JCM
Journals.ASM.org

Comparison of Four Chromogenic Culture Media for Carbapenemase-Producing *Enterobacteriaceae*

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

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

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 a 10-μg ertapenem or meropenem disc followed by subculturing on MacConkey agar (1). The aim of this study was to evaluate the suitability of available chromogenic media that have been recommended for isolation of CPE. Four chromogenic media were assessed for their ability to support the growth of 130 carbapenemase-producing *Enterobacteriaceae* and inhibit the growth of 70 other *Enterobacteriaceae*: Brilliance CRE, Colorex KPC, chromID ESBL, and a prototype chromogenic medium (chromID Carba) provided by bioMérieux, based on the same principles as a previously evaluated medium (ID Carba) (4). TSBs supplemented with ertapenem or meropenem, as recommended by CDC (1), were included for comparison. All media were assessed for their ability to inhibit other commensal microorganisms in stool samples by inoculation of 100 stool samples from hospitalized patients.

chromID Carba (reference 43861) and chromID ESBL (reference 43481) were provided by bioMérieux, La Balme-les-Grottes, France. Colorex KPC (reference PP3033) was provided by E&O laboratories, Bonnybridge, United Kingdom. Brilliance CRE (reference PO1226A) and all other culture media were purchased from Oxoid, Basingstoke, United Kingdom. A collection of 200 *Enterobacteriaceae* were obtained from diverse international sources and all possessed β-lactamases that had been defined at a molecular level by reference laboratories and/or recognized experts in the field. A total of 130 were carbapenemase producers, including 88 with NDM-1 (*Citrobacter freundii*, $n = 5$; other *Citrobacter* spp., $n = 4$; *Enterobacter cloacae*, $n = 22$; *E. coli*, $n = 30$; *Klebsiella pneumoniae*, $n = 24$; *Kluyvera* sp., $n = 1$; *Providencia rettgeri*, $n = 2$), nine with IMP (*K. pneumoniae*, $n = 6$; *E. coli*, $n = 2$; *Klebsiella oxytoca*, $n = 1$), 12 with KPC (*K. pneumoniae*, $n = 11$; *K. oxytoca*, $n = 1$), 15 with OXA-48 (*K. pneumoniae*, $n = 9$; *E. coli*, $n = 4$; *E. cloacae*, $n = 2$), and six with VIM (*K. pneumoniae*, $n = 4$; *E. cloacae*, $n = 1$; *Serratia marcescens*, $n = 1$). A further 70 isolates were non-carbapenemase-producing *Enterobacteriaceae*, of which 49 produced a diverse range of extended-spectrum β-lactamases (ESBLs) and 21 produced Amp C β-lactamases.

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 blood agar, and CLED agar using a multipoint inoculator. All suspensions were diluted 1/1,000 in saline, and 1-μl aliquots were inoculated onto the same media. All plates were incubated for 48 h in air at 37°C and interpreted for growth and colony color after 18, 24, and 48 h of incubation. Final inocula on plates were predicted to be approximately 10^5 CFU/spot (high inoculum) and 10^2 CFU/spot (low inoculum). To validate the method of inoculum preparation, 10 strains were selected at random, 1 μl of the diluted suspension was inoculated onto each of three Columbia blood agar plates, and the inoculum was spread over the whole surface of the plates. After incubation for 24 h in air at 37°C, the average number of colonies for each isolate was recorded.

Each of the suspensions described above was further diluted 1/20 in saline, and 100 μl of each was used to inoculate 5 ml of TSB containing a 10-μg ertapenem disc and 5 ml of TSB containing a 10-μg meropenem disc. The final inocula in broths were predicted to be 10^5 CFU/ml and 10^2 CFU/ml. The 800 broths were incubated for 18 h at 37°C, and 100 μl of broth was then cultured onto MacConkey agar, which was incubated for 18 h at 37°C. All experiments described above were repeated in full on a separate occasion 3 weeks later. This was done using the same lots of prepoured chromogenic media but using freshly prepared TSBs with carbapenem discs added. The efficiencies of the chromogenic media for isolation of CPE were compared using McNemar's test with the continuity correction applied.

