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Comparative Evaluation of a Prototype Chromogenic Medium (ChromID CARBA) for Detecting Carbapenemase-Producing *Enterobacteriaceae* in Surveillance Rectal Swabs

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Carbapenemase-producing *Enterobacteriaceae* (CPE) are an increasing problem worldwide, and rectal swab surveillance is recommended as a component of infection control programs. The performance of a prototype chromogenic medium (chromID CARBA) was evaluated and compared with media tested by four other screening methods: (i) overnight selective enrichment in 5 ml tryptic soy broth with a 10-μg ertapenem disk followed by plating onto MacConkey agar (CDC-TS), (ii) short selective enrichment in 9 ml brain heart infusion broth with a 10-μg ertapenem disk followed by plating onto chromID ESBL medium (ESBL-BH), (iii) direct plating onto chromID ESBL, and (iv) direct plating onto MacConkey agar supplemented with meropenem (1 μg/ml) (MCM). The screening methods were applied to detect CPE in 200 rectal swab specimens taken from different hospitalized patients. Identification and antimicrobial susceptibility were performed by the Vitek 2 system. Carbapenem MICs were checked by Etest. Carbapenemase production was confirmed using the modified Hodge test, combined-disk tests, and PCR assays. In total, 133 presumptive CPE strains were detected. Phenotypic and genotypic assays confirmed 92 strains to be CPE (56 KPC-positive *Klebsiella pneumoniae*, 29 VIM-positive *K. pneumoniae*, and 7 KPC-positive *Enterobacter aerogenes* strains) recovered from 73 patients, while the remaining 41 strains were confirmed to be CPE negative (19 ESBL producers and 22 nonfermenters). chromID CARBA, ESBL-BH, and chromID ESBL exhibited the highest sensitivity (92.4%), followed by CDC-TS and MCM (89.1%) ($P = 0.631$). The specificity was greater for chromID CARBA (96.9%) and ESBL-BH (93.2%) than for CDC-TS (86.4%), MCM (85.2%), and chromID ESBL (84.7%) ($P = 0.014$). In conclusion, chromID CARBA was found to be a rapid and accurate culture screening method for active CPE surveillance.

Carbapenems are used as a last-resort antibiotic class for the treatment of infections due to multidrug-resistant *Enterobacteriaceae*. However, during the last decade carbapenem resistance has been increasingly reported and carbapenemase-producing *Enterobacteriaceae* (CPE) are emerging as a growing challenge in health care facilities (22). These isolates produce different types of β-lactamases capable of hydrolyzing carbapenems. Among these carbapenemases, metallo-β-lactamases (MBLs; Ambler class B) and *Klebsiella pneumoniae* carbapenemase (KPC; Ambler class A) are prevailing in *Enterobacteriaceae* from countries or large geographic regions, including the United States, Israel, Italy, Greece, the Far East, and South America (9, 12, 14, 15, 17, 18, 22, 26, 27). Moreover, oxacillinase-48 (OXA-48; Ambler class D) has recently been isolated in *Enterobacteriaceae* from Turkey (6), and it has since been reported from other countries in the Mediterranean Basin and Western Europe (13, 22). Carbapenemase-producing pathogens have been associated with high rates of morbidity and mortality, particularly among critically ill patients with prolonged hospitalization (4, 21, 32, 37). Furthermore, CPE are usually multidrug-resistant pathogens, making them even more worrisome, since the treatment options are very restricted (4). It is also of note that the carbapenemase genes harbored by CPE are mostly transposon- and/or integron-encoded determinants that can easily disseminate to other enterobacterial strains and species (11, 30, 35). These facts suggest the need to implement adequate preventive measures, including active surveillance, in order to contain the spread of these pathogens.

Since gastrointestinal carriers of CPE are thought to be the reservoir of cross-transmission in health care settings, surveillance has been deemed necessary (2, 3, 7, 18). Therefore, collection of rectal swab specimens seems to be the most appropriate sampling method for microbiologic surveillance, which can be accomplished using either culture or molecular techniques. Although direct detection by molecular assays exhibits high sensitivity and has the advantage of rapid identification of CPE (16, 31), these methods are not available for daily use in many laboratories. It should also be noted that their use is limited to the detection of isolates that harbor the target β-lactamase gene (31), and the residue of swabs with low inocula may not be sufficient for successful DNA extraction (29). Even more, molecular methods do not give the possibility for further strain typing and susceptibility testing.

