Laboratory Detection of Carbapenemase-producers

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Carbapenemase-producing Gram-negative Microorganisms. Where are we now? Challenges in prevention, diagnosis, detection and therapy.
Thursday 4 June 2015
Royal College of Physicians, London
Detection of carbapenemase-producing Enterobacteriaceae by disc susceptibility testing.
Where is the difficulty?

• Zone diameters for carbapenems should be measured very carefully – some isolates have very low level resistance to carbapenems.

• Complications may arise with producers of ESBL or particularly AMPC β-lactamases.
Klebsiella pneumoniae

EUCAST

BSAC

Ertapenem (R)

Meropenem (R)

Meropenem (R)

Ertapenem (R)

OXA-48 carbapenemase
Klebsiella pneumoniae

EUCAST

BSAC

TEM R
MERO S (29 mm)
ERTA S (25 mm)
CXM S (25 mm)
TEM R (12 mm)
MERO S (32 mm)
ERTA S (28 mm)
CXM S (29 mm)

Klebsiella pneumoniae
Klebsiella pneumoniae

OXA-48 carbapenemase
EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance.

Version 1.0
December 2013

EUCAST subcommittee for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance:

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UK Standards for Microbiology Investigations

Laboratory Detection and Reporting of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases)

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Table 1. Clinical breakpoints and screening cut-off values for carbapenemase-producing Enterobacteriaceae (according to EUCAST methodology).

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>Disk diffusion zone diameter (mm) with 10 μg disks</th>
<th>S breakpoint</th>
<th>Screening cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>≥22 mm</td>
<td>&lt;25 mm</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥22 mm</td>
<td>&lt;23 mm</td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≥25 mm</td>
<td>&lt;25 mm</td>
<td></td>
</tr>
</tbody>
</table>
Detection of carbapenemase-producing *Enterobacteriaceae* by disc susceptibility testing.

- 248 isolates of *Enterobacteriaceae* (all with defined β-lactamases) were tested using EUCAST methodology.

<table>
<thead>
<tr>
<th>All CPE</th>
<th>166</th>
<th>NON-CPE</th>
<th>82</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP</td>
<td>14</td>
<td>ESBL</td>
<td>60</td>
</tr>
<tr>
<td>KPC</td>
<td>15</td>
<td>AMP-C</td>
<td>19</td>
</tr>
<tr>
<td>NDM-1</td>
<td>115</td>
<td>K1</td>
<td>3</td>
</tr>
<tr>
<td>OXA-48</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIM</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Detection of carbapenemase-producing *Enterobacteriaceae* by disc susceptibility testing.

- The 248 isolates of *Enterobacteriaceae* were tested with:

  - Temocillin 30 µg
  - Imipenem 10 µg
  - Doripenem 10 µg
  - Meropenem 10 µg
  - Ertapenem 10 µg
  - Faropenem 10 µg
Disc susceptibility testing with carbapenems against 248 Enterobacteriaceae as a predictor of carbapenemase activity using EUCAST criteria:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>99%</td>
<td>79%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>98%</td>
<td>88%</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>99%</td>
<td>69%</td>
</tr>
<tr>
<td>(Faropenem)</td>
<td>(99%)</td>
<td>(94%)</td>
</tr>
</tbody>
</table>
Use of Faropenem as an Indicator of Carbapenemase Activity in the Enterobacteriaceae

Kathryn M. Day,a,b Rachel Pike,c Trevor G. Winstanley,a Clare Lanyon,b Stephen P. Cummings,b Muhammad W. Raza,a Neil Woodford,c John D. Perry,a,b

Microbiology Department, Freeman Hospital, Newcastle upon Tyne, United Kingdom; School of Life Sciences, University of Northumbria, Newcastle upon Tyne, United Kingdom; Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMR-RAI), HPA Microbiology Services—Colindale, London, United Kingdom; Department of Laboratory Medicine, Northern General Hospital, Sheffield, United Kingdom

