ORIGINAL ARTICLE

Utility of CHROMagar mSuperCARBA for surveillance cultures of carbapenemase-producing *Enterobacteriaceae*

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Abstract

Culture of carbapenemase-producing *Enterobacteriaceae* (CPE) as part of active surveillance is one of the most useful strategies for successful infection control programmes. Our objective was to compare the recently introduced CHROMagar mSuperCARBA agar for CPE detection in surveillance cultures from perineal swabs with the US Centers for Disease Control and Prevention method. Our results showed that this agar is a useful and affordable alternative (sensitivity 93.05%, specificity 96.21%, diagnostic accuracy 95.2%) to detect CPE in hospital settings.

Keywords: Carbapenemases, chromogenic agar, *Enterobacteriaceae*, performance, surveillance cultures
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Introduction

Carbapenemase-producing *Enterobacteriaceae* (CPE) were detected about 20 years ago in the United States and are currently a global epidemic [1]. The infections caused by these multidrug-resistant microorganisms have high rates of morbidity and close to 50% mortality, mostly due to the few therapeutic options available, such as tigecycline, polymyxins, fosfomycin and aminoglycosides [2,3].

More than 2000 genetic variants of carbapenemases have been described. The predominant enzymes are as follows: *Klebsiella pneumoniae* carbapenemase (KPC; serin carbapenemase, Ambler group A), New Delhi metallo-β-lactamase (NDM; metallo-β-lactamase, Ambler group B) and OXA-48 (oxacillinase, Ambler group D). KPC carbapenemase predominates in endemic countries such as Greece, Italy and Israel, with incidences exceeding 30% [4], while Turkey and other Mediterranean countries report the OXA-48 variant as predominant [5]. NDM carbapenemase predominates in countries such as India, where it has a prevalence of 50% [6]. Regarding Latin America, KPC carbapenemase is considered endemic in some countries such as Colombia, Argentina and Brazil, while Chile only reports outbreaks caused by this microorganism. NDM variant predominates in countries such as Guatemala. However, the latter variant and OXA-48 carbapenemase are usually reported with less frequency in Latin America [7].

The US Centers for Disease Control and Prevention (CDC) and the European Society of Clinical Microbiology and Infectious Diseases recommend early detection to prevent the spread of CPE [8–10]. The early identification of these microorganisms is frequently carried out through rectal swab cultures. Given the fact that sensitive and specific methodologies with good turnaround times are required, several investigators have supported the use of chromogenic agars for identification [10]. The sensitivity of these agars varies according to the type of carbapenemase studied and the brand.

The CDC recommends the use of Landman's protocol for the detection of intestinal colonization of carbapenemase-producing *Enterobacteriaceae* [11]. The CHROMagar mSuperCARBA agar (CHROMagar) was marketed in 2016 as an

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appropriate alternative for the detection of KPC, NDM and OXA-48 like carbapenemases in *Enterobacteriaceae*. This chromogenic agar is based on a previous culture medium designed by Nordmann et al. in 2012 [32], which had shown the best performance for CPE detection in many studies [10,12,13]. However, to date, there is little scientific literature demonstrating its usefulness in surveillance cultures with patient samples.

Our objective was to compare the performance of CHROMagar mSuperCARBA agar with the method recommended by the CDC for detection of intestinal colonization by carbapenemase-producing *Enterobacteriaceae*.

Materials and methods

We carried out a prospective and multicentre study from February to April 2016. We included seven adult intensive care units from Guayaquil (Ecuador). Informed consent was obtained from each patient or their relatives for the sampling.

Patient selection

Perineal swabs were collected weekly in each patient who had more than 48 hours of hospitalization.

Microbiologic surveillance cultures

Amies media was used to transport samples to the laboratories. All the swabs were processed by the CDC method as previously described [11]. The samples were suspended in 5 mL of trypticase soy broth (Oxoid), and subsequently a 10 μg ertapenem (Oxoid) disc was added. The broth was incubated in air atmosphere for 24 hours at 35°C. After incubation, 100 μL of suspension was inoculated on MacConkey II agar (Becton Dickinson) [11]. It was incubated for 24 hours at 35°C in air atmosphere. The CHROMagar mSuperCARBA agar (SC) (CHROMagar) processing was performed in the bacteriology laboratory of the National Institute of Public Health Research 'Dr Leopoldo Izquieta Pérez.' The swab was plated in the agar prepared according to the manufacturer's instructions (≤72 hours of preparation) and incubated for 24 hours at 35°C in air atmosphere.

