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# Comparison of Chromogenic Media for Recovery of Carbapenemase-Producing *Enterobacteriaceae* (CPE) and Evaluation of CPE Prevalence at a Tertiary Care Academic Medical Center

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**We evaluated the performance characteristics of chromID CARBA and HardyCHROM Carbapenemase for the detection of carbapenemase-producing *Enterobacteriaceae* (CPE). A CPE prevalence study was conducted using chromID CARBA; this demonstrated that in low-prevalence settings, CPE screening agars may lack specificity, and confirmation of putative isolates is necessary.**

Over the last decade, carbapenem-resistant *Enterobacteriaceae* (CRE) have disseminated worldwide (1–4). Carbapenem resistance can be mediated by multiple mechanisms, including enzymes that can inactivate carbapenems (carbapenemases) or the presence of efflux or porin mutations. Perhaps the most alarming are the carbapenemase-producing isolates, which are frequently housed on mobile genetic elements, are resistant to most (if not all)  $\beta$ -lactams, and are often resistant to multiple other classes of drugs as well.

Due to the lack of therapeutic options for these organisms, tremendous efforts have been focused on preventing the spread of carbapenemase-producing *Enterobacteriaceae* (CPE). In order to identify and isolate asymptomatic carriers, active surveillance for fecal carriage in hospitalized patients is becoming increasingly common.

The objective of this study was to compare the limit of detection (LOD) of two commercially available chromogenic agars, chromID CARBA (bioMérieux, Durham, NC) and HardyCHROM Carbapenemase (recently renamed HardyCHROM CRE; Hardy Diagnostics, Santa Maria, CA), for the recovery of CPE from clinical specimens. In addition, a prevalence study was conducted to determine the rate of fecal and respiratory carriage of CPE at Barnes-Jewish Hospital (St. Louis, MO), a tertiary care academic medical center.

(This work was presented in part at the 114th General Meeting of the American Society for Microbiology, Boston, MA, 17 to 20 May 2014.)

For the LOD analysis, CPE-negative remnant stool specimens from *Clostridium difficile* testing were pooled, divided into 5-ml aliquots, and frozen at  $-20^{\circ}\text{C}$  until use. Bacteria were inoculated into stool specimens, resulting in a series of specimens with a final concentration of  $10^1$  to  $10^4$  CFU/ml. To mimic a rectal swab specimen, an ESwab (Copan Diagnostics, Murrieta, CA) was placed into the stool and subsequently into ESwab transport media. After 1 h at room temperature, the swab was vortexed, and 1 drop of the specimen was inoculated onto chromID CARBA and HardyCHROM Carbapenemase agars. After 24 h, the plates were evaluated, and LOD was determined for each isolate ( $n = 12$ ) (Table 1). A total of three replicates were performed.

For comparison, 1 drop of the ESwab specimen was also added to 5 ml of tryptic soy broth (TSB) containing a 10- $\mu\text{g}$  meropenem disk (BD Diagnostics, Franklin Lakes, NJ; CDC method) (5). The

next day, 1 drop of the TSB was inoculated onto chromID CARBA and HardyCHROM Carbapenemase agars. After 24 h, the plates were examined, and the LOD was determined for each isolate. Two replicates were performed. A third replicate was performed using a larger volume, 100  $\mu\text{l}$  of TSB, which was inoculated onto both chromogenic agars.

The Barnes-Jewish Hospital prevalence study was carried out over 12 weeks, from October 2013 to January 2014. This study was approved by the Washington University School of Medicine Human Research Protection Office. Remnant stool and rectal swab specimens submitted for *C. difficile* testing, *C. difficile* testing plus enteric culture, or vancomycin-resistant enterococci (VRE) screening, as well as tracheal aspirate specimens, were deidentified by an honest broker. A 10- $\mu\text{l}$  loop of specimen was inoculated onto chromID CARBA agar, with the exception of VRE swabs, which were inoculated directly onto the agar. Multiple lot numbers of chromID CARBA were used. The plates were examined for growth after 24 and 48 h of incubation. All growth was subcultured to blood agar plates (BD Diagnostics) and incubated overnight. The next day, isolates were Gram stained and subsequently analyzed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on the Vitek MS (IVD v.2.0; bioMérieux) (6–9). If identification was not achieved, the isolates were analyzed by the Bruker Biotyper (library version 3.4; Bruker Daltonics, Billerica, MA) (10, 11).