The aim of this study was to determine the ability of a disc susceptibility test using faropenem (10 μg) to predict carbapenemase activity in Enterobacteriaceae. A collection of 166 isolates of carbapenemase-producing Enterobacteriaceae (CPE) and 82 isolates of Enterobacteriaceae that produced other β-lactamases was compiled from diverse sources. Disc susceptibility testing was performed using the CLSI/EUCAST methodology with discs of faropenem (10 μg), temocillin (30 μg), and four carbapenems (each 10 μg). A further prospective evaluation of the faropenem disc susceptibility test was performed using 205 consecutive isolates referred to a United Kingdom reference laboratory in parallel with molecular methods for carbapenemase detection. Of 166 isolates of CPE, 99% showed growth up to the edge of a 10-μg faropenem disc compared with only 6% of other β-lactamase producers (sensitivity, 99%; specificity, 94%). A “double zone” around 10-μg faropenem discs was frequently associated with OXA-48 producers. Of the carbapenems, the most useful agent was imipenem, where a zone diameter of ≥23 mm as a predictor of carbapenemase activity had a sensitivity of 99% and a specificity of 85%. The presence of no zone of inhibition around a 30-μg temocillin disc was a consistent feature of strains producing OXA-48 carbapenemase. For 205 isolates of Enterobacteriaceae referred to a United Kingdom reference laboratory, growth up to a 10-μg faropenem disc correctly identified 84 of 86 carbapenemase producers (98% sensitivity), with a specificity of 87%. Disc susceptibility testing using faropenem (10 μg) is a simple, convenient, and highly predictive screening test for carbapenemase-producing Enterobacteriaceae.

Carbapenems are relied upon as one of the few effective options for treatment of Enterobacteriaceae that produce extended-spectrum and/or AmpC β-lactamases. However, their utility is becoming compromised by the increasing occurrence of Enterobacteriaceae that produce carbapenemases (1, 2). Clinical laboratories must have access to simple phenotypic screening tests for the accurate detection of carbapenemase-producing Enterobacteriaceae (CPE). A number of tests have been devised for detection of carbapenemases (3–8), but these are performed only if there is sufficient suspicion based on the results of antimicrobial susceptibility testing. However, inferring carbapenemase activity from an antibiogram is not always straightforward because some isolates of CPE appear susceptible to carbapenems, while isolates producers, including 115 with NDM-1 (Citrobacter freundii, n = 5; other Citrobacter spp., n = 6; Enterobacter cloaceae, n = 27; Escherichia coli, n = 52; Klebsiella pneumoniae, n = 22; Pseudomonas aeruginosa, n = 1; Providencia rettgeri, n = 2); 14 with IMP (K. pneumoniae, n = 11; E. coli, n = 2; Klebsiella oxytoca, n = 1); 15 with KPC (K. pneumoniae, n = 12; E. coli, n = 1; E. cloaceae, n = 1; K. oxytoca, n = 1); 13 with OXA-48 (K. pneumoniae, n = 9; E. coli, n = 2; E. cloaceae, n = 2); and 9 with VIM (K. pneumoniae, n = 5; E. coli, n = 3; E. cloaceae, n = 1). Most of the carbapenemase producers coproduced extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase (AmpC), but these are not documented here for the sake of clarity. Eighty-two isolates were non-carbapenemase producers, of which 60 produced ESBLs, including 25 isolates producing 10 varieties of CTX-M (K. pneumoniae, n = 14; E. coli, n = 5; K. oxytoca, n = 1; Proteus mirabilis, n = 1).

Temocillin and piperacillin/tazobactam resistance by disc diffusion as antimicrobial surrogate markers for the detection of carbapenemase-producing Enterobacteriaceae in geographical areas with a high prevalence of OXA-48 producers.