Bacterial identification and antimicrobial susceptibility

For the CDC method, we considered presumptive CPE colonies to be those growing as lactose positive, following CDC recommendations. Additionally, we also considered lactose-negative colonies (Hardy Diagnostics). In the SC method, according to the manufacturer's instructions, red colonies were considered *Escherichia coli,* metallic blue the remainder of the coliforms and colorless colonies other Gram-negative bacteria. All presumptive CPE colonies cultured in the two agars were identified by the API 20E system (bioMérieux). We performed antimicrobial susceptibility testing by the disc diffusion method [14]. Imipenem (IMP) 10 μg and meropenem 10 μg were used. Carbapenem-resistant *Enterobacteriaceae* (CRE) and nonresistant strains were defined according to Clinical Laboratory Standards Institute breakpoints. CRE strains were those with zone diameters ≤22 mm, and those with no resistance to carbapenem had zone inhibition diameters ≥23 mm [14].

Phenotypic detection of carbapenem resistance mechanisms

We tested all CRE with the modified Hodge test (MHT) [14]. Combined-disc tests of meropenem with and without phenylboronic acid (PBA) (Liofilchem) to detect serin carbapenemases and imipenem with and without EDTA (Liofilchem) to detect metallobetalactamase was performed as described elsewhere [4,15]. Carbapenemase production was confirmed if MHT was positive and the combined-disc test with PBA or EDTA was positive. CRE with a negative or undetermined MHT and/or a combined-disc–negative test were also tested with the carbapenem inactivation method [16] and MHT with Müller-Hinton agar (Becton Dickinson) supplemented with 250 mg/mL cloxacillin sodium salt (MHT-C) (MilliporeSigma) and meropenem disc (10 μg) according to previously described protocols [17]. We defined isolates with negative carbapenem-inactivation method and MHT-C as CRE–non-producing carbapenemase (CRE non-PC). These isolates were also studied for extended-spectrum β-lactamase (ESBL) and AmpC production. An ESBL phenotype was defined in *Enterobacteriaceae* with a synergy effect observed among cefepime (30 μg), cefotaxime (30 μg) and ceftazidime (30 μg) discs and the amoxicillin/clavulanic acid disc (20 μg/10 μg), which was placed at 15 mm from centre to centre of ceftazidime, cefepime and cefotaxime [14]. An AmpC phenotype was considered in CRE non-PC isolates, with a synergistic effect observed with the double-disc method with ertapenem (10 μg) and phenylboronic acid (300 μg) (PBA) discs (Kirby-Bauer method with ertapenem and PBA discs placed 15 mm from centre to centre in MHT-C) [14].

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *K. pneumoniae* ATCC BAA-1705 were used as quality control strains for the described tests.

Molecular detection of carbapenemases

All CRE were subcultured on trypticase soy agar (Oxoid) for 24 hours at 35°C in air atmosphere. DNA was extracted using the Wizard Genomic DNA purification kit (Promega) following the manufacturer's recommendations. We assessed the DNA quality by analysing the ratio of the absorbance at 260 nm/

280 nm. A previously described multiplex PCR protocol was used to detect KPC, OXA-48, VIM, IMP and NDM genes [17].

Interpretation of results

A CPE isolated with any of the two methodologies and molecularly confirmed was considered as a true-positive result. A true-negative result was defined when no CPE were recovered with any of the two methods studied. Samples with carbapenem-susceptible *Enterobacteriaceae* or CRE non-PC were considered as false-positive results. If one methodology recovered a CPE that was molecularly confirmed and the other did not, we considered the sample to be falsely negative. The discordant results were defined as any one that showed a different result regarding the positivity or negativity of the sample or the type or number of CPE isolated in one of the two methodologies. Concordance was defined as having the same result with the two methodologies.

