluations of this microarray (Naas et al., 2011; Woodford et al., 2011), it has been reported before (Platteel et al., 2011) in an evaluation of another microarray from the same manufacturer (Check KPC ESBL). A limitation of this evaluation is the fact that the capacity to detect TEM ESBLs or IMP carbapenemases has not been tested. Another limitation is that unknown carbapenemase genes may not have been detected by both the microarray and the reference method, i.e. PCR and sequencing.

We conclude that this microarray assay is a practical and rapid tool for detection of genes encoding NDM, OXA-48, VIM and KPC carbapenemases in a routine clinical setting.

Finally, because the microarray is designed to analyse three isolates at a time, it is costly to analyse a single isolate.

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**REFERENCES**