1354 consecutive isolates of Enterobacteriaceae with reduced susceptibility to carbapenems were referred to Belgian / French Reference Laboratories. These were evaluated using disc susceptibility testing:

<table>
<thead>
<tr>
<th>All CPE</th>
<th>435</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>60</td>
</tr>
<tr>
<td>NDM-1</td>
<td>20</td>
</tr>
<tr>
<td>OXA-48</td>
<td>323</td>
</tr>
<tr>
<td>VIM</td>
<td>32</td>
</tr>
<tr>
<td>NON-CPE</td>
<td>919</td>
</tr>
</tbody>
</table>

Distribution of meropenem 10 µg disc inhibition zone and of carbapenemases for Enterobacteriaceae isolates referred to the NRLs in 2012 (n=1354).
Results:

~ 50% of CPE were susceptible to meropenem using disc testing.

20% of CPE could not be detected using the EUCAST recommended cut-off of < 25 mm for meropenem. (including 80/323 with OXA-48 and 6/32 with VIM).

Ertapenem was not tested.
Is ertapenem (10 µg) better for detecting OXA-48?

• 270 isolates of *K. pneumoniae* with OXA-48 were collected from patients in Turkey and tested against 18 antimicrobials including meropenem, imipenem, and ertapenem using EUCAST disc diffusion methodology.

  [Perry et al. Unpublished. 2015]
Results for 270 *K. pneumoniae* with OXA-48.

<table>
<thead>
<tr>
<th>Drug</th>
<th>% susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>49</td>
</tr>
<tr>
<td>Meropenem</td>
<td>43</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.7</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>54</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>54</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>43</td>
</tr>
<tr>
<td>Temocillin</td>
<td>0 (all &lt; 10 mm)</td>
</tr>
</tbody>
</table>

Sensitivity of EUCAST *meropenem* cut-off (< 25 mm) for detecting carbapenemase activity = 92%.

Sensitivity of EUCAST *ertapenem* cut-off (< 25 mm) for detecting carbapenemase activity = 99.3%.
Conclusions:

- If only a single carbapenem is to be routinely tested against Enterobacteriaceae, test ertapenem and perform confirmation tests on any isolates that are non-susceptible (i.e. resistant or intermediate).

- This provides maximum sensitivity for detection with acceptable specificity.
Acute trust toolkit for the early detection, management and control of carbapenemase-producing Enterobacteriaceae
CDC Broth enrichment method for isolation of CPE.

Inoculate stool sample into:
5 mL nutrient broth plus
a 10 µg disc of ertapenem or meropenem

Incubate broth overnight and look for lactose-fermenters – confirm presence of CPE.
A Comparison of Four Chromogenic Culture Media and the CDC broth method for isolation of Carbapenemase-producing *Enterobacteriaceae*. 
Table 1: Number of *Enterobacteriaceae* with different β-lactamases recovered on various culture media recommended for isolation of carbapenemase-producing *Enterobacteriaceae* (CPE), after 18 h incubation.

<table>
<thead>
<tr>
<th>β-Lactamase/Carbapenemase</th>
<th>Brilliance CRE High&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CRE Low&lt;sup&gt;b&lt;/sup&gt;</th>
<th>chromID CARBA High</th>
<th>chromID CARBA Low</th>
<th>chromID ESBL High</th>
<th>chromID ESBL Low</th>
<th>COLOREX KPC High</th>
<th>COLOREX KPC Low</th>
<th>TSB / ertapenem High</th>
<th>TSB / meropenem Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>KPC</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>NDM</td>
<td>88</td>
<td>77</td>
<td>85</td>
<td>83</td>
<td>87</td>
<td>86</td>
<td>76</td>
<td>50</td>
<td>87</td>
<td>71</td>
</tr>
<tr>
<td>OXA</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>VIM</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>106</td>
<td>101</td>
<td>125</td>
<td>118</td>
<td>126</td>
<td>125</td>
<td>114</td>
<td>73</td>
<td>129</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other β-lactamases</th>
<th>TSB / ertapenem High</th>
<th>TSB / meropenem Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL</td>
<td>49</td>
<td>19</td>
</tr>
<tr>
<td>Amp C</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>28</td>
</tr>
</tbody>
</table>

