Evaluation of the Oxoid Brilliance™ CRE Agar for detection of carbapenemase producing Enterobacteriaceae

James Cohen Stuart, Guido Voets, Sebastiaan Voskuil, Wouter Rottier
Jelle Scharringa, Ad Fluit, Maurine Leverstein-van Hall.

Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands

University Medical Center Utrecht

Introduction

The emergence and spread of carbapenemase producing Enterobacteriaceae (CPE) poses a considerable threat to clinical patient care and public health. Invasive infections with these strains are associated with high rates of morbidity and mortality. Swift and accurate detection of CPE is essential for adequate antibiotic therapy, and for infection control purposes, especially in an outbreak setting. Selective agars play an essential role in the detection of such isolates in clinical specimens.

Objectives

1.To determine the test characteristics of the Oxoid Brilliance™ CRE Agar (Thermo Fisher Scientific, United Kingdom)(CRE Agar) for detection of CPE.
2. To determine the capacity of the CRE agar to detect carbapenem non-susceptible Enterobacteriaceae.
3. To investigate the species-specificity of the colony colours.

Methods

Test collection: included 234 well characterized non-repeat Enterobacteriaceae isolates. PCR and sequencing of beta-lactamases was used as the reference test

74 carbapenemase *positive* isolates: 56 *Klebsiella pneumoniae*, 8 *Escherichia coli*, 6 *Enterobacter* spp., 2 *Proteus mirabilis*, and 2 *Serratia marcescens isolates* producing the following carbapenemases: 36 KPC -2/3, 4 KPC plus VIM, 4 NDM-1, 6 GIM, 20 VIM, 4 OXA-48.

160 carbapenemase *negative* control isolates included 87 *E. coli*, 42 *Enterobacter* spp, 16 *K. pneumoniae*, 8 *Klebsiella oxytoca*, 4 *P. mirabilis*, one *Citrobacter freundii*, one *P. stuartii*, and one *S. marcescens,* of which 97 produced an ESBL (63 CTX-M, 14 TEM, 12 SHV, 8 other ESBL genes or combinations), 37 an AmpC (13 plasmid mediated, 24 chromosomal), 7 co-produced AmpC and ESBL, 8 isolates were K1 hyperproducing *K. oxytoca*, and 11 isolates were beta-lactamase negative.

The isolates (one per patient) originated from the following sources: 51 isolates were collected at a Dutch beta-lactamase reference centre (University Medical Centre Utrecht) in 2010 for detection of carbapenemase genes, 29 were from Greece, 20 from New York, 118 were from Dutch ESBL surveillance studies, 6 isolates were from Germany, and 10 were ATCC or NTCC reference strains.

The selective plates were inoculated with 10 micro-litres of a 0.5 McFarland suspension, corresponding to approximately 10e6 colony forming units (CFU). Plates were incubated under aerobic conditions and read after 24 and 48 hours
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The ertapenem and meropenem MICs were determined using broth micro-dilution (Merlin, Germany). Imipenem MICs were determined using Etest (Biomerieux, France). MICs were interpreted according to CLSI and EUCAST breakpoints .

Results

SENSITIVITY AND SPECIFICITY FOR DETECTION OF CPE

The *sensitivity* for detection of CPE after both 24 and 48 hours was 72/74 (97%)(**Table 1)**.

The growth of two VIM-1 positive isolates was inhibited on the CRE agar (an *E. coli*, and a *P. mirabilis* with ertapenem MICs of 0.25 and <0.0625 mg/L, respectively, with meropenem MICs of 0.5 and 2 mg/L, respectively, and both with an imipenem MIC of 6 mg/L.

The *specificity* for detection of CPE after 24 hours was only 71% **(Table 2).** This low specificity may be explained by the large number (of negative control isolates with an increased ertapenem MIC.
Of the 46 isolates growing on the agar, 43 (96%) had an ertapenem MIC ≥ 0.125 mg/L (MIC50 1 mg/L, MIC 90 > 8mg/L). The majority of these 46 control isolates were predominantly *E. cloacae (*24 (52%)) with an derepressed AmpC gene, and furhterrmore *E. coli* and *K. pneumoniae* with ESBL genes (CTX-M, SHV) or (plasmidal or chromosomal) AmpC genes.