Cost analysis

Cost of each method was calculated in US dollars at 2016 prices. Laboratory technologist charge was estimated at $7.50 per hour, based on a monthly salary of $1212 (scale remuneration of public service officers) [18].

Statistical analysis

We used Microsoft Office Excel 365 (2015) by frequencies and percentages. Sensitivity, specificity, positive and negative predictive values (PPV, NPV), diagnostic accuracy, and positive and negative likelihood ratio of each method were calculated. The agreement between the two tests was established by Cohen's kappa index. The kappa index was interpreted as follows: 0.01–0.20, poor agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, good agreement; and 0.81–1, very good agreement [19]. Statistical significance was calculated by Pearson's chi-square test, and values of $p \leq 0.05$ were considered statistically significant.

Results

We processed 950 swabs corresponding to 605 patients (one to six samples per patient, with a mean of two samples per patient). We obtained 633 negative results and 317 positive results (228 patients), detecting a total of 330 CPE. A total of 269 of the 317 samples were recovered using the CDC method and 292 using the SC method (Tables 1 and 2). Thirteen samples had more than one CPE, with 12 detected with the SC method and one with the CDC method. CHROMagar mSuperCARBA was statistically more sensitive (p 0.003) than the CDC method, allowing us to detect more than one CPE in the samples studied.

TABLE 1. Results obtained from two methods in samples with one carbapenemase producing-*Enterobacteriaceae*

Microorganism	*N* (%)	Concordant result	Discordant result
Klebsiella pneumoniae KPC	277 (90.78)	224	53 (25 CDC^+/SC^-, 27 CDC^-/SC^+)
Enterobacter aerogenes KPC	4 (1.31)	4	0
Enterobacter cloacae KPC	7 (2.30)	2	5 (CDC^-/SC^+)
Escherichia coli KPC	1 (0.32)	1	0
Klebsiella oxytoca KPC	3 (0.98)	2	1 (CDC^-/SC^+)
Proteus mirabilis NDM	13 (4.27)	0	13 (CDC^-/SC^+)
Total	304	233	72

CDC, Centers for Disease Control and Prevention method; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; SC, CHROMagar mSuperCARBA agar method.

KPC was the predominating carbapenemase (92.24%). NDM carbapenemase was also detected in 5.75% of the samples, and OXA-48 carbapenemase was detected in none.

Concordance

A total of 91.05% (865) of the results were concordant. We observed considerable agreement between the two methods for CPE detection (kappa index 0.79; 95% confidence interval, 0.75–0.84). The best concordance was found for KPC variant (kappa index 0.87; 95% confidence interval 0.82–0.90), and there was no agreement at all for NDM detection (kappa index 0)

Samples with discordant results occurred in 63 patients (including those with more than one CPE); 37 subjects (58.73%) had more than one sample collected during the study period. Eleven patients (31 samples) had the same discordant results more than once and could be corroborated (Table 3).

False-positive and -negative results

We obtained 24 (2.52%) false-positive results with the SC method, of which 14 were CRE non–carbapenem resistant and ten were susceptible to imipenem and meropenem. With the CDC method, we obtained 198 (20.84%) false-positive results, of which six were CRE non–carbapenem-resistant isolates and 192 samples had *Enterobacteriaceae* with susceptibility to imipenem and meropenem (129 *E. coli,* 54 *K. pneumoniae* and nine *Enterobacter cloacae*). The most frequent mechanism of resistance to carbapenems detected in non-CPE isolates was extended-spectrum β-lactamases combined with porin mutations.

We obtained 22 (2.31%) false-negative results with the SC method and 47 (4.94%) false-negative results with the CDC method (Table 4).

Sensitivity, specificity, PPV, NPV and diagnostic accuracy

SC showed the best results in all statistical analyses (Table 5).