A subset of 60 specimens from the prevalence study, submitted for *C. difficile* testing, were inoculated onto both chromID CARBA

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TABLE 1 Limits of detection for chromID CARBA and HardyCHROM carbapenemase media using spiked stool specimens<sup>a</sup>

Strain	Limit of detection (CFU/ml) with direct inoculation		Limit of detection (CFU/ml) with CDC method	
	ChromID CARBA	HardyCHROM Carbapenemase	ChromID CARBA	HardyCHROM
KPC isolates				
<i>Klebsiella pneumoniae</i> BAA-1705	10 <sup>3</sup>	10 <sup>4</sup>	Not recovered	Not recovered
<i>Klebsiella pneumoniae</i>	10 <sup>2</sup>	10 <sup>3</sup>	Not recovered	Not recovered
<i>Escherichia coli</i> 1	10 <sup>3</sup>	Not recovered	Not recovered	Not recovered
<i>Escherichia coli</i> 2	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>4</sup>
<i>Enterobacter cloacae</i> complex 1	10 <sup>3</sup>	Not recovered	Not recovered	Not recovered
<i>Enterobacter cloacae</i> complex 2	10 <sup>2</sup>	Not recovered	Not recovered	Not recovered
<i>Citrobacter freundii</i> complex 1	10 <sup>3</sup>	Not recovered	Not recovered	Not recovered
<i>Citrobacter freundii</i> complex 2	10 <sup>2</sup>	Not recovered	Not recovered	Not recovered
NDM-1 isolates				
<i>Klebsiella pneumoniae</i> BAA-2146	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>2</sup>
<i>Escherichia coli</i>	10 <sup>3</sup>	10 <sup>3</sup>	Not recovered	Not recovered
<i>Enterobacter cloacae</i> complex	10 <sup>2</sup>	10 <sup>3</sup>	Not recovered	Not recovered
<i>Providencia rettgeri</i>	Not recovered	Not recovered	Not recovered	Not recovered

<sup>a</sup> Tested serial dilutions, 10<sup>1</sup> to 10<sup>4</sup> CFU/ml.

and HardyCHROM Carbapenemase media, as described above, for direct comparison of the two media.

Antimicrobial susceptibility testing was performed on all Gram-negative organisms recovered on chromID CARBA agar using Kirby-Bauer disk diffusion. Categorical interpretation of zone sizes for all antimicrobials was based on the 2013 Clinical and Laboratory Standards Institute (CLSI) disk diffusion criteria (12). Although carbapenem disk diffusion breakpoints do not exist for nonfermenting Gram-negative bacilli, disk diffusion was used as an initial screen for carbapenem resistance. All *Enterobacteriaceae* and *Pseudomonas putida* isolates and carbapenem-nonsusceptible *Pseudomonas aeruginosa* isolates were tested for the *bla*<sub>KPC</sub> (13), *bla*<sub>NDM-1</sub> (14), *bla*<sub>IMP</sub>, and *bla*<sub>VIM</sub> (15) genes by real-time PCR on the Cepheid SmartCycler II (Cepheid, Sunnyvale, CA). In addition, all carbapenem-nonsusceptible *Enterobacteriaceae* were tested for the presence of the *bla*<sub>CTX-M</sub> gene using conventional PCR detected by agarose gel electrophoresis (15).

Six (50%) of the 12 isolates did not grow at the highest concentration tested (10<sup>4</sup> CFU/ml) on HardyCHROM carbapenemase agar, including *Klebsiella pneumoniae* carbapenemase (KPC)-producing strains of *Escherichia coli* and *Citrobacter freundii* complex, whereas a single isolate did not grow on the chromID CARBA agar (a *Providencia rettgeri* NDM-1-producing isolate). For the isolates that did grow, the limit of detection was determined to be 10<sup>2</sup> to 10<sup>4</sup> CFU/ml (Table 1).