| Sensitivity (%)           | 82                  | 78                  | 96                 | 91                 | 97                 | 96                 | 88               | 56               | 99                  | 78                  |
| Specificity (%)           | 60                  | 66                  | 76                 | 89                 | 6                  | 19                 | 70               | 77               | 10                  | 69                  |
| PPV (%)                   | 79                  | 81                  | 88                 | 94                 | 66                 | 69                 | 84               | 82               | 67                  | 82                  |
| NPV (%)                   | 64                  | 61                  | 91                 | 84                 | 50                 | 72                 | 75               | 49               | 88                  | 63                  |

<sup>a</sup>High inocula were approximately 100 000 CFU/spot for chromogenic media or 100 000 CFU/ml for broth media.

<sup>b</sup>Low inocula were approximately 100 CFU/spot for chromogenic media or 100 CFU/ml for broth media.

Table 1: Number of *Enterobacteriaceae* with different β-lactamases recovered on various culture media recommended for isolation of carbapenemase-producing *Enterobacteriaceae* (CPE), after 18 h incubation.

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th><em>Brilliance</em> CRE</th>
<th>chromID CARBA</th>
<th>chromID ESBL</th>
<th>COLOREX KPC</th>
<th>TSB / ertapenem</th>
<th>TSB / meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>n</strong></td>
<td><strong>High</strong></td>
<td><strong>Low</strong></td>
<td><strong>High</strong></td>
<td><strong>Low</strong></td>
<td><strong>High</strong></td>
</tr>
<tr>
<td>IMP</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>KPC</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>NDM</td>
<td>88</td>
<td>77</td>
<td>75</td>
<td>85</td>
<td>83</td>
<td>87</td>
</tr>
<tr>
<td>OXA</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>VIM</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>106</td>
<td>101</td>
<td>125</td>
<td>118</td>
<td>126</td>
</tr>
<tr>
<td>Other β-lactamases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESBL</td>
<td>49</td>
<td>19</td>
<td>16</td>
<td>11</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Amp C</td>
<td>21</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>28</td>
<td>24</td>
<td>17</td>
<td>8</td>
<td>66</td>
</tr>
</tbody>
</table>

**Sensitivity (%)**

- 82
- 78
- 96
- 91
- 97
- 96
- 88
- 56
- 99
- 78
- 97
- 47

**Specificity (%)**

- 60
- 66
- 76
- 89
- 6
- 19
- 70
- 77
- 10
- 69
- 27
- 79

**PPV (%)**

- 79
- 81
- 88
- 94
- 66
- 69
- 84
- 82
- 67
- 82
- 71
- 80

**NPV (%)**

- 64
- 61
- 91
- 84
- 50
- 72
- 75
- 49
- 88
- 63
- 83
- 44

---

*a* High inocula were approximately 100 000 CFU/spot for chromogenic media or 100 000 CFU/ml for broth media.

*b* Low inocula were approximately 100 CFU/spot for chromogenic media or 100 CFU/ml for broth media.

Table 2: Isolates recovered on various selective media from 100 stool samples from patients in Newcastle, UK.