TABLE 2. Results obtained from two methods in samples with more than one carbapenemase producing-*Enterobacteriaceae*

	CDC		SC	
Strain	**Microorganism 1**	**Microorganism 2**	**Microorganism 1**	**Microorganism 2**
28098V	*Enterobacter cloacae* bla_{KPC}	*K. pneumoniae* bla_{KPC}	*E. cloacae* bla_{KPC}	—
27037M	*Klebsiella pneumoniae* bla_{KPC}	—	*E. cloacae* bla_{KPC}	*K. pneumoniae* bla_{KPC}
27920L	*E. cloacae* bla_{KPC}	—	*E. cloacae* bla_{KPC}	*K. pneumoniae* bla_{KPC}
27902N	Negative	—	*Escherichia coli* bla_{KPC}	*K. pneumoniae* bla_{KPC}
27411R	*E. coli* bla_{KPC}	—	*E. coli* bla_{KPC}	*K. pneumoniae* bla_{KPC}
27405J	Negative	—	*E. coli* bla_{KPC}	*K. pneumoniae* bla_{KPC}
28001S	*E. coli* bla_{KPC}	—	*E. coli* bla_{KPC}	*K. pneumoniae* bla_{KPC}
27301G	*K. pneumoniae* bla_{KPC}	—	*Proteus mirabilis* bla_{NDM}	*K. pneumoniae* bla_{KPC}
27306M	*K. pneumoniae* bla_{KPC}	—	*P. mirabilis* bla_{NDM}	*K. pneumoniae* bla_{KPC}
27046P	*K. pneumoniae* bla_{KPC}	—	*P. mirabilis* bla_{NDM}	*K. pneumoniae* bla_{KPC}
27302P	*K. pneumoniae* bla_{KPC}	—	*P. mirabilis* bla_{NDM}	*K. pneumoniae* bla_{KPC}
27303V	*K. pneumoniae* bla_{KPC}	—	*P. mirabilis* bla_{NDM}	*K. pneumoniae* bla_{KPC}
27694V	*K. pneumoniae* bla_{KPC}		*P. mirabilis* bla_{NDM}	*K. pneumoniae* bla_{KPC}

CDC, Centers for Disease Control and Prevention method; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; SC, CHROMagar mSuperCARBA agar method.

TABLE 3. Discordant results in patients with more than one sample

Patient no.	Total samples/no. of samples with discordant results repeated	Discordant result, CDC/SC
1	5/2	Negative/*Klebsiella pneumoniae* KPC
2	5/3	Negative/*K. pneumoniae* KPC
3	3/2	Negative/*K. pneumoniae* KPC
4	5/2	Negative/*K. pneumoniae* KPC
5	3/3	Negative/*K. pneumoniae* KPC
6	4/4	Negative/*K. pneumoniae* KPC
7	5/2	Negative/*K. pneumoniae* KPC
8	2/2	*K. pneumoniae* KPC/Negative
9	3/3	Negative/*Proteus mirabilis* NDM
10	4/4	Negative/*P. mirabilis* NDM[a]
11	4/4	Negative/*P. mirabilis* NDM[a]

CDC, Centers for Disease Control and Prevention method; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; SC, CHROMagar mSuperCARBA agar method.
[a]Two samples of each patient had *K. pneumoniae* KPC by CDC method and *P. mirabilis* NDM and *K. pneumoniae* KPC by SC method.

Cost analysis

We found that the CDC method was more expensive than the SC method (SC $8990.72 vs. CDC $ 10307.92). Although the total cost of the supplies used was estimated to be higher for the SC method (SC $8477.32 vs. CDC $7866.21) the cost of laboratory technologist time increased the total cost of the CDC method (SC $513.40 vs. CDC $2441.71), mainly due to the lack of specificity, in addition to the fact that 39.91% of the cost of the inputs of the CDC method was used to work samples with false-positive results (Table 6).

Discussion

One of the main strategies to avoid transmission of CPE in healthcare settings is prompt detection of its intestinal carriers. In order for this strategy to be successful, the method used must be accurate and have quick turnaround times.