For stool samples, approximately 0.5 g of stool sample was suspended in 1 ml of 0.85% saline, and after vortexing, 50-μl aliquots were used to inoculate the four chromogenic media and

Received 18 June 2012 **Accepted** 28 June 2012
Published ahead of print 3 July 2012
Address correspondence to John D. Perry, john.perry@nuth.nhs.uk.

doi:10.1128/JCM.01613-12

TABLE 1 Numbers of *Enterobacteriaceae* with different ß-lactamases recovered on various culture media recommended for isolation of CPE after 18 h of incubation

Medium	Inoculum[a]	No. of isolates obtained producing:										
		Carbapenemases						Other β-lactamases				
		IMP (9[b])	KPC (12)	NDM (88)	OXA (15)	VIM (6)	Total (130)	ESBL (49)	AmpC (21)	Total (70)	Sensitivity (%)	Specificity (%)
Brilliance CRE	High	1	11	77	12	5	106	19	9	28	82	60
	Low	1	10	75	10	5	101	16	8	24	78	66
chromID Carba	High	9	12	85	13	6	125	11	6	17	96	76
	Low	8	12	83	10	5	118	5	3	8	91	89
chromID ESBL	High	9	12	87	12	6	126	46	20	66	97	6
	Low	9	12	86	12	6	125	43	14	57	96	19
Colorex KPC	High	9	12	76	12	5	114	14	7	21	88	70
	Low	1	10	50	9	3	73	9	7	16	56	77
TSB plus ertapenem	High	9	12	87	15	6	129	44	19	63	99	10
	Low	6	10	71	11	4	102	12	10	22	78	69
TSB plus meropenem	High	9	12	85	14	6	126	35	16	51	97	27
	Low	0	8	45	6	2	61	10	5	15	47	79

[a] High inocula were approximately 100,000 CFU/spot for chromogenic media and 100,000 CFU/ml for broth media. Low inocula were approximately 100 CFU/spot for chromogenic media and 100 CFU/ml for broth media.
[b] Numbers in parentheses are total numbers of isolates.

TSBs supplemented with ertapenem or meropenem discs. These media were incubated and processed as described above. All colonies recovered on all media were identified using MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry (Bruker Daltonics, Coventry, United Kingdom) with complementary biochemical tests where required. Any isolates of *Enterobacteriaceae* were screened phenotypically for the presence of β-lactamases using commercial kits (KPC and MBL Confirm ID kit plus temocillin and ESBL/AmpC screening kit; Bioconnections, Wetherby, United Kingdom). A cloverleaf assay (Hodge test) was also performed.

All 200 isolates grew well and produced >10 colonies when low inocula were inoculated onto Columbia blood agar and CLED agar on separate occasions. Colony counts performed on 10 isolates to validate the method of inoculum preparation revealed an average count of 89 CFU/spot for the low inoculum (range, 31 to 163 CFU/spot). The high and low inocula referred to in Table 1 were therefore regarded as approximately 10^5 CFU/spot and 10^2 CFU/spot, respectively. Table 1 shows that 96 to 97% of the CPE tested grew on chromID ESBL after 18 h of incubation and the only isolates inhibited at low inocula were three isolates of *K. pneumoniae* with the OXA-48 enzyme and two isolates of *P. rettgeri* with NDM-1. As expected, the specificity of this medium was very low, and it allowed growth of the vast majority of ESBL producers as well as isolates producing plasmidic AmpC β-lactamase. All four chromogenic media were subjected to extended incubation for up to 48 h. This had no impact on the sensitivity of chromID Carba and negligible impact (sensitivity improved by ≤1%) on Brilliance CRE and chromID ESBL. Sensitivity was improved on Colorex KPC, however, after 48 h of incubation (91% for high inocula and 63% for low inocula) with no negative impact on specificity. This resulted in the recovery of nine additional isolates of CPE that failed to grow within 18 h at low inocula (6 isolates with NDM-1, 2 with KPC, and 1 with OXA).