Thus, several culture techniques for screening carbapenem-resistant *Enterobacteriaceae* have been tested, including methods that use in-house-prepared selective media, such as MacConkey agar or tryptic soy broth containing a 10-μg carbapenem disk (3, 8, 19, 20), or commercial chromogenic agar media, like CHROMagar KPC (Hy-Labs, Rehovot, Israel) (1, 23, 29) and chromID ESBL medium (bio-

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Mérieux, Marcy l'Etoile, France) (5). However, these screening methods are designed to detect carbapenem-resistant *Enterobacteriaceae* and not specifically CPE. chromID CARBA (bioMérieux) is a chromogenic solid medium particularly designed for CPE detection and supplemented with specific agents that inhibit the growth of Gram-positive and noncarbapenemase producers (24). The aim of the present study was to evaluate the performance of chromID CARBA and compare it to that of four other culture-based screening methods for CPE detection directly from rectal swabs.

MATERIALS AND METHODS

Patients and specimens of the study. Rectal swab specimens were collected from 200 different patients at high risk for colonization with CPE (admission from other institutions or periodic surveillance of high-risk units) hospitalized from February to April 2011 in the intensive care unit and medical wards of an 800-bed tertiary care hospital in Piraeus, Greece. Almost half of the patients enrolled ($n = 93$) had been hospitalized during the preceding year in several hospitals and long-term-care facilities located in the broader region of Athens and Piraeus. As per survey protocol, rectal swab sample collection was performed using a nylon flocked swab system with 5 ml of Amies gel transport medium. The tip of the sterile swab, premoistened with sterile saline, was inserted approximately 1 in. beyond the anal sphincter and carefully rotated, in order to sample the external rectal orifice, withdrawn, and placed in the tube with the gel transport medium. Samples were immediately transferred to the laboratory and processed.

Culture screening methods. The swab containing the sample was transferred into 1 ml phosphate-buffered saline buffer and was agitated to release the microorganisms from the swab tip. An inoculum volume of 100 μl was transferred onto each of five culture media for testing by different methods: (i) overnight selective enrichment in 5 ml tryptic soy broth with a 10-μg ertapenem disk (final ertapenem concentration, 2 μg/ml) followed by plating onto MacConkey agar (CDC protocol) (CDC-TS) (8), (ii) short selective enrichment (4 to 6 h) in 9 ml brain heart infusion broth with a 10-μg ertapenem disk (final ertapenem concentration, 1 μg/ml) followed by plating onto chromID ESBL medium (ESBL-BH) (bioMérieux), (iii) direct plating onto chromID ESBL medium (bioMérieux), (iv) direct plating onto chromID CARBA prototype medium (bioMérieux), which consists of a nutrient base combining different peptones, three chromogenic substrates enabling the detection of activities of specific metabolic enzymes for *Escherichia coli*, *Klebsiella*/*Enterobacter*/*Serratia*/*Citrobacter*, and *Proteeae*, and a proprietary mixture of antibiotics favoring the selective growth of carbapenemase-producing *Enterobacteriaceae*, and (v) direct plating onto MacConkey agar plate supplemented with meropenem at 1 μg/ml (MCM). The last medium was used within 48 h after preparation. On each medium, evaluation of bacterial growth was made after 18 to 24 h of incubation at 37°C in ambient air by two different observers. All chromID agar plates were also inoculated with the following control strains: carbapenemase-negative *Klebsiella pneumoniae* ATCC 700603, carbapenemase-positive *K. pneumoniae* ATCC BAA-1705, ESBL-positive *Escherichia coli* CIP 103982, ESBL-negative *E. coli* ATCC 25922, and ESBL-positive *Proteus mirabilis* ATCC BAA-856.

Detection and identification of CPE colonies. On chromogenic agar plates, presumptive CPE colonies were considered those with a color appearance according to the manufacturer's instructions (green-blue to browny green, pink to burgundy, or brown colonies). With the CDC-TS and MCM methods, presumptive CPE colonies were considered those growing as lactose-fermenting or lactose-nonfermenting colonies on MacConkey plates. Suspected CPE colonies were subcultured from respective screening plates onto MacConkey plates and were submitted to identification and susceptibility testing using the Vitek 2 automated system using GN-ID and GN09 cards, respectively (bioMérieux). Imipenem, meropenem, and ertapenem MICs were verified with Etest (bioMérieux) using CLSI guidelines (10).