<table>
<thead>
<tr>
<th></th>
<th>Brilliance CRE</th>
<th>chromID CARBA</th>
<th>chromID ESBL</th>
<th>COLOREX KPC</th>
<th>TSB plus ertapenem</th>
<th>TSB plus meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acinetobacter spp.</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Pseudomonas spp.</strong></td>
<td>2</td>
<td>9</td>
<td>12</td>
<td>3</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td><strong>S. maltophilia</strong></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>2 (1)</td>
<td>3 (1)</td>
<td>22 (18)</td>
<td>1 (1)</td>
<td>39 (10)</td>
<td>43 (8)</td>
</tr>
<tr>
<td><strong>Candida spp.</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Enterococcus spp.</strong></td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>99</td>
<td>82</td>
</tr>
<tr>
<td><strong>Staphylococcus spp.</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><strong>Streptococcus sp.</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total isolates</strong></td>
<td><strong>6</strong></td>
<td><strong>14</strong></td>
<td><strong>37</strong></td>
<td><strong>4</strong></td>
<td><strong>167</strong></td>
<td><strong>155</strong></td>
</tr>
</tbody>
</table>

*No CPE were detected from 100 routine stool samples. Numbers in parenthesis indicate isolates that were found to produce ESBL or AmpC ß-lactamase or both.
Conclusions:

- CDC broth method lacks sensitivity and specificity (and takes a day longer than chromogenic media to obtain results). Don’t use it!

- chromID ESBL and chromID CARBA had the best sensitivity for detection of CPE, but the latter is much more specific.

- All media showed weakness for detection of CPE with OXA-48 carbapenemase.

- Due to the low prevalence in the UK, clinical trials in other countries would be necessary for accurate assessment of methods.
Comparison of Four Chromogenic Culture Media for Carbapenemase-Producing Enterobacteriaceae

Kathryn M. Wilkinson, Trevor G. Winstanley, Clare Lanyon, Stephen P. Cummings, Muhammad W. Raza, and John D. Perry

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Four chromogenic media for carbapenemase-producing Enterobacteriaceae (CPE) and two selective broths were challenged with a collection of Enterobacteriaceae with well-defined β-lactamases and 100 stool samples. With low inocula of 130 isolates of CPE, the sensitivities of the four chromogenic media were as follows: Brillance CRE, 78%; chromID Carba, 91%; chromID ESBL, 96%; and Colorex KPC, 56%. The corresponding sensitivities of Trypticase soy broth plus ertapenem or meropenem were 78% and 47%, respectively.

The global dissemination of Enterobacteriaceae harboring carbapenemases is a major public health concern (6). In the United States, the Centers for Disease Control and Prevention (CDC) provided guidance on the isolation of carbapenemase-producing Escherichia coli and Klebsiella spp. from rectal swabs by advising the use of 5 ml Trypticase soy broth (TSB) supplemented with 10% horse blood. Each isolate was subcultured on cystine lactose electrolyte deficient (CLED) agar and incubated for 24 h in air. Colonies were then suspended in sterile saline (0.85%) to a density equivalent to 0.5 McFarland unit, as determined with a densitometer (Densimat; bioMérieux). One-microliter aliquots of these suspensions were delivered onto the four chromogenic media. Columbia
Pakistan Study I: Armed Forces Institute for Pathology, Rawalpindi.

- 200 stool samples cultured onto MacConkey agar in Pakistan. Growth harvested from each sample and referred to the UK.

- Cultures derived from MacConkey were plated onto:
  - Colorex KPC (CHROMagar formulation)
  - chromID CARBA
Findings:

• 37 / 200 patients were colonised with CPE – all with NDM-1 carbapenemase (prevalence: 18.5%).

• 56 isolates of CPE were recovered on chromID CARBA compared with 41 on Colorex KPC ($P = 0.012$).

• Lack of recovery on Colorex KPC was associated with meropenem MIC < 4 mg/L.
Pure growth of blue colonies of *C. freundii* with NDM-1 enzyme on Colorex KPC medium (left). On ID Carba the same specimen yields *C. freundii* as green colonies and *E. coli* as red colonies; both species produced NDM-1 carbapenemase.
Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media


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Objectives: To determine the prevalence and antimicrobial susceptibility of carbapenemase-producing Enterobacteriaceae among hospitalized patients and outpatients attending two military hospitals in Rawalpindi, Pakistan, and to compare the performance of two chromogenic culture media for the isolation of these organisms.

