Isolation of carbapenemase-producing *Enterobacteriaceae* (CPE) from stool samples.


John Perry
Freeman Hospital Microbiology Department

Transparency declaration: Freeman Hospital Microbiology Department receives sponsorship from bioMérieux for development and evaluation of culture media.
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
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</th>
<th>Brilliance 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><em>n</em></td>
<td>High&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Low&lt;sup&gt;b&lt;/sup&gt;</td>
<td>High</td>
<td>Low</td>
<td>High</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>
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<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>
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**Other β-lactamases**

<table>
<thead>
<tr>
<th></th>
<th>ESB L</th>
<th>Amp C</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><em>n</em></td>
<td>49</td>
<td>21</td>
<td>High&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Low&lt;sup&gt;b&lt;/sup&gt;</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
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<td>ESBL</td>
<td>49</td>
<td>19</td>
<td>16</td>
<td>11</td>
<td>5</td>
<td>46</td>
<td>43</td>
<td>14</td>
<td>9</td>
<td>44</td>
<td>12</td>
<td>35</td>
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<td></td>
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<tr>
<td>Amp C</td>
<td>21</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>20</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>19</td>
<td>10</td>
<td>16</td>
<td>5</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>28</td>
<td>24</td>
<td>17</td>
<td>8</td>
<td>66</td>
<td>57</td>
<td>21</td>
<td>16</td>
<td>63</td>
<td>22</td>
<td>51</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></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          |     |     |     |     |     |     |

<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>
<thead>
<tr>
<th></th>
<th>Brilliance</th>
<th>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><em>Acinetobacter</em> spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>2</td>
<td>9</td>
<td>12</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td><em>S. maltophilia</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></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>
<td></td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>99</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp.</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus</em> sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total isolates</td>
<td>6</td>
<td>14</td>
<td>37</td>
<td>4</td>
<td>167</td>
<td>155</td>
<td></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 β-lactamase or both.*
Conclusions:

- CDC broth method lacks sensitivity and specificity (and takes a day longer than chromogenic media to obtain results).

- 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

Microbiology Department, Freeman Hospital, Newcastle upon Tyne, United Kingdom; Microbiology Department, Royal Hallamshire Hospital, Sheffield, United Kingdom; and School of Life Sciences, University of Northumbria, Newcastle upon Tyne, United Kingdom

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: Brilliance 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

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
Pakistan Study II: Armed Forces Institute for Pathology, Rawalpindi.

- 175 stool samples cultured directly onto:
  - chromID CARBA (bioMerieux)
  - 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.
Risk factor analysis: Association between length of hospitalization and CPE carriage at a military hospital in Pakistan:
Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media


Abstract

The aim of this study was to assess the frequency and genotypic diversity of carbapenemase-producing Enterobacteriaceae (CPE) in stool samples from patients attending a military hospital in Pakistan. Further aims included the identification of factors that might predispose to faecal carriage and evaluation of 2 chromogenic culture media: Buffalo CPE and chromID CARBA. Of 155 patients, 72 (46.3%) had faecal carriage of CPE and all
Pakistan Study III: National Institute of Health, Islamabad.

- 152 stool samples from outpatients with diarrhoea were cultured directly onto:
  - chromID CARBA (bioMerieux)
  - Brilliance CRE (Oxoid)

- All recovered colonies were stored and sent to Freeman Hospital for processing.
Findings:

- 13 / 152 outpatients had faecal carriage of CPE (prevalence: 8.6%).
- CPE were recovered from 12 / 13 patients using chromID CARBA (sensitivity: 92%).
- CPE were recovered from 7 / 13 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.
For further details see:

Original Article

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

K.M. Day¹, M. Salman², B. Kazi², H.E. Sidjabat³, A. Silvey³, C.V. Lanyon⁴, S.P. Cummings⁴, M.N. Ali², M.W. Raza¹, D.L. Paterson³ and J.D. Perry¹

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2 Public Health Laboratories Division, National Institute of Health, Islamabad, Pakistan
3 University of Queensland Centre for Clinical Research, Brisbane, Qld, Australia
4 School of Life Sciences, University of Northumbria, Newcastle upon Tyne, UK