Among the three chromogenic media designed for detection of CPE, chromID Carba showed the best sensitivity and specificity and was at least as good as Brilliance CRE and Colorex KPC for recovering *Enterobacteriaceae* that produced any of the five commonest types of carbapenemase. The number of isolates of CPE recovered using either chromID Carba or chromID ESBL using low inocula was significantly higher ($P < 0.001$) than that obtained with any of the other four media. A weakness of Brilliance CRE was the recovery of only 1 of 9 IMP-producing *Enterobacteriaceae*, even when a high inoculum was used, and a weakness of Colorex KPC was its relatively poor sensitivity overall (56%) when challenged with low inocula of CPE. Selective TSB showed excellent recovery of CPE when high inocula were used (10^5 CFU/ml), but sensitivity of detection was severely impacted by challenge with low inocula (10^2 CFU/ml), and over half of all CPE were inhibited by TSB plus 2 mg/liter meropenem. Both of the selective broths showed poor specificity and allowed growth of most ESBL and AmpC producers when high inocula were used. When data were compared with those from repeat experiments performed 3 weeks later, all sensitivity values were within 3% of those reported above, with most values within 1%.

No CPE were recovered from 100 stool samples. As might be predicted from the data above, chromID ESBL and both selective broths showed relatively poor selectivity, and *Enterobacteriaceae* that lacked carbapenemases were frequently isolated (Table 2). Brilliance CRE and Colorex KPC appeared superior to chromID Carba for inhibition of *Pseudomonas* spp.

CDC guidelines recommend the use of TSB supplemented with either a meropenem disc or an ertapenem disc for isolation of CPE (1). Evidence from this study suggests that ertapenem is significantly better than meropenem for this purpose, but even so, 22% of CPE chosen for this study were inhibited by TSB plus ertapenem when low inocula of approximately 100 CFU/ml were used. Moreover, both types of broth showed poor specificity (Table 2). Nord-

TABLE 2 Isolates recovered on various selective media from 100 stool samples

Organism	No. of isolates recovered on:					
	Brilliance CRE	chromID Carba	chromID ESBL	Colorex KPC	TSB plus ertapenem	TSB plus meropenem
Acinetobacter spp.	0	0	0	0	2	0
Pseudomonas spp.	2	9	12	3	15	5
S. maltophilia	2	0	0	0	0	1
Enterobacteriaceae[a]	2 (1)	3 (1)	22 (18)	1 (1)	39 (10)	43 (8)
Candida spp.	0	0	0	0	5	12
Lactobacilli	0	0	0	0	0	1
Enterococcus spp.	0	2	3	0	99	82
Staphylococcus spp.	0	0	0	0	7	9
Streptococcus sp.	0	0	0	0	0	1
Total isolates	6	14	37	4	167	155

[a] No CPE were detected from 100 routine stool samples. Numbers in parentheses are the numbers of isolates that were found to produce ESBL or AmpC β-lactamase or both.

mann et al. compared chromID ESBL with CHROMagar KPC for their ability to support the growth of *Enterobacteriaceae* that produced NDM-1 carbapenemase (3). They observed that chromID ESBL showed excellent detection levels, whereas CHROMagar KPC often required higher inocula for successful detection. Colorex KPC is reported to have a formulation identical to that of CHROMagar KPC (4), and our findings are consistent with these data, as 98% of NDM-1 producers were recovered on chromID ESBL using low inocula compared with only 57% using Colorex KPC. As chromID ESBL is primarily designed to isolate ESBL producers, this clearly limits its specificity, and use of this medium for screening for CPE may be highly labor-intensive in areas where ESBL-producing isolates are very common.

