Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria

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Objectives: To assess the performance of three commercial molecular assays for detecting major families of carbapenemases in pure bacterial isolates.

Methods: A panel of 450 isolates with previously defined carbapenem resistance mechanisms was tested using the Check-Direct CPE kit, the eazyplex SuperBug complete A kit and the Xpert Carba-R kit. Isolates included 438 Enterobacteriaceae and 12 Pseudomonas spp. comprising 100 isolates each with KPC, NDM, VIM or OXA-48-like enzymes, two isolates producing both an NDM and an OXA-48-like enzyme, 24 IMP producers and 24 isolates without a known carbapenemase gene. Discordant results (commercial versus in-house) were investigated using in-house PCR and amplicons were sequenced to define the carbapenemase allele present.

Results: All three commercial assays detected all isolates with KPC, VIM, NDM and classic OXA-48 carbapenemases (no false-negatives). Isolates producing the OXA-181 variant (n = 18) were not detected by the Xpert Carba-R kit or the eazyplex SuperBug complete A kit, but were subsequently detected with modified versions of these kits. Only the Xpert Carba-R kit could detect IMP carbapenemases, although this was limited to the IMP-1 subgroup. Invalid or false-positive results were either not observed when following the manufacturer’s protocols or were eliminated by making simple interpretative adjustments to allow use with bacterial isolates rather than clinical samples.

Conclusions: Commercial assays offer a reliable means of detecting bacteria with clinically significant carbapenemases. Coverage of some assays required expansion to maximize the sensitivity for OXA-48-like carbapenemases. Choice will ultimately depend on preferred gene coverage, intended throughput, cost and ability to fit into local workflows.

Keywords: MBL, KPC, OXA-48, NDM, Enterobacteriaceae

Introduction

Resistance to carbapenem antibiotics is recognized internationally as one of the most pressing aspects of the growing public health threat posed by antimicrobial resistance.1 Although carbapenem resistance can arise by several mechanisms, greatest emphasis is placed on those bacteria that have acquired transferable carbapenemases. These diverse enzymes confer resistance or reduced susceptibility to carbapenems, and most producers are multiresistant due to the combination of carbapenemase activity and other co-resident resistance mechanisms affecting other antibiotic classes.2

The Enterobacteriaceae are widely reported as hosts of carbapenemases, especially Klebsiella pneumoniae and Escherichia coli, although the carbapenemase genes and their associated mobile genetic elements have host ranges that extend beyond these species.3,6 The ‘big five’ carbapenemase families include the KPC and OXA-48-like non-metallo-enzymes and the IMP, NDM and VIM metallo-enzymes. There are significant regional and country differences in the relative importance of these families. In Europe, the KPC, OXA-48-like, NDM and VIM enzymes dominate;5 IMP enzymes are more prevalent in the Far East and Australia.6,7

The spread of these carbapenemases involves both successful ‘high-risk’ lineages (e.g. K. pneumoniae ST258 with KPC) and successful plasmids (e.g. IncL/M OXA-48 plasmids). The big five carbapenemase families have each been associated with outbreaks of infection and colonization in healthcare settings.6,8–11 The range of carbapenem MICs displayed by carbapenemase producers means that laboratories must use supplementary tests to distinguish genuine carbapenemase producers from those isolates resistant to carbapenems through other mechanisms (e.g. porin loss combined with either ESBL or AmpC activity).
Rapid confirmation of carbapenemase production is essential not only for appropriate management of infected patients, but also for prompt deployment of infection prevention and control measures to minimize onward transmission of the resistant bacteria.

Molecular diagnostics allow rapid screening for carbapenemase genes either in cultured bacteria found to have resistance or reduced susceptibility to carbapenems or directly in clinical samples, screening swabs or faeces. Reported molecular solutions make use of various assay formats, although many are based on real-time PCR, and comprise both in-house and commercial options. Here, we compared the performance of three commercial assays for detecting major clinically relevant carbapenemases in a large panel of bacterial isolates with previously defined carbapenem resistance mechanisms.