In our study, the sensitivity of the SC method for CPE detection (KPC and NDM) was higher than that of the CDC method (93.05% vs. 84.7%). However, it is lower than that reported by García-Fernández et al. [20] in 2016 (100%), in a study that used 210 rectal swabs from patients colonized with CPE carrying NDM, KPC and OXA-48 carbapenemases. Sensitivity reported by other authors is higher for the SC

TABLE 4. Sample results with non–carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* classified as falsely positive

Microorganism	Laboratory code	CDC	SC	Imipenem (mm)	Meropenem (mm)
Klebsiella pneumoniae AmpC + porin mutations	27111T	−	+	28	23/16
K. pneumoniae AmpC + porin mutations	27416T	−	+	24	19
K. pneumoniae AmpC + porin mutations	27740T	−	+	20	18
Enterobacter aerogenes AmpC + porin mutations	25428C	+	−	23	17
E. aerogenes AmpC + porin mutations	25729C	+	−	16	18
E. cloacae AmpC + porin mutations	28384A	−	+	13	23
Enterobacter cloacae AmpC + porin mutations	27909R	−	+	6	12
K. pneumoniae ESBL + porin mutations	25339R	+	+	24	14
K. pneumoniae ESBL + porin mutations	28736Z	+	+	15	17
K. pneumoniae ESBL + porin mutations	25337Z	+	+	18	17
K. pneumoniae ESBL + porin mutations	26745P	−	+	25	20
K. pneumoniae ESBL + porin mutations	27104A	+	+	22	13
K. pneumoniae ESBL + porin mutations	27185A	−	+	25	17
Escherichia coli ESBL + porin mutations	26422S	−	+	25	17

CDC, Centers for Disease Control and Prevention method; ESBL, extended-spectrum β-lactamase; SC, mSuperCARBA agar method.

TABLE 5. Results of statistical analysis

	CDC		SC	
Result	**%**	**95% CI**	**%**	**95% CI**
Sensitivity	84.7	80.3–88.3	93.05	89.6–95.3
Specificity	68.9	65.2–72.3	96.21	94.4–97.5
Positive predictive value	57.3	52.8–61.7	92.4	88.9–94.8
Negative predictive value	90.1	87.1–92.5	96.5	94.8–97.7
Diagnostic accuracy	74.1	71.2–76.6	95.2	93.6–96.4

CDC, Centers for Disease Control and Prevention method; CI, confidence interval; SC, mSuperCARBA agar method.

method as well. Garcia-Quintanilla et al. [21] in 2017 demonstrated sensitivity and specificity of 100% for this medium when they tested 113 *Enterobacteriaceae* strains, including KPC, NDM, VIM, IMP and even OXA-48 compared to noncommercial Supercarba agar. Nonetheless, these studies mostly used strains previously characterized, not patient samples [20,22]. The lower sensitivity obtained in this study could be due to the use of the same swab for the two procedures. In addition, it has been previously reported that enrichment of the sample in broth, conducted in the CDC method, increases the sensitivity of methodologies [23].

We found that the sensitivity of the CDC method was relatively high (84.7%) despite its inability to detect NDM carbapenemase and the difficulty in detecting more than one CPE in the same sample (kappa index 0). The high sensitivity is probably due to the predominance of KPC carbapenemase (94.24%) in the analysed samples. This is in agreement with other authors reporting 98.8% sensitivities when processing rectal swab samples with KPC carbapenemase [24]. However, a meta-analysis published in 2016 shows a lack of sensitivity of this method compared to other commercial agars for the detection of CPE in surveillance rectal swab samples [4]. Nonetheless, this method is still routinely used because of its low costs [25].

The literature describes specificities ranging from 49.6% to 100% [26,27] for the CDC method. The specificity reported by us was mainly caused for the high number of false-positive results obtained; the majority (96.96%) were isolates that were not classified as CRE according to our definition (resistance to imipenem and meropenem). However, we do not know the mechanism of resistance implicit in these isolates and their susceptibility to ertapenem, which is a limitation of our study. However, there is a small chance for these isolates to harbour an OXA-48 like carbapenemase, which confers lower minimum inhibitory concentrations to carbapenems because it has not been reported in our country so far. It has been also described that the presence of OXA-48 carbapenemase is uncommon in strains with susceptibility to imipenem [28]. However, it has been shown that SC has the best sensitivity for the detection of OXA-48 [21].

