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Comparison of two chromogenic media for the detection of extended-spectrum  $\beta$ -lactamase producing *Enterobacteriaceae* stool carriage in nursing home residents

Beth Blane, Hayley J. Brodrick, Theodore Gouliouris, Kirsty E. Ambridge, Angela D. Kidney, Catherine M. Ludden, Direk Limmathurotsakul, M. Estée Török, Sharon J. Peacock

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**Note****Comparison of two chromogenic media for the detection of extended-spectrum  $\beta$ -lactamase producing *Enterobacteriaceae* stool carriage in nursing home residents**

Running Title: Chromogenic media for ESBL-positive *Enterobacteriaceae*

Beth Blane,<sup>1</sup> Hayley J. Brodrick,<sup>1</sup> Theodore Gouliouris,<sup>1,2,3</sup> Kirsty E. Ambridge,<sup>1</sup> Angela D. Kidney,<sup>1</sup> Catherine M. Ludden,<sup>1</sup> Direk Limmathurotsakul,<sup>4</sup> M. Estée Török,<sup>1,2,3</sup> Sharon J. Peacock.<sup>1,2,3</sup>

<sup>1</sup>Department of Medicine, University of Cambridge, Cambridge, United Kingdom

<sup>2</sup>Public Health England, Clinical Microbiology and Public Health Laboratory, Cambridge, United Kingdom

<sup>3</sup>Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom

<sup>4</sup>Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand

**Corresponding author:** Beth Blane. Department of Medicine, University of Cambridge, Level 5, Box 157, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, United Kingdom.  
Tel: +44 (0)1223 336839; Fax: +44(0)1223 336846; Email: eb544@medschl.cam.ac.uk

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**Abstract**

ChromID ESBL agar and *Brilliance* ESBL agar were compared for the isolation of extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae* from 298 stools. These had comparable sensitivity and selectivity for the 116 positive samples. Pre-enrichment with cefpodoxime and extending incubation to 48 hours after direct plating both significantly increased sensitivity but reduced selectivity of both agars.

**Keywords** Extended-spectrum  $\beta$ -lactamase, *Enterobacteriaceae*, detection, chromogenic agar, sensitivity, selectivity

**Text**

The incidence of bloodstream infections caused by extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* is rising in Europe and the United States of America (European Centre for Disease Prevention and Control, 2013; Castanheira *et al*, 2013). This trend is mirrored by the global dissemination of other ESBL-producing species, particularly *Klebsiella* spp. (World Health Organisation, 2014). Infections caused by ESBL-producing *Enterobacteriaceae* are associated with increased mortality, length of stay, and hospital costs compared to non-ESBL producing *Enterobacteriaceae* (Schwaber & Carmeli, 2007; Tumbarello *et al*, 2010). Screening for rectal carriage of ESBL-producing organisms has been advocated as an infection control strategy particularly during outbreaks (Siegel *et al*, 2006), and is routinely performed in some healthcare settings (Murk *et al*, 2009). The advent of chromogenic media has improved the detection of ESBL-producing *Enterobacteriaceae* (Glupczynski *et al*, 2007; Huang *et al*, 2010; Réglie-Poupet *et al*, 2008). However, few studies have assessed the yield of target organisms using different culture methodologies with conflicting results reported on the value of prolonging direct incubation to 48 h (Réglie-Poupet *et al*, 2008; Willems *et al*, 2013) or of using a pre-enrichment step (Murk *et al*, 2009; Diederer *et al*, 2012). The aim of this method comparison study was to assess the sensitivity and selectivity of two chromogenic media with or without pre-enrichment to detect ESBL-producing *Enterobacteriaceae* in stool during a longitudinal carriage survey of ESBL-producing organisms in residents of a nursing home in Cambridgeshire. The study protocol was approved by the National Research Ethics Service East of England Ethics Committee (NRES ref: 13/LO/1278), and the Cambridge University Hospitals NHS Foundation Trust Research and Development Department. Written informed consent was obtained from participants or consultees, as appropriate.

A total of 298 stool samples were collected from 37 residents over a 14-week period, commencing in March 2014. Stools were refrigerated and processed within 24 hours, unless collected over the weekend when they were processed within 72 hours. We performed direct plating onto chromID ESBL agar (bioMérieux, Marcy l'Etoile, France) and *Brilliance* ESBL agar (Oxoid, Basingstoke, UK). In addition, we plated onto both media after a selective pre-enrichment step using cefpodoxime [Health Protection Agency, 2012]. For direct plating, approximately 0.2 g of stool was plated onto each agar. For selective pre-enrichment, approximately 0.2 g of stool was added to 10ml of Tryptic Soy Broth (Sigma, Dorset, UK) containing cefpodoxime 1 µg/ml (Oxoid, Basingstoke, UK), vortexed, and incubated with shaking at 150 rpm at 37°C in air overnight. The following day, 200 µl of suspension was inoculated onto each agar. All agar plates were incubated at 37°C in air and inspected after 24 and 48 hours. Presumptive *Enterobacteriaceae* were selected from chromID and *Brilliance* ESBL agars based on colony colour as defined by the manufacturers' instructions. Sub-cultured isolates were initially identified using MATRIX-ASSISTED LASER DESORPTION/IONISATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS, Biotyper version 3.1, SR\_Clinical library) (Bruker Daltonics, Bremen, Germany) with a MALDI-TOF MS score of 2.0 or higher. Bacterial species and the presence of genes encoding ESBL were subsequently confirmed from whole genome sequence data. DNA was extracted using the QIAextractor (QIAGEN), according to the manufacturer's instructions. Library preparation was conducted according to the Illumina protocol, and sequencing was performed on an Illumina HiSeq2000 with 100-cycle paired-end runs. Sequence reads were mapped using SMALT (<http://www.sanger.ac.uk/resources/software/smalt/>) to relevant reference genomes (*Klebsiella*

