Evaluation of two commercial real-time PCR assays for detection of carbapenemase genes in Enterobacteriaceae

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Abstract
The implementation of PCR for the detection of carbapenemase genes enables rapid results with significant epidemiological implications. Two commercial real-time PCR assays, the Hylabs Hy-CRE and Sacace MDR MBL+KPC/OXA, were evaluated for the detection of the genes blaKPC, blaNDM, blaOXA-48, blaVIM and blaIMP-1 carbapenemase in a collection of 96 carbapenem-resistant Enterobacteriaceae strains with different resistant mechanisms. Both assays exhibited excellent diagnostic performance, with 100% sensitivity and specificity.

The increasing prevalence of carbapenem-resistant Enterobacteriaceae (CRE) is a major clinical and public health concern [1]. Detection of carbapenemase-producing Enterobacteriaceae (CPE) is of major importance for implementing infection control measures in a timely manner [2] and possibly also in guiding antibacterial therapy. Moreover, identification of the specific resistance mechanism (i.e. the carbapenemase gene) is valuable regarding the tracing and understanding of local transmission patterns [3]. Some of the phenotypic methods that were commonly used for the above purpose, such as the Modified Hodge Test, suffer from insufficient sensitivity and specificity [4]. The newer commercial phenotypic tests derived from the CARBA NP assay, based on Nordmann’s protocol, have better analytical performance but may be influenced by factors such as the type of media [5] and are incapable of identifying the exact carbapenemase gene.

For these reasons, the use of molecular tests for the detection of the common carbapenemase genes is probably the most informative, accurate and rapid method for carbapenemase detection. Although many different carbapenemase genes have been reported, the globally predominant ones are blaKPC, blaNDM, blaOXA-48, blaVIM and the blaIMP [6]. Therefore, assays designed to tests these five genes are likely to successfully distinguish between carbapenemase-producing and -non-producing CRE in practically every country in the developed world. As a result, there is a need for reliable and affordable commercial assays and for comparative studies to evaluate their performance in routine use.

This study’s objective was to assess the diagnostic performance of two commercial real-time (RT)-PCR assays, the Hylabs Hy-CRE and the Sacace MDR MBL+KPC/OXA (Sacace MDR) tests, for the detection and differentiation of carbapenemase genes in CRE. The study aimed to evaluate the tests’ performance in terms of specificity, sensitivity, total turnaround time and hands-on time.

A collection of 96 clinical Enterobacteriaceae strains was examined in the Clinical Microbiology Laboratory, Tel Aviv Sourasky Medical Center, Israel. The strains were isolated from surveillance and clinical cultures from 2012 to 2014. The strain collection comprised 62 carbapenemase-producing CRE (KPC-11, VIM-13, NDM-17, IMP-2 and OXA-48-18) and 34 non-carbapenemase-producing CRE and included a diversity of bacterial genera of the Enterobacteriaceae family: Serratia marcescens (n=1), Raoultella planticola (n=1), Morganella morganii (n=1), Citrobacter sp. (n=7), Proteus sp. (n=3), Providencia sp. (n=8), Escherichia coli (n=24), Klebsiella sp. (n=24) and Enterobacter sp. (n=27). Co-production of NDM and OXA-48 was seen in one isolate. All of the carbapenemase-producing CRE isolates were confirmed by in-house PCR and the CARBA NP test, as previously described [3, 7]. The results of these analyses served as the reference standard for methodological comparison.

DNA was extracted directly from bacterial colonies by boiling and centrifugation. Briefly, bacteria were collected by a 1 µl loop from three colonies grown on MacConkey agar and suspended in 100 µl of Tris-EDTA (TE) buffer. The suspension was incubated at 100°C for 10 min and then...
centrifuged at 12,000 r.p.m. for 1 min. The supernatant was used for the RT-PCR reaction. The total turnaround time for DNA preparation was 15 min.