Methods: Stool samples from 200 distinct patients were cultured on MacConkey agar and subsequently on two chromogenic media ('Oxoid CM 0385', 'Oxoid CM 2193').
Pakistan Study II: Armed Forces Institute for Pathology, Rawalpindi.

- 175 stool samples cultured directly onto:
  - chromID CARBA (bioMérieux)
  - Brilliance CRE (Oxoid)

- All recovered colonies were stored and sent to Freeman Hospital for processing.
Fig. 1: *E. coli* (pink/red colonies) and *K. pneumoniae* (blue/green colonies) on chromID CARBA (left) and *Brilliance* CRE (right) isolated
Findings:

- 32 / 175 patients had faecal carriage of CPE (prevalence: 18.3%).
- For all 32 patients, CPE were recovered using chromID CARBA (sensitivity: 100%).
- CPE were recovered from 20 / 32 patients using *Brilliance* CRE (sensitivity: 62.5%).
- A large number of Enterobacteriaceae with ESBL were recovered on *Brilliance* CRE that may have hindered the isolation on CPE.
- It is likely that deterioration of selective agents in *Brilliance* CRE occurred during transportation of media to Pakistan.
Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media


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ORIGINAL ARTICLE

Prevalence of NDM-1 carbapenemase in patients with diarrhoea in Pakistan and evaluation of two chromogenic culture media


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Abstract
Aims: To evaluate two chromogenic media, Brilliance CRE and chromID CARBA, with stool samples referred to the Public Health Laboratories Division of the National Institute of Health in Islamabad, and assess the prevalence of
E. coli and K. pneumoniae (both with OXA-48 carbapenemase) on chromID OXA-48.
Turkey Study I: Hacettepe Üniversitesi Tıp Fakültesi, Ankara.

- Rectal swabs from 302 distinct hospitalized patients samples were cultured using:
  - chromID CARBA (bioMérieux)
  - chromID OXA-48 (bioMérieux)
  - TSB plus ertapenem + subculture on MacConkey agar (CDC method).

- All recovered colonies were stored and sent to Freeman Hospital for processing.
Evaluation of chromID OXA-48 for the recovery of carbapenemase-producing *Enterobacteriaceae* from rectal swabs from hospitalized patients in Ankara, Turkey

<p>| Table 1: Total number of colonized patients detected by each method and by combinations of methods. |
|--------------------------------------------------|---------------------------------|-----------------|-------------|-------------|-------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tr>
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<td>75.8</td>
<td>94.4</td>
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</tr>
</tbody>
</table>

302 distinct patients were screened.
Evaluation of a new chromogenic medium, chromID OXA-48, for recovery of carbapenemase-producing Enterobacteriaceae from patients at a university hospital in Turkey


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DOI 10.1007/s10096-014-2255-z
Don’t expect to isolate CPE from stool samples using disc susceptibility testing!

*E. coli* with VIM carbapenemase.
*K. pneumoniae* (with CTX-M and DHA-1) mixed with *E. coli* (with VIM-1 carbapenemase)

chromID CPS
(plus ertapenem disc)  chromID CARBA
Conclusions / Opinions for screening media:

- Don’t use the CDC broth method.
- Don’t rely on disc testing direct from clinical samples.
- Don’t use chromogenic media designed for detection of ESBL producers.
- Do use chromogenic agars specifically designed for the isolation of CPE. (or a validated ‘molecular’ method)
Confirmation of carbapenemase activity is necessary from colonies isolated on any medium.