CAPACITY TO DETECT CARBAPENEM NON-SUSCEPTIBLE ENTEROBACTERIACEAE

The sensitivity and specificity of the CRE agar to detect Enterobacteriaceae isolates non-susceptible to at least one carbapenem was 85% (111/130) and 93% (97/104) using the CLSI breakpoints, versus 89% (106/119) and 90 % (103/115),respectively, using the higher EUCAST carbapenem breakpoints.

The sensitivity to detect carbapenem I/R isolates (CLSI) depended on the presence of a carbapenemase gene. CPE isolates (all non-susceptible to at least one carbapenem) were detected with a sensitivity of 97% (Table 1), versus a sensitivity of only 70% (39/56) for isolates non-susceptible to at least one carbapenem due to other beta-lactamases (p<0.001).
This difference may be explained by the selective compounds in the CRE agar, which seem to inhibit the ESBL and AmpC expressing isolates, but not the CPE.
The 17 carbapenemase negative isolates with increased carbapenem MICs that failed to grow on the agar were 12 chromosomal AmpC producing *E. cloacae* isolates (of which one ESBL positive), one K1 hyperproducing *K. oxytoca* and 4 ESBL producers (one *K. pneumoniae*, two *E. coli* and one *P. mirabilis*).

COLONY COLORS

The colony colours of all *K. pneumoniae*, *Enterobacter* spp., *P. mirabilis* and *S. marcesens* isolates were blue, whereas *E. coli* colonies were brownish.

Table1 Growth characteristics of 74 CPE isolates

Carbapenemase positive	Species	n	Growth at 24 hrs
KPC 2/3	*E. coli* *K. pneumoniae*	4 32	4 32
KPC+VIM-1	*K. pneumoniae*	4	4
NDM-1	*K. pneumoniae* *E. coli*	3 1	3 1
GIM-1	*E. cloacae* *S. marcesens* *E. coli*	3 2 1	3 2 1
VIM-1	*K. pneumoniae* *E. coli* *E.cloacae* *P. mirabilis*	13 2 3 2	13 1* 3 1*
OXA-48	*K. pneumoniae*	4	4
Total		**74**	**72 (97%)**

Table 2 Growth characteristics of 160 carbapenemase negative Enterobacteriaceae isolates on the Oxoid CRE Brilliance agar.

Carba-penemase negative	Species	n	Growth at 24 hrs	ESBL/AmpC genes of isolates growing at 24 hrs (n)
ESBL	*E. coli* *K. pneumoniae* *P. mirabilis* *P. stuartii* *S. marcesens*	61 13 2 1 1	10 7 0 0 0	CTX-M (10) CTX-M (5) SHV (2)
Derepressed chromosomal AmpC* plus ESBL	*E. cloacae* *C. freundii*	21 1	18 0	CTX-M (13) SHV (5)
Plasmid AmpC	*E. coli*	11	2	CMY (2)
Derepressed chromosomal AmpC*	*E. cloacae* *E. coli*	20 6	6 0	Chromosomal AmpC (6)
ESBL plus plasmid AmpC	*K. pneumoniae* *E. coli*	3 1	2 1	CMY+SHV (1) MIR+SHV(1) MIR+CTX-M (1)
K1, non-ESBL TEM/SHV,no BL	*K. oxytoca*	19	0	
Total		**160**	**46 (29%)**	

* inferred from a cefoxitin MIC ≥ 16mg/L, a cefotaxime and ceftazidime MIC >32, and absence of synergy between clavulanic acid and cefotaxime and ceftazidime.

Conclusions

This first evaluation of the Oxoid Brilliance CRE Agar shows that it is a highly sensitive method for detection of CPE available within 24 hrs.

However, phenotypic or molecular confirmation of the carbapenemase gene is needed since the CRE agar can not differentiate between resistance mechanisms underlying the increased carbapenem MIC.

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