Based on our initial findings demonstrating a superior LOD, chromID CARBA was selected for the prospective prevalence study. A total of 611 specimens, including 561 fecal samples and 50 tracheal aspirates, were inoculated directly onto chromID CARBA agar. Of the 561 fecal specimens, 450 were submitted for *C. difficile* toxin testing and 111 were submitted for VRE screening. Forty-nine of the isolates submitted for *C. difficile* testing were also submitted for enteric culture. The specimens represented multiple patient age groups and hospital units, including intensive care and oncology.

Of the 611 specimens inoculated onto chromID CARBA, 139 isolates were recovered from 131 specimens. The breakdown of organisms recovered is shown in Table 2. Of the 9 *Enterobacteria-*

*ceae* isolates, 6 were recovered after 24 h of incubation, and 3 isolates were recovered after 48 h of incubation. After 24 h, the overall specificity was 70%, and the specificity was 95.3% based upon interpretation of the chromogenic medium.

A subset of 60 specimens from the point prevalence study were inoculated onto both chromID CARBA and HardyCHROM Carbapenemase to directly compare the performance of the two media using clinical specimens. After 24 h, 2 isolates, *P. aeruginosa* and *Pseudomonas* sp., were recovered on chromID CARBA (96.7% overall specificity; 100% specificity based upon direct interpretation of the chromogenic medium). From the same 60 specimens, 12 *Enterococcus* spp. were recovered on HardyCHROM carbapenemase (80% overall specificity; 100% specificity based upon direct interpretation of the chromogenic medium). After 48 h of incubation, 10 additional isolates were recovered on

TABLE 2 Isolates recovered on chromID CARBA agar after 24 and 48 h of incubation

Isolate	No. of isolates after:		Total no. of isolates	No. of PCR-positive isolates <sup>a</sup>
	24 h	48 h		
<i>Aeromonas</i> sp.	1	0	1	NA
<i>Burkholderia multivorans</i>	0	1	1	NA
<i>Candida</i> sp.	1	35	36	NA
<i>Citrobacter freundii</i> complex	1	1	2	0/2
<i>Enterobacter</i> sp.	2	1	3	0/3
<i>Escherichia coli</i>	1	0	1	0/1
<i>Enterococcus</i> sp.	1	22	23	NA
<i>Klebsiella oxytoca</i>	1	1	2	1/2
<i>Pseudomonas aeruginosa</i>	27	23	50	0/10
<i>Pseudomonas</i> sp.	3	6	9	0/3
<i>Stenotrophomonas maltophilia</i>	4	6	10	NA
<i>Serratia marcescens</i>	1	0	1	0/1
Total	43	96	139	1/22

<sup>a</sup> Isolates were tested for the presence of *bla*<sub>KPC</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>IMP</sub>, and *bla*<sub>VIM</sub>. NA, PCR not performed.

TABLE 3 Antimicrobial susceptibility profiles of *Enterobacteriaceae* recovered on chromID CARBA

Isolate	Antimicrobial susceptibility to <sup>a</sup> :									Carbapenemase PCR result <sup>b</sup>	CTX-M PCR result
	CAZ	CRO	FEP	TZP	IMI	MEM	ERT	DOR	ATM		
<i>Citrobacter freundii</i>	S	S	S	S	S	S	S	S	S	Negative	Negative
<i>Citrobacter sedlakii</i>	R	R	R	R	S	S	S	S	R	Negative	Negative
<i>Enterobacter cloacae</i> complex	R	R	S	I	S	S	R	S	R	Negative	Negative
<i>Enterobacter cloacae</i> complex	R	R	S	R	S	I	R	I	R	Negative	Negative
<i>Enterobacter</i> sp.	R	R	S	S	S	S	I	S	R	Negative	Negative
<i>Escherichia coli</i>	R	R	R	R	I	S	S	S	R	Negative	Positive
<i>Klebsiella oxytoca</i>	R	R	R	R	S	S	S	S	R	Negative	Negative
<i>Klebsiella oxytoca</i>	S	R	S	I	S	S	S	S	S	Positive (KPC)	Positive
<i>Serratia marcescens</i>	R	R	R	R	I	R	R	S	R	Negative	Negative

<sup>a</sup> Antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion. CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; TZP, piperacillin-tazobactam; IMI, imipenem; MEM, meropenem; ERT, ertapenem; DOR, doripenem; ATM, aztreonam; S, susceptible; R, resistant; I, intermediate.