Materials and methods

Bacterial isolates, identification and carbapenemase status

The 450 isolates included in this study comprised 438 Enterobacteriaceae and 12 Pseudomonas spp. (Table 1). They had been submitted to PHE’s Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit from laboratories across the UK for the investigation of resistance mechanisms. Most had been submitted between January 2012 and April 2014, with the exception of the IMP producers, which were received between July 2009 and February 2014. The isolates were not consecutive referrals, but were rather selected for geographical, temporal (within the above timeframes) and carbapenem resistance status.

Isolates were cultured from –80°C freezer storage or from the sender’s original slopes on MacConkey agar plates with a 10 μg carbenapem disc (Oxoid, Basingstoke, UK); growth from this plate was used for the tests without further subculture. They had previously been identified using either API-20E tests (bioMérieux SA, Marcy-l’Étoile, France) or, since August 2012, by MALDI-TOF MS (Bruker Microflex LT, Bruker Daltonik GmbH, Bremen, Germany). Antibiotic susceptibilities (MICs) had been determined by BSAC agar dilution using AMRHAI’s standard Gram-negative antibiotic panel, which includes enterobacteria, imipenem (tested with/without EDTA, 320 mg/L to detect likely MBL producers) and meropenem. MICs were interpreted using BSAC breakpoints, where available.

The challenge panel of 450 isolates comprised 100 each of KPC, NDM, VIM and OXA-48-like producers, 24 isolates producing both NDM and an OXA-48-like enzyme, 24 IMP producers and 24 isolates that were carbapenem resistant, but did not contain a known carbapenemase gene (Table 1). The carbapenemase genes had been previously detected using in-house PCR assays as previously described and/or with a commercial microarray (Check-MDR CT102; Check-Points, Wageningen, The Netherlands). These data were regarded as the gold standard against which the commercial assays were compared.

Commercial assays

The systems compared were: the Check-Direct CPE kit (Check-Points), tested on both an Applied Biosystems 7500 Real-Time PCR (Life Technologies Ltd, Paisley, UK) and a BD MAXTM (Becton Dickinson, Oxford, UK) platform; the eazyplex® SuperBug complete A kit (Amplex, Gießen, Germany), tested on a Genie® II (OptiGene, Horsham, UK); and the Xpert® Carba-R kit, tested on a GeneXpert GX XVI (Cepheid, Sunnyvale, CA, USA). On the ABI 7500 platform, the Check-Direct CPE kit does not distinguish between VIM and NDM producers, but these are distinguished on the BD MAX™ platform.

All assays were run according to manufacturers’ standard protocols except where indicated.

DNA preparation

Check-Direct CPE on the ABI 7500

A single colony was stabbed using a Sterile Colony Sampler (toothpicks) and suspended in 200 μL of Extraction Buffer (Check-Points) before 10 μL of IC (internal control) solution (Check-Points) was added and the sample was mixed by vortexing for 30 s. Suspensions were heated at 98°C for 10 min, vortexed for 30 s and then centrifuged at 16100 g for 2 min. A 10 μL aliquot of this suspension was used in each reaction.

Check-Direct CPE on the BD MAX™

A 5 μL aliquot of a 0.5 McFarland standard cell suspension was diluted 1 in 100 in 495 μL of sterile distilled water and mixed by pipetting. This 500 μL suspension was added directly to the ABI 7500 platform.

eazyplex® SuperBug complete A

A single colony was touched lightly and growth suspended in 500 μL of resuspension and lysis fluid (RALF) (Amplex) and heated in a heating block at 99°C for 2–3 min. Then 25 μL of the suspension was added to each of the eight tubes in the eazyplex® SuperBug complete A test strip and knocked gently to remove air bubbles, before loading into the Genie II machine.