Correspondence
John D. Perry, Department of Microbiology, Freeman Hospital, Freeman Road, High Heaton, Newcastle upon Tyne NE7 7DN, UK.
E-mail: john.perry@nuth.nhs.uk

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 (bioMerieux)
  - chromID OXA-48 (bioMerieux)
  - 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

**TABLE 1: Total number of colonized patients detected by each method and by combinations of methods.**

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC method</td>
<td>19</td>
<td>57.6</td>
<td>95.2</td>
<td>59.4</td>
<td>94.8</td>
</tr>
<tr>
<td>chromID OXA-48</td>
<td>25</td>
<td>75.8</td>
<td>99.3</td>
<td>92.6</td>
<td>97.1</td>
</tr>
<tr>
<td>chromID Carba</td>
<td>19</td>
<td>57.6</td>
<td>98.9</td>
<td>86.4</td>
<td>95</td>
</tr>
<tr>
<td>chromID OXA-48 plus CDC method</td>
<td>30</td>
<td>90.9</td>
<td>94.8</td>
<td>68.2</td>
<td>98.8</td>
</tr>
<tr>
<td>chromID OXA-48 plus chromID Carba</td>
<td>30</td>
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<td>75.8</td>
<td>94.4</td>
<td>62.5</td>
<td>96.9</td>
</tr>
</tbody>
</table>

302 distinct patients were screened. For further information:

Evaluation of chromID OXA-48 for the recovery of carbapenemase-producing Enterobacteriaceae from rectal swabs from hospitalized patients in Ankara, Turkey

P. Zarakolu,1 K.M. Day,2,3 C. V. Lanyon,2 S. P. Cummings,2 M. Akova,1 J. D. Perry,3*
1Hacettepe University School of Medicine, Ankara, Turkey, 2Northumbria University, Newcastle upon Tyne, UK
3 Freeman Hospital, Newcastle upon Tyne, UK (*john.perry@nuth.nhs.uk)

Background: There is a pressing need to define robust standardized screening methods for the effective detection of carbapenemase-producing Enterobacteriaceae (CPE) in order to control their spread.1 To address this need the Centers for Disease Control recommended a broth enrichment method that could be used in almost any laboratory.2 This method entails the inoculation of a rectal swab into 5 ml of trypticase soy broth (TSB) to which a 10 µg carbapenem disc has been added (meropenem or ertapenem). The broth is then subcultured after overnight incubation at 37°C onto MacConkey agar. However, it is increasingly recognized that chromogenic culture media may have a useful role to play in the efficient detection of CPE.3,4

Purpose of the Study: The aim of this study was to critically assess three culture methods for screening hospitalized patients in Ankara, Turkey, for potential gut colonization with CPE. These three methods comprised: enrichment culture using TSB plus 2 mg/L ertapenem as recommended by CDC; direct culture on an established chromogenic agar designed for detection of CPE (chromID CARBA) and, direct culture on a chromogenic agar specifically designed for isolation of CPE that produce OXA-48 carbapenemase (chromID OXA-48).

Materials and Methods

Rectal swabs were obtained from 302 distinct patients as part of routine screening for CPE. Material from swabs was suspended in 0.5 ml sterile saline (0.85%) and 50 µl inoculated onto chromID OXA-48, chromID Carba and 5mL TSB containing a 10µg ertapenem disc. All media were incubated for 18-20 h at 37°C. After incubation, 10 µL of broth was inoculated onto MacConkey agar and incubated overnight at 37°C. All isolates were identified by MALDI-TOF MS. Any Enterobacteriaceae isolated on any of the three media were screened for possible carbapenemase production in accordance with UK national guidelines using the KPC, MBL & OXA48 confirm ID kit (Rosco) and confirmed using PCR for the five most common carbapenemase genes (OXA-48, KPC, VIM, IMP and NDM-1).

