Introduction

The worldwide dissemination of extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae has resulted in greater dependence on carbapenems for the treatment of infections by these organisms.

The emergence of acquired carbapenemases in such isolates is of major concern as these confer resistance to most β-lactams and are usually associated with other non-β-lactam resistance determinants, leaving limited treatment options.

Early detection of these isolates is beneficial for appropriate patient management and for infection control purposes.

In New Zealand, the isolation of carbapenemase-producing Enterobacteriaceae (CPE) is relatively infrequent and all CPE have been isolated from patients returning from overseas. Currently we screen isolates that have reduced susceptibility to carbapenems by the Modified Hodge Test (MHT) and detection of metallo-β-lactamases (MBLs) using the chelating agents dipyridylamine (DPA) and ethylenediaminetetraacetic acid (EDTA). Confirmation requires referral to a reference laboratory.

Recently, a number of phenotypic and molecular tests have become available, including several chromogenic agars, biochemical tests and commercial molecular systems.

Objective

To evaluate and compare phenotypic and molecular methods for the detection of carbapenemases in Enterobacteriaceae.

Method

30 Enterobacteriaceae clinical isolates, including 12 characterised strains were tested in a blinded fashion. Identification was confirmed by MALDI-TOF MS (bioMérieux). All isolates had reduced susceptibility to ertapenem and/or the VITEK 2 XL (bioMérieux) indicated the presence of a carbapenemase phenotype.

The 12 strains had been characterized by the National Reference Laboratory, ESR. see Table 1.

Phenotypic Tests

The Modified Hodge Test (MHT) was carried out according to CLSI M100-S24 Guidelines.

Detection of MBL using the chelating agents DPA and EDTA available from Rosco Diagnostica.

Two chromogenic screening agars: Brilliance™ CRE Agar (Oxoid) and ChromID® CARBA SMART Agar (bioMérieux)

Three Biochemical Tests: Neo-Rapid CARB DIATABS™ (Rosco Diagnostica) – commercial assay. Carba NP – in-house test with phenol red solution used as the colour indicator. Blue-Carba – in-house variant of the Carba NP with bromothymol blue selected as the indicator of acidification.


Results

The MHT detected 10 (83%) CPE producing isolates, however 2 carbapenemase producing P. mirabilis gave indeterminate results. Four false positives (22%) were observed amongst the ESCHaPPM isolates with chromosomal AmpC.

DPA and EDTA detected all the MBL isolates but yielded 1 false positive result.

Brilliance™ CRE and chromID® CARBA SMART Brilliance™ CRE grew 11 (92%) of the CPE isolates. chromID® CARBA SMART gave similar sensitivity (83%). The bi-plate allows screening for CPE on one half and for OXA-48-like producers on the other, improving the specificity.

Neo-Rapid CARB DIATABS™, Carba NP and Blue-Carba The Blue-Carba had the highest sensitivity of the three tests. However all 3 failed to detect the OXA-181 isolates. When comparing both in-house assays, the Blue-Carba was the easier to perform. The latest CLSI M100-S25 has introduced the Carba NP as a confirmatory test for suspected carbapenemase production in Enterobacteriaceae.

Xpert CARBA-R.

Xpert CARBA-R detected all NDM-1, KPC, VIM-2 and IMP-1 isolates but its target was limited to OXA-48 and IMP-1.

Conclusion

For Enterobacteriaceae, the Blue-Carba is an easy, rapid, inexpensive biochemical test for detection of diverse carbapenemase producers from bacterial cultures. However it failed to detect OXA-181 isolates.

The Brilliance™ CRE and the chromID® CARBA SMART media detected the OXA-181 isolates. Either media in combination with the Xpert CARBA-R or the Blue-Carba would provide good sensitivity.

False positive MHT can occur with high-level AmpC producers and strains producing CTX-M.

At present, there isn’t a single “best” detection method.

References:

9. Woodford, S. N. 2016. The PHE CPE Toolkit and the need to detect Carbapenemase Producing Enterobacteriaceae. JRCV

Acknowledgements:

1. Funding provided by the Microbiology Department Research and Education Fund.
2. National Reference Laboratory, ESR.

Table 1

<table>
<thead>
<tr>
<th>Carbenemase producers</th>
<th>n=12</th>
<th>MHT*</th>
<th>MBL (DPA/EDTA)</th>
<th>Brilliance</th>
<th>CARBA SMART</th>
<th>Neo-Rapid DIATABS</th>
<th>Carba NP</th>
<th>Blue-Carba</th>
<th>Xpert Carba-R</th>
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<tbody>
<tr>
<td>KPC (1) KPC-357273</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>NDM-1 (4) COL (1), P. melli (1), KPC (2)</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>6</td>
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<tr>
<td>IMP (3) IMP-532181</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>OXA-181 (3) COL (2), KPC (1)</td>
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<td>3</td>
<td>3</td>
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<td>Non-carbenemase producers</td>
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<tr>
<td>Total # carbenemase producers</td>
<td>12/10/12</td>
<td>11/12</td>
<td>10/12</td>
<td>6/12</td>
<td>7/12</td>
<td>9/12</td>
<td>8/12</td>
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<tr>
<td>Total # non-carbenemase producers</td>
<td>18</td>
<td>4/18</td>
<td>1/18</td>
<td>11/18</td>
<td>6/18</td>
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<td>Sensitivity %</td>
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<td>67</td>
<td>92</td>
<td>83</td>
<td>50</td>
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<tr>
<td>Specificity %</td>
<td>78</td>
<td>94</td>
<td>39</td>
<td>67</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>82</td>
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</tr>
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</table>

* Includes weak positive results.

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