Table 1. Challenge panel of 450 isolates with defined carbapenem resistance mechanisms

<table>
<thead>
<tr>
<th>Species</th>
<th>KPC</th>
<th>NDM</th>
<th>VIM</th>
<th>OXA-48-like</th>
<th>NDM + OXA-48-like</th>
<th>IMP</th>
<th>none</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>62</td>
<td>52</td>
<td>55</td>
<td>59</td>
<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>6</td>
<td>0</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>9</td>
<td>28</td>
<td>8</td>
<td>29</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>18</td>
<td>14</td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Others†</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>2</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

†23 isolates comprising *Citrobacter* spp. (n = 15), *Raoultella* spp. (n = 3), *Leclercia adecarboxylata* (n = 2), *Serratia marcescens* (n = 2) and *Kluyvera georgiana* (n = 1).
Xpert® Carba-R

A 10 μL aliquot of a 0.5 McFarland standard cell suspension was diluted 1 in 10 in 90 μL of PBS, and 10 μL of this dilution was mixed into a vial of Sample Reagent (Cepheid). Approximately 1.7 mL was added to the Xpert® Carba-R Assay Cartridge, which was loaded into the GeneXpertGX XVI.

Data analysis

Results from the Check-Direct CPE, eazyplex® SuperBug complete A and Xpert® Carba-R assays were all interpreted initially according to the manufacturers’ guidelines. Subsequently, a cut-off value (Ct ≤ 31) was applied to identify carbapenemase-positive isolates with the Check-Direct CPE kit on the ABI 7500 platform, and all Ct values after 31 cycles were disregarded. Where there were mismatches between the results of one or more commercial assays and the result that was recorded in our laboratory information management system from prior in-house assays, the discordance was investigated by retesting isolates using the in-house PCR to ensure that the expected carbapenemase gene was still present and by sequencing the PCR amplicon to define the precise allele present, and using the commercial method to ensure negativity.

Results

Analytical sensitivity of assays and investigation of discordant results

Each of the commercial assays successfully detected the correct carbapenemase gene in all 302 isolates with a KPC, NDM or VIM enzyme and so achieved 100% sensitivity for these targets (Table 2).

Results from the Check-Direct CPE, eazyplex® SuperBug complete A and Xpert® Carba-R assays were all interpreted initially according to the manufacturers’ guidelines. Subsequently, a cut-off value (Ct ≤ 31) was applied to identify carbapenemase-positive isolates with the Check-Direct CPE kit on the ABI 7500 platform, and all Ct values after 31 cycles were disregarded. Where there were mismatches between the results of one or more commercial assays and the result that was recorded in our laboratory information management system from prior in-house assays, the discordance was investigated by retesting isolates using the in-house PCR to ensure that the expected carbapenemase gene was still present and by sequencing the PCR amplicon to define the precise allele present, and using the commercial method to ensure negativity.

Analytical specificity of commercial assays

No false-positive results were observed with the eazyplex® SuperBug complete assays and Xpert® Carba-R assays, or with Xpert® Carba-R.

The Check-Direct CPE assay detected correctly the 102 isolates with an OXA-48-like carbapenemase, whereas 18 of them (17 with the OXA-48-like enzyme alone and 1 with an OXA-48-like enzyme in combination with an NDM enzyme) were not detected by either the eazyplex® SuperBug complete A or the Xpert® Carba-R assay (Table 2). PCR and sequencing identified a blaOXA-181 allele in each of the 18 false-negative isolates. A modified eazyplex® SuperBug complete B kit (Amplex) and Xpert® Carba-R v2 kit (Cepheid) were subsequently provided and these modified assays both correctly identified the 18 OXA-181 producers.

Genes encoding IMP-type carbapenemases are not detected by the Check-Direct CPE or eazyplex® SuperBug complete A assays, but the Xpert® Carba-R is designed to detect blalmp-1-like alleles (IMP-1, -3, -6, -10, -25 and -30). In this evaluation, 17 of 24 IMP producers were detected by the Xpert® Carba-R assay. PCR confirmed the presence of a blalmp gene in the seven false-negative isolates, and amplicon (partial gene) sequencing identified carbapenemases most closely matching IMP-4, IMP-7, IMP-8, IMP-13 and IMP-14; the assay does not cover these alleles.