The RT-PCR tests were performed according to the manufacturer’s instructions, with minor modifications. For the Hy-CRE assay, 2 µl of each DNA extract were added to two PCR tubes containing 15 µl of the ready-to-use RT-PCR premixes: (1) KPC/OXA/NDM; (2) VIM/IMP. Each premix contained all reagents (buffer, enzyme, probes, primers and internal control). Positive and negative controls supplied in the kit (5 µl) were tested in each RT-PCR run. The RT-PCR programme was set as instructed by the manufacturer, using the RotorGene Q RT-PCR machine. RT-PCR analysis was performed by RotorGene Q software as follows: threshold settings were adjusted to maintain the positive control at Ct values between 20 and 25. Samples were considered positive if the signal was detected at a threshold of 30 < Ct.

For the Sacace MDR assay, two RT-PCR mixes were prepared for each DNA sample as follows: 10 µl of PCR-mix-FRT MBL or PCR-mix-FRT KPC/OXA-48; 5 µl of RT-PCR-mix-2; 0.5 µl of Polymerase; and 1 µl of internal control. Two microlitres of DNA extract were added to the mix. Positive and negative controls supplied with the kit (10 µl) were tested in each RT-PCR run. PCR water was added to achieve a total volume of 25 µl for each reaction. The RT-PCR programme was set as instructed by the manufacturer, using the Rotor Gene Q RT-PCR machine. RT-PCR analysis was performed by Rotor Gene Q software as follows: threshold level was set at 0.1, the outlier removal on 10 % and the slope correct was turned on. Samples were considered positive if the signal was detected at a threshold of 30 < Ct.

The sensitivities and specificities of the Hy-CRE and the Sacace MDR assays were excellent, ~100 and 100 %, respectively, as indicated in Table 1. Positive samples were detected with Ct values of 20–26 and 12–14 in the Hy-CRE and the Sacace MDR assays, respectively. All 34 of the carbapenem-resistant, carbapenemase-negative strains were concordantly tested negative by both kits (Ct value >30). To further evaluate the assays, we analysed 10 artificially mixed samples that harboured all the different pair combinations of the five carbapenemase genes (blaKPC, blaVIM, blaNDM, blaIMP and blaOXA-48). The Hy-CRE and the Sacace MDR assays were able to simultaneously detect the two carbapenemase genes in all of these samples. A noteworthy limitation of the Sacace MDR assay was the low signal of the positive controls, detected at Ct values of 26–28. Specifically, the IMP-positive control was consistently negative (Ct value >35 in different lots), posing significant limits on the applicability of the assay.

In addition to the excellent analytic performance, both assay techniques tested were rapid, providing final results in under two hours. Both assays required little handling by laboratory staff. This is particularly true for the Hy-CRE kit, in which the PCR pre-mix contained all the reagents including the enzymes, buffer, probes and primers. The Hy-CRE and Sacace MDR kits required approximately 1 and 2 min of hands-on time per sample (in an 18-sample run), respectively, and the assay run times were 87 and 122 min, respectively. Analysis of test results required <1 min per sample for each assay. Hence the total turnaround times were 103 and 139 min for the Hy-CRE and Sacace MDR assays, respectively (including DNA extraction).

Containment of the spread of carbapenem-resistant Enterobacteriaceae is dependent on their accurate and rapid diagnosis [8]. Compared to phenotype-based diagnostics, contemporary molecular approaches, such as RT-PCR, seem to hold great potential as faster diagnostic tests with increased sensitivity and specificity that also allow precise identification of the carbapenemase type. Several in-house PCR assays for carbapenemase genes have been described [9]. However, these involve a complex development and validation process and therefore may not be suitable for all laboratories. Hence, the use of commercial assays may overcome these limitations [10].

The two novel commercial multiplex RT-PCR assays investigated in this study, Hy-CRE and Sacace MDR, showed excellent diagnostic accuracy and a rapid turnaround in detecting the five most important carbapenemases, blaKPC, blaVIM, blaNDM, blaIMP and blaOXA-48. Furthermore, we found that each of the assays was capable of simultaneously amplifying two carbapenemase genes from mixed samples. The Hy-CRE assay was slightly superior to the Sacace MDR assay in terms of workload and performance, due to the negative IMP positive control in the Sacace assay.