The specificity of SC was 96.21%, which was better than the CDC method as well as other methods described in other studies that used rectal swabs [10]. However, it is important to emphasize that the specificity will vary depending on local epidemiology of presence of ESBL and AmpC enzymes as the mechanism causing carbapenem resistance. In our study, the ESBL with porin mutations (70%, $n = 10$) predominated in those CREs that did not produce carbapenemases.

Comparison of the sensitivity and specificity between different studies is difficult because of the limitations of each study, the differences in methods and the lack of a reference method. Additionally, the predominant CPE in swabs will depend on local epidemiology, which makes it difficult to extrapolate the results to other contexts.

TABLE 6. Cost analysis

			SC				CDC			
Method	**Minutes**	**Unitary value (US$)**	**Positive ($n = 292$)**	**Negative ($n = 634$)**	**Falsely positive, CSE ($n = 10$)**	**Falsely positive, CRE non-PC ($n = 14$)**	**Positive ($n = 269$)**	**Negative ($n = 483$)**	**Falsely positive, CSE ($n = 192$)**	**Falsely positive, CRE non-PC ($n = 6$)**
CDC, broth–MacConkey	12	0.58					$156.02	$280.14	$111.36	$3.48
Chromogenic agar	1	3.62	$1057.04	$2295.08	$36.20	$50.68				
API 20E identification system	3	14.50	$4234.00		$145.00	$203.00	$3900.50		$2784.00	$87.00
Susceptivity test, imipenem/meropenem	1	0.59	$172.28		$5.90	$8.26	$158.71		$113.28	$3.54
Phenotypic confirmatory method (boronic acid disc/EDTA disc)	1	0.71	$207.32			$9.94	$190.99			$4.26
Hodge test	2	0.15	$43.80			$2.10	$40.35			$0.90
Carbapenemase inactivation method (incubation time excluded)	2	0.28				$3.92				$1.68
Hodge test with cloxacillin	5	0.20				$2.80				$1.20
Total			$5714.44	$2295.08	$187.10	$280.70	$4446.57	$280.14	$3008.64	$102.06
Total cost of supplies			$8477.20				$7866.21			
Technologist time		$0.13	$513.40				$2441.71			
Total cost			$8990.72				$10<thinsp>307.92			

CDC, Centers for Disease Control and Prevention method, CRE, carbapenem-resistant *Enterobacteriaceae*, CSE, carbapenem-susceptible *Enterobacteriaceae*, SC, CHROMagar mSuperCARBA agar method.

We had good agreement (kappa index 0.79) for the detection of CPE and an almost perfect agreement for the detection of KPC carbapenemase (kappa index 0.87). The discordant results in 27 samples could be because the detection limit of the SC method is 10 CFU/mL [20] lower than the CDC method. There was no agreement observed for the detection of NDM carbapenemase (kappa index 0).

The SC method had the better predictive values (PPV 92.4%, NPV 96.5%) than the CDC method (PPV 57%, NPV 90.1%). Although the CDC method is described as being more affordable [25], its low PPV causes additional technical work in order to confirm suspicious isolates, which makes this protocol more expensive. As a consequence, it increases the workload in the laboratory, and lengthens the turnaround time and the time the patient needs to stay in isolation [25,29].

Ecuador imports laboratory reagents, which increases the costs of testing and limits the options, with laboratories often having to resort to less specific and noncommercial tests. In this study, we used the MHT, which has moderate specificity but is cheap; the Clinical and Laboratory Standards Institute recommended it until 2017 [14,30,31]. The selection of cheaper tests is a common practice in our country. However, we suggest that the tests for CPE surveillance should be based on local epidemiologic data after performing cost analysis.

In conclusion, the SC method is a robust, useful and affordable laboratory diagnostic method for CPE detection in surveillance programmes in regions with high CPE prevalence, particularly when several CPEs are circulating.

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Conflict of interest

Ca. Soria Segarra is a speaker for Merck and 3M. The other authors declare no conflict of interest.

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