Of note, the internal control in the Check-Direct CPE assay failed to amplify for 277 (62%) of 450 tests on the ABI 7500 and for 27 (6%) tests on the BD MAX™, most likely because it was out-competed by the carbapenemase gene(s). As indicated clearly in the manufacturer’s protocol, amplification of the internal control was deemed critical only for carbapenemase-negative isolates to prove absence of inhibitors.

Analytical specificity of commercial assays

No false-positive results were observed with the eazyplex® SuperBug complete assays and Xpert® Carba-R assays, or with

Table 2. Performance of three commercial kits for detecting carbapenemase genes in bacterial isolates

<table>
<thead>
<tr>
<th>Assay coverage</th>
<th>Check-Direct CPE on the ABI 7500</th>
<th>Check-Direct CPE on the BD MAX™</th>
<th>eazyplex® SuperBug complete A</th>
<th>Xpert® Carba-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay coverage</td>
<td>KPC, OXA-48-like, NDM/VIM™</td>
<td>KPC, OXA-48, NDM, VIM</td>
<td>KPC, OXA-48, NDM, VIM</td>
<td>KPC, OXA-48, NDM, VIM, IMP-1-like</td>
</tr>
<tr>
<td>Big five carbapenemases NOT detected</td>
<td>IMP family</td>
<td>IMP family</td>
<td>some OXA-48-like; IMP family</td>
<td>some OXA-48-like; some IMP subgroups</td>
</tr>
<tr>
<td>Hands on time per sample</td>
<td>&lt;5 min</td>
<td>&lt;5 min</td>
<td>~3–4 min</td>
<td>~1–2 min</td>
</tr>
<tr>
<td>Assay run time</td>
<td>~1.75 h</td>
<td>~2.5 h</td>
<td>20 min</td>
<td>~50 min</td>
</tr>
<tr>
<td>Sample throughput</td>
<td>up to 94 tests in a batch</td>
<td>up to 22 tests in a batch</td>
<td>1 or 2 independent tests</td>
<td>1–80 independent tests</td>
</tr>
<tr>
<td>Assay performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPC (n = 100)</td>
<td>100%</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>OXA-48-like (n = 100)</td>
<td>100%</td>
<td></td>
<td>83%</td>
<td>83%</td>
</tr>
<tr>
<td>NDM (n = 100)</td>
<td>100%</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>VIM (n = 100)</td>
<td>100%</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>IMP (n = 24)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>71% (17/24)</td>
</tr>
<tr>
<td>NDM + OXA-48-like (n = 2)</td>
<td>2 x NDM; 2 x OXA-48</td>
<td>2 x NDM; 2 x OXA-48</td>
<td>2 x NDM; 1 x OXA-48</td>
<td>2 x NDM; 1 x OXA-48</td>
</tr>
<tr>
<td>non-carba (n = 24)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

N/A, not applicable; non-carba, carbapenem-negative, but carbapenem-resistant, isolates.

*The Check-Direct CPE kit does not distinguish between NDM and VIM producers on the ABI 7500 platform, but does so on the BD MAX™ platform.

*Capacity of GeneXpert machines varies with model.

*Sequencing identified OXA-181 in 18 isolates where the OXA-48-like gene was not detected.

*Sequencing identified closest matches with IMP-4, IMP-7, IMP-8, IMP-13 and IMP-14 in seven isolates where an IMP-1-like gene was not detected.
the Check-Direct CPE assay on the BD MAX™ platform; no carbapenemase genes were detected in 24 carbapenemase-negative isolates and no incorrect carbapenemase genes were detected in the 426 carbapenemase-producing isolates (100% specificity).

However, when data from the Check-Direct CPE assay on the ABI 7500 platform were interpreted according to the manufacturer’s protocol (any Ct value indicates a positive result), there were 173 false-positive results (overall specificity of 82%). These false-positive results were all eliminated by applying a threshold of Ct value of ≤31 to indicate a positive result (100% specificity), and the manufacturer’s protocol has been revised accordingly.