References


Results

Table 1: Total number of colonized patients detected by each method and by combinations of methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
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</tr>
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<tr>
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<td>86.4</td>
<td>95</td>
</tr>
<tr>
<td>chromID OXA-48 plus CDC method</td>
<td>30</td>
<td>90.9</td>
<td>94.8</td>
<td>68.2</td>
<td>98.8</td>
</tr>
<tr>
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<td>25</td>
<td>75.8</td>
<td>94.4</td>
<td>62.5</td>
<td>96.9</td>
</tr>
</tbody>
</table>

A total of 33 patients (11%) were found to be colonized with CPE out of 302 distinct patients who were screened. Klebsiella pneumoniae was by far the most prominent species of CPE and was isolated from 31 of the 33 colonized patients. All isolates of CPE were confirmed as harboring OXA-48 carbapenemase as confirmed by both phenotypic testing and PCR. No other carbapenemases were detected in the isolates of Enterobacteriaceae. Table 1 shows the sensitivity of each method (and combinations of the three methods) for detection of colonized patients.

Direct culture of stool samples onto chromID OXA-48 was the most sensitive single method in this setting (sensitivity: 75.8%; P = 0.2) and the use of a combination of chromogenic media increased the sensitivity to 90.9%. Use of the CDC broth enrichment method resulted in a relatively poor positive predictive value (due to the recovery of 18 false positives) and had the disadvantage of requiring 2 days for isolation of colonies.

Conclusion

• chromID OXA-48 was the best single method for screening for CPE in this setting where only OXA-48 producers were encountered, and was more sensitive than the CDC broth method.

• A combination of chromID OXA-48 with chromID CARBA offered isolation of presumptive carbapenemase-producing Enterobacteriaceae within 18-20 h with high sensitivity (90.9%) and specificity (98.5%). All CPE recovered on either chromogenic medium formed coloured colonies allowing easy discrimination from other organisms e.g. Acinetobacter species.

Acknowledgements

The authors are grateful to bioMérieux, for sponsorship of this study.
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)
*K. pneumoniae* (with CTX-M and DHA-1) mixed with *E. coli* (with VIM-1 carbapenemase)
Confirmation of carbapenemase activity is necessary from colonies isolated on any medium.

Further investigations could include:
• Molecular methods (e.g. PCR, Microarrays)
• Hodge Test (poor sensitivity and specificity)
• Colorimetric tests (e.g. CARBA NP test)
• MALDI-TOF Mass spectrometry.
• Inhibitor disc combinations (e.g. from MAST or Rosco).
• If in doubt: Refer for molecular investigations.
MRP = Meropenem;
MRPCX = Meropenem + cloxacillin  
MRPBO = Meropenem + boronic acid.
MRPDP = Meropenem + dipicolinic acid  
TEMOC = Temocillin
A Meropenem
B Meropenem + Boronic
C Meropenem + Cloxacillin
D Meropenem + DPA
E Temocillin

<table>
<thead>
<tr>
<th>Reference</th>
<th>Comments</th>
<th>A (mm)</th>
<th>B (mm)</th>
<th>C (mm)</th>
<th>D (mm)</th>
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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
UK Standards for Microbiology Investigations

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

Issued by the Standards Unit, Microbiology Services, PHE UK Protocols | P 8 | Issue no: 1.1 | Issue date: 08.05.14 | Page: 1 of 25
UK Standards for Microbiology Investigations:

“In light of the limited available evidence we would currently recommend that if stool samples or rectal swabs require screening for CPE, the method chosen should have demonstrated performance at least equivalent to plating on to a commercially-prepared chromogenic agar medium specifically recommended for this purpose. It is essential that suspect colonies are then subjected to confirmatory tests as previously described”

http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317138520481
Take-home messages:

• 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 stool samples has been applied successfully and may be useful in outbreak situations.
Main collaborators:

- Freeman Hospital; **Kathryn Day**, Muhammad Raza, John Perry

- AFIP, Rawalpindi; Shahid Abbasi, Sakeenah Naqvi, Aamir Hussain, Irfan Ali Mirza, Shamshad Ali

- PHE Colindale; Jiancheng Zhang, David M. Livermore, Neil Woodford.

- NIH, Islamabad; Muhammad Salman, Birjees Kazi, Mehrun N. Ali.

- University of Queensland; Hanna E. Sidjabat, Anna Silvey, Witchuda Kamolvit, David L. Paterson.

- Hacettepe University School of Medicine, Ankara; Pinar Zarakolu, Murat Akova.

- bioMérieux, La Balme-les-Grottes; Sandrine Ghirardi, Gilles Zambardi, Sylvain Orenga.