Phenotypic and molecular identification of carbapenemase and other ESBL genes. In all presumptive CPE isolates, carbapenemases were detected using combined-disk tests of meropenem without and with phenylboronic acid (PBA), EDTA, or both (33, 34) and confirmed by PCR assays for genes for KPC, IMP, and VIM (33), NDM (24), and OXA-48 (25). PCR-negative isolates were further tested by the modified Hodge test (MHT) according to CLSI guidelines (10). PCR assays were also used to detect plasmidic AmpC and expanded-spectrum β-lactamase (ESBL) genes, including SHV, TEM, CTX-M, and GES/IBC genes (33).

LOD of screening methods. Six previously well-characterized CPE strains from our collections (34) consisting of four KPC-positive *K. pneumoniae* clinical strains, one VIM-positive *K. pneumoniae* clinical strain, and one KPC-positive *E. coli* clinical strain as well as an NDM-positive *K. pneumoniae* strain (ATCC BAA-2146) and an OXA-48-positive *K. pneumoniae* strain (NCTC 13442) were included as reference CPE strains in experiments to assess the limit of detection (LOD) of the five screening methods. Reference strains were thawed and subcultured onto MacConkey agar plates before use. Strains were suspended in normal saline to the density of a 0.5 McFarland standard ($\sim 2 \times 10^8$ CFU/ml), followed by serial 10-fold dilutions. An aliquot of 100 μl from each dilution of each study strain was tested by the five different screening methods described above, as well as on standard MacConkey agar plates for performing viable colony counts. Viable bacteria were counted after 24 h at 37°C, and growth on screening media was compared to growth on MacConkey agar plates. The experiments were performed in triplicate. The LOD of each screening method was the lowest concentration of the reference strain that resulted in recovery of viable colonies.

Sensitivity and specificity. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated for each of the screening methods. True-positive strains were defined as all presumptive CPE growing on the media and genotypically confirmed to be CPE positive. False-positive strains were defined to be all presumptive CPE growing on the media that were genotypically confirmed to be CPE negative. No growth by all screening methods was characterized as a true-negative result. No recovery of a genotypically confirmed CPE-positive strain using a particular screening method was characterized as a false-negative result for this specific screening method. Differences in sensitivity and specificity among the various screening methods were analyzed using the chi-square test. A P value of <0.05 was considered statistically significant.

RESULTS

Detection limit experiment of the five screening methods. The reference CPE strains, their carbapenem MICs, and the LODs of the five screening methods are presented in Table 1. All 8 CPE strains grew on CDC-TS, MCM, and chromID CARBA, and 7 CPE strains grew on chromID ESBL and ESBL-BH; the OXA-48-producing reference strain was not recovered by the last two methods. Using CDC-TS and MCM, the LOD was less than 10^2 CFU/ml in 3/8 strains, while using chromID ESBL, ESBL-BH, and chromID CARBA, the LOD was lower than 10^2 CFU/ml in 6/8 strains.

All five screening methods successfully detected the KPC- and VIM-producing strains with carbapenem MICs of >32 μg/ml at inocula of 5.9×10^0 to 2.3×10^1 CFU/ml, while the NDM-producing strain (carbapenem MICs, >32 μg/ml) was detected at higher inocula (1.3×10^2 to 8.5×10^3) by all methods. KPC-positive strains with lower carbapenem MICs were detected at inocula of 7×10^0 to 6.5×10^1 CFU/ml by chromogenic methods but in all cases were detected at higher inocula (9.5×10^0 to 5×10^2) by the CDC-TS and MCM methods. In addition, the OXA-48-producing strain was detected at a high inoculum by chromID CARBA medium (1.1×10^7 CFU/ml), as well as CDC-TS and MCM methods (5.2×10^7 to 5.5×10^7 CFU/ml), despite its low carbapenem MICs. As was expected, the OXA-48-producing strain was not recovered using ESBL-BH and chromID ESBL,

TABLE 1 LODs of the five screening methods for recovery of the reference CPE strains