*pneumoniae* NTUH K2044 (GenBank accession number NC\_006625.1), and *Escherichia coli* EC958 (GenBank accession number NZ\_HG941718)). Molecular confirmation of the presence of at least one gene known to encode ESBL was sought by *in silico* PCR using the ResFinder database (Zankari *et al*, 2012). Sequence data for all isolates have been submitted to the European Nucleotide Archive (ENA) ([www.ebi.ac.uk/ena](http://www.ebi.ac.uk/ena)) with the accession numbers shown in Supplementary Table 1. Antimicrobial susceptibility testing was performed using the N206 card on the Vitek 2 instrument (bioMérieux) calibrated against EUCAST breakpoints. ESBL-production was confirmed using the AmpC and ESBL detection disc test D68C (Mast Group, Merseyside, UK) as per the manufacturer's instructions. Colonies with a colour/morphology combination that were not consistent with *Enterobacteriaceae* growing on either media were examined by Gram stain and microscopy with additional identification to species level using MALDI-TOF MS, as required.

Sensitivity of detection of ESBL-producing *Enterobacteriaceae* was calculated by comparing the number of positive plates for a given method with the cumulative yield of ESBL-producing organisms from all four methods (direct plating onto two media, and plating onto both following a pre-enrichment step). Selectivity for each method was defined as the number of plates that suppressed the growth of organisms that were not ESBL-producers. Statistical analysis was performed using STATA, version 12.1 (STATA, College Station, Texas, USA). Sensitivity and selectivity were compared using the exact McNemar test.

ESBL-producing organisms were isolated from 116/298 (39%) stool samples. Fifteen of the 37 residents tested were positive on at least one occasion (range 2 to 15 positive samples, median 8).

All 116 isolates were positive for the *bla*<sub>CTX-M-15</sub> gene and two isolates also carried the *bla*<sub>SHV-28</sub> gene. The majority were *E. coli* (111/116, 96%), and the remainder were *Klebsiella pneumoniae* (5/116, 4%). No stool samples grew more than one ESBL-producing species. Sensitivity of detection following direct plating was higher when incubated for 48 hours versus 24 hours (75% versus 63% for chromID agar,  $p=0.0001$ ; 68% versus 59% for *Brilliance* agar,  $p=0.001$ ). Using pre-enrichment with 24 hours incubation further increased the sensitivity of detection of ESBL-producing organisms (from 75% to 97% for chromID agar,  $p<0.0001$ ; and from 68% to 98% for *Brilliance* agar,  $p<0.0001$ ). We speculate that pre-enrichment improved sensitivity due to carriage of low levels of ESBL-producing organisms, although bacterial load was not formally quantified. Increasing the duration of incubation to 48 hours after a pre-enrichment step did not increase the detection rate further ( $p>0.99$  for both agars). Although the sensitivity to detect ESBL producers using chromID agar was higher than *Brilliance* agar for the direct plating method with 48 hours incubation (75% versus 68%,  $p=0.04$ ), the sensitivity was comparable for the enrichment method either with 24 hours or 48 hours incubation (both  $p$  values = 0.50) (table 1).

Selectivity was lower at 48 hours versus 24 hours after direct plating for both media (from 70% versus 85% for chromID agar,  $p<0.0001$ ; 64% versus 87% for *Brilliance* agar,  $p<0.0001$ ).

Selectivity further declined when a pre-enrichment step was added followed by plating and 24 hours incubation for chromID agar (70% to 63%,  $p=0.02$ ) and a similar trend was observed for *Brilliance* agar (64% to 61%,  $p=0.26$ ). Increasing the duration of incubation to 48 hours after a pre-enrichment step significantly decreased the selectivity for both agars (from 63% to 55% for chromID agar,  $p<0.0001$  and from 61% to 58% for *Brilliance* agar,  $p=0.002$ ). Although

selectivity of chromID agar was higher than *Brilliance* agar for the direct plating method with 48 hours incubation (70% versus 64%,  $p=0.02$ ), their selectivity was comparable for the enrichment method either with 24 hours or 48 hours incubation (both  $p$  values  $> 0.20$ ).