With sensitivities of 100 %, these results are in line with those of other commercially available molecular assays capable of detecting these five carbapenemase genes (blaKPC, blaVIM, blaNDM, blaIMP and blaOXA-48) such as the Xpert Carba-R (Cepheid, Sunnyvale, CA) and hyplex SuperBug ID (AmplexDiagnostics GmbH, Gars-Bahnhof, Germany). The Hy-CRE assay was slightly superior to the Sacace MDR in terms of accuracy and rapid turnaround time.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>Carbenapenase genes</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>blaKPC blaNDM blaOXA-48 blaVIM blaIMP blaNDM blaOXA-48</td>
</tr>
<tr>
<td>Hy-CRE</td>
<td>Positive</td>
<td>11 17 18 13 2 1 0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0 0 0 0 0 0 34</td>
</tr>
<tr>
<td>Sacace MDR</td>
<td>Positive</td>
<td>11 17 18 13 2 1 0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0 0 0 0 0 0 34</td>
</tr>
</tbody>
</table>
Germany), for which sensitivities of 96.6-100% [11, 12] and 97% [13], respectively, have been reported. However, the Hy-CRE and Sacace MDR assays are significantly cheaper compared to the Xpert Carba-R assay [12], although the latter has the advantage of being an automated system with no requirement for DNA extraction and a shorter hands-on-time. The Hy-CRE and Sacace MDR assays have time and sensitivity advantages over the hypxler SuperBug ID assay, which failed to detect one of the five carbapenemase genes, as all three blaoIM forge harbouring strains were missed [13].

Another commercial RT-PCR kit, the Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands) [14, 15], includes the carbapenemase genes blaKPC, blavIM, blaoNDM and blaoXA-48 but lacks the blaoIMP gene. The failure to detect the blaoIMP gene may be a key disadvantage in geographical areas where these carbapenemases are prevalent, such as in the Far East and Australia [6]. Additional disadvantages of this assay include the lack of differentiation between blavIM and blaoNDM harbouring isolates using certain RT-PCR platforms (Applied Biosystems 7500, Life Technologies Ltd, Paisley, UK or Light Cycler 480 II, Roche, Brussels, Belgium) [14]. Its reported sensitivity in the literature varies according to the PCR mix used – from 93 to 100% [15].

The isolates included in this study were selected in order to ‘challenge’ the assays with the widest possible variety of CPE genes and species. Hence, the relative rates of the different CPE’s were different from those found at our institution (KPC, 58% OXA-48, 25%; NDM, 16%; VIM, 1%) [16]. In addition, an intrinsic limitation of molecular assays for carbapenemase detection is that only a pre-specified set of known carbapenemase genes can be identified, while new variants of known carbapenemases or new carbapenemases might not be detected. Also, rare carbapenemase genes not included in the assay used might be present (e.g. blaoIM and blaoSIM) [17, 18]. However, these genes are exceedingly rare in comparison to those such as blavKPC or blavIM, they are not located on mobile genetic elements (e.g. plasmids) and are mostly restricted to specific species (e.g. Enterobacter cloacae). Thus, the potential risk for significant dissemination of these genes is probably very limited. Notwithstanding, phenotypic tests may still play a complementary role in detection of these rare cases that have limited epidemiological significance.

The availability of sensitive, simple and accessible molecular diagnostic tools for the detection of carbapenemase genes in routine practice, such as the multiplex assays evaluated, is important for effectively combatting antibiotic resistance and improving patient care. Thus, our study provides important data for clinical laboratories that are looking for implementation of molecular assays for the detection of CPE.

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**Conflicts of interest**

As declared in ‘Funding’ above. The study was designed, analysed and written by the authors independent of the supporting company.

**Ethical statement**

Ethics Committee approval was not required (laboratory study conducted on bacterial isolates).

**References**


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