<sup>b</sup> Isolates were tested for the presence of *bla*<sub>KPC</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>IMP</sub>, and *bla*<sub>VIM</sub>.

chromID CARBA, decreasing the overall specificity to 80%. An additional 18 isolates were recovered on HardyCHROM Carbapenemase after 48 h of incubation, resulting in an overall specificity of 50%.

Three of the 9 *Enterobacteriaceae* isolates recovered on chromID were susceptible to all carbapenems tested (Table 3). In addition, 40 of 50 *P. aeruginosa* isolates recovered were susceptible to imipenem and meropenem, while 10 isolates were nonsusceptible to both of these carbapenems.

Eight *Enterobacteriaceae* ( $n = 9$ ), all *P. putida* ( $n = 3$ ), and all carbapenem-nonsusceptible *P. aeruginosa* ( $n = 10$ ) isolates were negative according to PCR for the *bla*<sub>KPC</sub> (13), *bla*<sub>NDM-1</sub> (14), *bla*<sub>IMP</sub>, and *bla*<sub>VIM</sub> genes (15). One isolate of *Klebsiella oxytoca* was positive for the *bla*<sub>KPC</sub> gene. However, this isolate was susceptible to all four carbapenems as tested by disk diffusion. Additionally, the *K. oxytoca* isolate and the *E. coli* isolate were positive for the *bla*<sub>CTX-M</sub> gene (15).

Our findings contrast with those of Papadimitriou-Olivergeris et al. (16), who recently published a report evaluating chromID CARBA for detection of CPE from rectal swabs at a hospital in Greece. Their study evaluated fewer specimens ( $n = 177$ ) and included only patients in intensive care, and the recovery of CPE was significantly higher (89 CPE from 86 specimens). A single isolate of *Enterobacter cloacae* was the only *Enterobacteriaceae* isolate recovered on chromID CARBA that did not produce a carbapenemase. The sensitivity of chromID CARBA at the patient level was 96.5%, while the specificity was 91.2% based upon the direct interpretation of the chromogenic medium and increased to 100% when Gram stain characteristics were included in the interpretation algorithm.

Day and colleagues (17, 18) recently published two articles investigating the prevalence of CPE in Pakistan using chromogenic media. Both studies had fewer subjects than our study ( $n = 175$  and 152) and had higher rates of CPE (18.3% and 8.6%). The reported sensitivities of the chromogenic media were 100% and 92% in these studies, and both studies demonstrated recovery of non-CPE *Enterobacteriaceae* and significant numbers of *Pseudomonas* spp. While the analytical performance characteristics of chromID CARBA are favorable in areas of high prevalence (16–18), the positive predictive value of this medium for screening decreases in low-prevalence settings.

Both chromID CARBA and HardyCHROM carbapenemase are subject to breakthrough with non-CPE organisms. The colony

morphology of non-*Enterobacteriaceae* isolates was distinct from that of *Enterobacteriaceae*, with the exception of the *Aeromonas* isolate, which was burgundy and resembled *E. coli*. In addition to the breakthrough of non-*Enterobacteriaceae*, chromID CARBA is also subject to growth of non-CPE *Enterobacteriaceae*. As a result of the proportion of non-CPE isolated on this medium, we recommend that all *Enterobacteriaceae* be confirmed as carbapenemase producing before reporting the presence of a CPE in the specimen. This can add to the turnaround time of the testing. Although *Enterobacteriaceae* isolates were morphologically distinct from other species recovered on the agar, there were no morphological differences between CPE and non-CPE isolates to guide this analysis prior to the subsequent characterization of the isolates.

Overall, chromID CARBA has analytical performance characteristics for CPE superior to those of HardyCHROM Carbapenemase agar. During initial testing, LODs for chromID CARBA were typically a log or more sensitive than those observed for the HardyCHROM Carbapenemase medium. While it is unclear if this difference is clinically significant, due to a paucity of data on the typical fecal burden of CPE, it is not uncommon for laboratories to select a screening approach with the best analytical sensitivity. Based on our low prevalence, it is unlikely that routine screening for CPE would be justified or cost-effective at our hospital. Health care institutions may want to conduct prevalence studies to gain a snapshot of local or institutional CPE carriage and inform the need for screening and preventive measures to control the spread of these important pathogens in their institution.

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