Further investigations could include:
- Hodge Test
- Colorimetric tests (e.g. CARBA NP test)
- Inhibitor disc combinations (e.g. from MAST or Rosco).
- MALDI-TOF Mass spectrometry.
- Molecular methods (e.g. PCR, Microarrays)
- If in doubt: Refer for molecular investigations.
Hodge test:

- Can be useful if you obtain a strong positive result but sensitivity and specificity are not great and better tests are available.
- e.g. Sensitivity for detection of MBLs has been found to be as low as 12%. ¹

¹Doyle et al. JCM 2012; 50: 3877-80.
Colorimetric tests:

- CARBA NP test.
  - (e.g. J Med Microbiol. 2014: 63: 772-6)
- Rapid CARB screen test.
  - (e.g. J Clin Microbiol. 2014: 52: 3060-3)
- Blue-CARBA
  - (e.g. J Clin Microbiol. 2013: 51: 4281-3)

[Image of colorimetric test results]
CARBA NP test: last 6 publications:

- Sensitivity = 88% Osterblad et al. AAC. 2014 58:7553-6.

- 5/6 report… “consistent problems with detection of OXA-carbapenemases (e.g. OXA-48)”
- Specificity is consistently excellent for the CARBA NP test.
Disc susceptibility testing with carbapenemase inhibitors:

- Examples;
- Rosco Diagnostica Neo-Sensitabs
- Mastdiscs ID inhibitor combination discs
- KPC
- NDM-1
- OXA-48
- VIM / IMP

JCM. 2012; 50: 3877-80
MRP = Meropenem;  
MRPCX = Meropenem + cloxacillin  
MRPBO = Meropenem + boronic acid.  
MRPDP = Meropenem + dipicolinic acid  
TEMOC = Temocillin
Interpretation:

Meropenem

- Synergy with Boronic acid (BA)
  - Class A carbapenemase (e.g. KPC)

- Synergy with BA AND cloxacillin.
  - Amp C / porin loss

- Synergy with DPA.
  - Metallo-carbapenemase. (e.g. NDM, IMP, VIM)

Temocillin

- High level resistance. (MIC > 32 mg/L)
  - OXA-48-like.

High probability of CPE: refer for PCR testing to confirm.
A Meropenem
B Meropenem + Boronic
C Meropenem + Cloxacillin
D Meropenem + DPA
E Temocillin

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Result key:
0 = Incomplete Input
1 = KPC
2 = MBL
3 = AMP C and Porin Loss
4 = Indeterminate
-1 = Error
5 = Oxa-48
MALDI-TOF Mass Spectrometry:
MALDI-TOF Mass Spectrometry:

- Excellent results achieved within 30 minutes (sensitivity 98-100%)
- Inexpensive (after purchase of instrument)
- Some expertise / experience required.
- At least one commercial kit should be launched in 2015.

Molecular methods:

- These include real-time PCR and Microarray methods.
- Sensitivity and specificity are close to 100% although ‘gene-coverage’ of assays may vary.

- Jeong et al. *J Microbiol Methods.* 2015;113:4-9

- The use of PCR methods for direct testing of clinical samples is very promising:

Culture positive rectal swabs on an appropriate medium to identify CPE.

- Real-time PCR
- Detection of NDM, VIM, KPC, OXA-48, IMP-1 in less than 1 hour.
- Minimal hands-on time.
- Expensive.
- May not detect rare carbapenemases.
Ertapenem (< 25 mm) (Except *Enterobacter* fully susceptible to meropenem)

2 h Colorimetric test (e.g. Carba NP) plus inhibitor discs (overnight).

Positive in either assay = presumptive CPE

PCR (and refer positives to reference lab)
Take-home messages:

- Ertapenem is a sensitive screening agent for CPE using disc susceptibility testing with acceptable specificity in routine practice.
- For screening stool samples, use a commercially available chromogenic medium designed for the isolation of CPE.
- Some media are better than others! Look at a range of literature sources to decide on the best choice.
- If you are not following National Guidelines, ensure that you have good evidence for whatever method you are using.
- PCR testing of rectal swabs has been applied successfully and may be useful in outbreak situations or if prompt results are essential.
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Thank you for listening!