Discussion

Molecular diagnostics for detecting major carbapenemase genes may be deployed at two points in laboratory workflow. They can be used to detect the genes directly in a clinical or screening sample or may be used post-culture to seek the genes in isolates with resistance or reduced susceptibility to one or more carbapenems. We evaluated the performance of three commercial kits suited to both of these purposes,17–19 using a panel of isolates with defined carbapenem resistance mechanisms.

All of the assays detected all isolates with KPC, VIM, NDM and classic OXA-48 carbapenemases; no false-negative results were observed with any kit. However, only the Check-Direct CPE assay identified OXA-181 producers in initial tests. This OXA-48-like carbapenemase can currently only be distinguished by gene sequencing. It was identified here in 18 of 102 isolates with an OXA-48-like enzyme, in 17 it was the sole carbapenemase present and in 1 it was co-produced with NDM-1. As originally supplied to us, neither the eazyplex® SuperBug complete A nor the Xpert® Carba-R assay detected the OXA-181 variant. A subsequent rapid modification to the eazyplex® SuperBug complete B kit allowed detection of the blaOXA-181 gene. The eazyplex® assays use loop-mediated isothermal amplification (LAMP) technology rather than real-time PCR, which is the basis of the Check-Direct CPE and Xpert® Carba-R assays. Each target in the LAMP assay is sought in an individual test, rather than in the multiplexed format of the real-time PCRs. This may offer greater flexibility to respond rapidly if gene coverage needs to be altered, e.g. if a rare or unknown carbapenemase becomes prevalent. It may be more challenging and slower (though <1 year) to revise and re-validate a multiplex real-time assay to cover a new target. Nevertheless, a modified Xpert® Carba-R v2 assay was also provided to us that did detect OXA-181.

Two of the assays (Check-Direct CPE and eazyplex® SuperBug complete A/B) were unable to detect IMP carbapenemase genes, whereas the third (Xpert® Carba-R) detected only the IMP-1 subgroup. While this diminishes overall sensitivity for identifying carbapenemase producers, gene coverage must always be balanced against local or national prevalence of different enzymes. IMP variants are not as prevalent in Europe and many other parts of the world as are KPC, OXA-48-like, NDM and VIM enzymes. In England, for example, IMP enzymes are detected in only ~1% of carbapenemase producers confirmed by the national reference laboratory (N. Woodford and K. Hopkins, unpublished results). Inability to detect some or all IMP producers becomes a more serious consideration if an assay is to be used in areas where these carbapenemases are more prevalent, such as in the Far East and Australia.6,7 No currently available commercial or in-house assays are comprehensive for all known families of metallo- and non-metallo-carbapenemases; they all compromise on coverage and so there is some innate risk that a negative isolate might produce an uncommon or genuinely novel carbapenemase. Any laboratory considering a molecular diagnostic must assess whether its target coverage might be expected to affect sensitivity adversely and this must be based on detailed knowledge of the carbapenemases that are circulating locally, regionally or nationally; coverage requirements will vary with geography. Assessing the concordance of molecular data with phenotypic susceptibility results remains essential to identify isolates with unexplained resistance to carbapenems and which warrant further investigation.

This evaluation also served unintentionally to provide evidence of the robustness of the in-house PCR assays. The 426 carbapenemase producers had all been detected by the reference laboratory, including the 18 OXA-181 producers and 24 IMP producers. Although difficult, it is possible to design primers that can provide greater sensitivity for the OXA-48-like and IMP carbapenemase families.

In summary, commercial assays offer a reliable means of detecting bacteria with clinically significant carbapenemases, especially KPC, NDM and VIM types. The coverage of some assays has recently been expanded to detect OXA-181 producers and so improve their sensitivity for detecting isolates with OXA-48-like carbapenemases. Whether any given laboratory chooses to use a molecular test and the subsequent choice of test will depend on cost, intended throughput, preferred gene coverage and ability to fit into local workflows.

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