Bacterial strain	Carbapenemase	MIC (μg/ml)			Lowest limit of detection (CFU/ml) of the culture screening methods[a]				
		Meropenem	Imipenem	Ertapenem	CDC-TS	ESBL-BH	chromID ESBL	chromID CARBA	MCM
K. pneumoniae 1	KPC	>32	>32	>32	9.2×10^0	9×10^0	5.9×10^0	8.9×10^0	6.7×10^0
K. pneumoniae 2	KPC	8	16	16	9.5×10^0	8.5×10^0	7×10^0	9.3×10^0	1.2×10^1
K. pneumoniae 3	KPC	4	8	16	1.1×10^2	4.2×10^1	8.4×10^0	1.8×10^1	1.5×10^2
K. pneumoniae 4	KPC	2	8	16	4.2×10^2	5.7×10^1	4.3×10^1	4.8×10^1	5×10^2
E. coli 1	KPC	2	8	16	3.8×10^2	6.5×10^1	5.5×10^1	5.8×10^1	3.9×10^2
K. pneumoniae 5	VIM	>32	>32	>32	9.5×10^0	1.4×10^1	8.9×10^0	2×10^1	2.3×10^1
K. pneumoniae ATCC BAA-2146	NDM	>32	>32	>32	7.9×10^3	2.2×10^2	1.3×10^2	4.1×10^3	8.5×10^3
K. pneumoniae NCTC 13442	OXA-48	1	2	2	5.2×10^7	ND	ND	1.1×10^7	5.5×10^7

[a] Lowest limit of detection values are the mean values of the three counts. CDC-TS, inoculation of swabs in tryptic soy broth with a 10-μg ertapenem disk followed by plating to MacConkey agar (100 μl of the selective TSB after overnight enrichment); ESBL-BH, inoculation of swabs in brain heart infusion broth with a 10-μg ertapenem disk followed by plating to a chromID ESBL plate (100 μl of the selective brain heart infusion broth after 4 to 6 h of enrichment); MCM, supplementation of MacConkey agar plates with meropenem (1 μg/ml); ND, no detection.

since this strain exhibits low expanded-spectrum cephalosporin MICs.

Surveillance rectal swab testing. The performances of the screening media to recover CPE strains are presented in Table 2 and Fig. 1. As many as 133 presumptive CPE strains were recovered from 87 of the 200 patient swab specimens by at least one screening method. Phenotypic and genotypic methods confirmed 92 distinct strains (85 *K. pneumoniae* and 7 *Enterobacter aerogenes* strains) recovered from 73 (36.5%) patient swab specimens to be CPE, while the remaining 41 strains were confirmed to be non-CPE.

Nonchromogenic screening methods. By the CDC-TS method, 75 of the 200 rectal swab specimens yielded 102 distinct presumptive CPE strains. Of these strains, 82 were confirmed to be CPE by phenotypic and molecular methods (52 KPC-positive *K. pneumoniae*, 23 VIM-positive *K. pneumoniae*, and 7 KPC-positive *E. aerogenes* strains), whereas the remaining 20 strains were identified to be non-CPE (1 ESBL-positive *P. mirabilis* strain and 19 nonfermenters). MCM medium from 78 swab specimens yielded 104 distinct presumptive CPE strains, of which the same 82 were genotypically confirmed to be CPE strains, whereas the remaining 22 were identified to be nonfermenters (Table 2).

Chromogenic screening methods. With the ESBL-BH method, 77 of the 200 rectal swab specimens yielded 94 distinct strains with a color appearance compatible with CPE (89 green-blue strains, 4 burgundy strains, and 1 brown strain); with chromID ESBL medium, 87 of the rectal swab specimens yielded 108 distinct strains with a color appearance compatible with CPE (89 green-blue, 15 burgundy, and 4 brown strains), while with chromID CARBA, 73 swab specimens yielded 89 green-blue strains that were presumptively considered to be CPE.

Phenotypic and molecular methods confirmed 85 CPE strains recovered by all the above-described chromogenic screening methods (54 KPC-positive *K. pneumoniae*, 25 VIM-positive *K. pneumoniae*, and 6 KPC-positive *E. aerogenes* strains). The 15 strains with a burgundy color were identified as ESBL-producing *E. coli*, while the 4 strains with a brown color were identified as ESBL-producing *P. mirabilis*. There were four samples in which growth of tiny green colonies on all tested chromogenic media was initially considered CPE, resulting in unnecessary laboratory workup. The implicated false-positive CPE isolates were identified as *Acinetobacter baumannii* (Table 2). It should be noted that white colonies recovered from the above-described chromogenic media were considered nonfermenting bacteria and did not result in unnecessary laboratory workup with phenotypic and molecular confirmatory methods.