Both media supported the growth of a range of species that were not ESBL-producing *Enterobacteriaceae*. The majority were *Pseudomonas* spp. (Table 2), although these were distinguishable based on colour and colonial morphology. The most problematic group were a small number of *Enterobacteriaceae* that displayed the expected phenotype but were not ESBL-producers, based on Vitek 2 testing. Further testing showed that the majority were AmpC hyper-producing *Citrobacter* spp., the remainder being *E. coli* or *K. pneumoniae* that appeared to lack ESBL or AmpC activity and for which the basis of growth on the media was not known (table 2). The *E. cloacae* did not contain a gene encoding an ESBL based on *in silico* PCR of whole genome sequence data for this isolate (data not shown).

In summary, plating after pre-enrichment followed by 24 hours incubation combines high sensitivity with a 48-hour turnaround time, and using this method both ESBL *Brilliance* and chromID agars had comparable sensitivity and selectivity for the isolation of ESBL-producing *Enterobacteriaceae* from stool. There was a loss of selectivity after 48 hours incubation for all methods and when using pre-enrichment due to increased growth of non-ESBL-producing organisms.



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**Table 1.** Sensitivity and selectivity of two chromogenic agars for the isolation of ESBL-producing *Enterobacteriaceae* from 298 stool samples

|  | <b>ChromID</b>             |     | <i>Brilliance</i>          |     | <b>ChromID</b>                       |     | <i>Brilliance</i>                    |     |
|--|----------------------------|-----|----------------------------|-----|--------------------------------------|-----|--------------------------------------|-----|
|  | <b>ESBL direct plating</b> |     | <b>ESBL direct plating</b> |     | <b>ESBL plating after enrichment</b> |     | <b>ESBL plating after enrichment</b> |     |
| Incubation time (hours)  | 24                         | 48  | 24                         | 48  | 24                                   | 48  | 24                                   | 48  |
| Number of plates growing ESBL-producing organisms                | 73                         | 87  | 68                         | 79  | 112                                  | 113 | 114                                  | 115 |
| Sensitivity*   | 63%                        | 75% | 59%                        | 68% | 97%                                  | 97% | 98%                                  | 99% |
| Number of plates NOT growing organisms other than ESBL-producers | 252                        | 210 | 258                        | 192 | 189                                  | 163 | 182                                  | 172 |
| Selectivity <sup>†</sup>   | 85%                        | 70% | 87%                        | 64% | 63%                                  | 55% | 61%                                  | 58% |

\* Sensitivity was calculated by comparing the number of positive plates for a given method, compared with the cumulative yield of ESBL-producing organisms from all four methods (direct plating onto two media, and plating onto both following a pre-enrichment step, n=116/298)

<sup>†</sup> Selectivity for each method was defined as the number of plates that suppressed the growth of organisms that were not ESBL-producers

**Table 2.** Details of non-ESBL-producing organisms growing on two chromogenic agars\*

|   | <b>ChromID</b>             |    | <i>Brilliance</i>          |    | <b>ChromID</b>                       |    | <i>Brilliance</i>                    |     |
|---|----------------------------|----|----------------------------|----|--------------------------------------|----|--------------------------------------|-----|
|   | <b>ESBL direct plating</b> |    | <b>ESBL direct plating</b> |    | <b>ESBL plating after enrichment</b> |    | <b>ESBL plating after enrichment</b> |     |
| Incubation time (hours)                   | 24                         | 48 | 24                         | 48 | 24                                   | 48 | 24                                   | 48  |
| <i>Pseudomonas</i> spp.                   | 17                         | 52 | 28                         | 83 | 65                                   | 86 | 91                                   | 102 |
| Other Gram-negative non-fermenters        | 4                          | 6  | 1                          | 3  | 13                                   | 15 | 2                                    | 2   |
| <i>Enterobacteriaceae</i>                 |                            |    |                            |    |                                      |    |                                      |     |
| AmpC <sup>+</sup> <i>Citrobacter</i> spp. | 12                         | 15 | 7                          | 13 | 21                                   | 22 | 21                                   | 21  |
| <i>E. coli</i>                            | 1                          | 1  | 0                          | 1  | 2                                    | 3  | 2                                    | 2   |
| <i>K. pneumoniae</i>                      | 0                          | 0  | 0                          | 0  | 0                                    | 1  | 1                                    | 1   |
| <i>E. cloacae</i>                         | 0                          | 0  | 0                          | 0  | 1                                    | 1  | 1                                    | 1   |
| Gram-positive cocci                       | 28                         | 34 | 4                          | 8  | 30                                   | 34 | 1                                    | 3   |
| Yeasts                                    | 0                          | 0  | 0                          | 0  | 0                                    | 1  | 0                                    | 0   |

\*Some plates grew more than one species and totals may not tally with sensitivity results shown in table 1, which used agar plate as the denominator

## Highlights

- Two chromogenic agars were compared for the isolation of ESBL *Enterobacteriaceae*
- Both agars had comparable sensitivity and selectivity
- Sensitivity after direct plating was higher when incubated for 48 versus 24 hours
- Using pre-enrichment with 24 hours incubation further increased the sensitivity
- There was a loss of selectivity after 48 hours incubation for all methods

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