In another study, Perry et al. compared a prototype version of chromID Carba (ID Carba) with Colorex KPC for the recovery of NDM-1 producers from cultures on MacConkey agar that had been derived from stool samples from patients attending Pakistan hospitals (4). They reported that ID Carba and Colorex KPC recovered 87.5% and 64.1% of CPE with NDM-1 carbapenemase, respectively. The relatively poor recovery of NDM-1 producers on Colorex KPC correlated with a low meropenem MIC. Vrioni et al. recently compared chromID ESBL with chromID Carba (and other nonchromogenic media) for the isolation of CPE from the rectal swabs of 200 hospitalized patients in Athens, Greece (5). Those authors found that chromID ESBL and chromID Carba had the same sensitivity (92.4%) but chromID Carba had a better specificity (96.9% versus 84.7%).

None of the three chromogenic media designed for detection of carbapenemase producers was particularly good at supporting the growth of OXA-48 producers, and at least a third of isolates were inhibited when tested at low inocula. Detection of such strains may be particularly problematic due to low carbapenem MICs and/or susceptibility to cephalosporins in the absence of a coproduced ESBL or AmpC enzyme (2). Eight of the 15 OXA-48 isolates tested here coproduced an ESBL and/or AmpC β-lactamases, but there was poor correlation between production of these enzymes and growth on any of the media.

In conclusion, we have provided some insight into the performances of six media that have been recommended for recovery of CPE. ChromID Carba showed optimal performance with the collection of isolates used in this study when both sensitivity and specificity were considered. Chromogenic media have the potential to provide useful tools for convenient and inexpensive screening of patients for CPE, thus necessitating further extensive evaluations of existing media and, if necessary, the development of new improved culture media.

ACKNOWLEDGMENTS

We are most grateful to the following collaborators, who generously shared isolates with characterized resistance mechanisms: Patrice Nordmann, Hôpital de Bicêtre, France; Neil Woodford, HPA Colindale, United Kingdom; Gilles Zambardi, bioMérieux, La Balme les Grottes, France; Enno Stürenburg, Universitätsklinikum Hamburg-Eppendorf, Germany; and Jaroslav Hrabák, Plzen Charles University, Prague, Czech Republic. We are also grateful to bioMérieux, La Balme les Grottes, France, for providing chromID Carba and for providing sponsorship to support this study. Finally, we are grateful to E&O Laboratories, Bonnybridge, United Kingdom, for provision of Colorex KPC medium.

The Freeman Hospital Microbiology Department (represented by K.M.W., M.W.R., and J.D.P.) receives funding from bioMérieux for the development and evaluation of culture media, and J.D.P. has performed paid consultancy work for the same company. The other authors have no conflicts to declare.

REFERENCES

1. **Centers for Disease Control and Prevention.** 2009. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, *Klebsiella* spp. and *E. coli* from rectal swabs. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoli.pdf.
2. **Nordmann P, Girlich D, Poirel L.** 13 February 2012, posting date. Detection of carbapenemase producers in *Enterobacteriaceae* using a novel screening medium. J. Clin. Microbiol. doi:10.1128/JCM.06477-11.
3. **Nordmann P, Poirel L, Carrër A, Toleman MA, Walsh TR.** 2011. How to detect NDM-1 producers. J. Clin. Microbiol. **49**:718–721.
4. **Perry JD, et al.** 2011. Prevalence of faecal carriage of *Enterobacteriaceae* with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. J. Antimicrob. Chemother. **66**:2288–2294.
5. **Vrioni G, et al.** 2012. Comparative evaluation of a prototype chromogenic medium (chromID Carba) for detecting carbapenemase-producing *Enterobacteriaceae* in surveillance rectal swabs. J. Clin. Microbiol. **50**:1841–1846.
6. **Walsh TR.** 2010. Emerging carbapenemases: a global perspective. Int. J. Antimicrob. Agents 36S. **3**:S8–S14.