Sensitivity and specificity of screening methods. According to our findings, 60 rectal swab specimens gave similar results by all tested methods, while 13 swab specimens showed discrepant re-

TABLE 2 CPE and non-CPE strains recovered from 200 rectal swab specimens using the five surveillance screening methods

Method[a]	No. of strains							
	CPE				Non-CPE			
	K. pneumoniae KPC positive	*K. pneumoniae* VIM positive	*E. aerogenes* KPC positive	Total of CPE	*E. coli* ESBL positive	*P. mirabilis* ESBL positive	Nonfermenting bacteria	Total of non-CPE
CDC-TS	52	23	7	82		1	19	20
ESBL-BH	54	25	6	85	4	1	4	9
chromID ESBL	54	25	6	85	15	4	4	23
chromID CARBA	54	25	6	85			4	4
MCM	52	23	7	82			22	22
Total	56	29	7	92	15	4	22	41

[a] CDC-TS, inoculation of swabs in tryptic soy broth with a 10-μg ertapenem disk followed by plating to MacConkey agar (100 μl of the selective tryptic soy broth after overnight enrichment); ESBL-BH, inoculation of swabs in brain heart infusion broth with a 10-μg ertapenem disk followed by plating to a chromID ESBL plate (100 μl of the selective brain heart infusion broth after 4 to 6 h of enrichment); MCM, supplementation of MacConkey agar plates with meropenem (1 μg/ml).

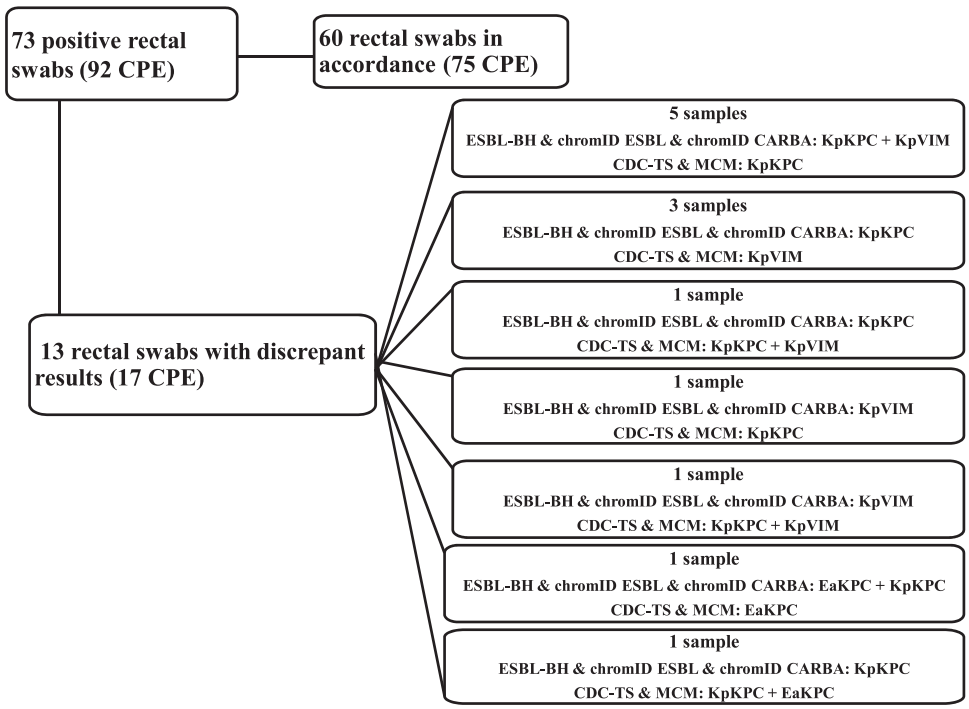

FIG 1 Recovery of 92 CPE strains from 73 positive rectal swab specimens and analysis of discrepant results. CDC-TS, inoculation of swabs in tryptic soy broth with a 10-μg ertapenem disk followed by plating to MacConkey agar (100 μl of the selective tryptic soy broth after overnight enrichment); ESBL-BH, inoculation of swabs in brain heart infusion broth with a 10-μg ertapenem disk followed by plating to a chromID ESBL plate (100 μl of the selective brain heart infusion broth after 4 to 6 h of enrichment); MCM, supplementation of MacConkey agar plates with meropenem (1 mg/liter); KpKPC, *K. pneumoniae* KPC positive; KpVIM, *K. pneumoniae* VIM positive; EaKPC, *E. aerogenes* KPC positive.

sults either in the number of CPE strains with different carbapenemase genes (11 samples) or in the number of different species of CPE that were identified (2 samples) (Fig. 1).

Recovery of CPE strains was enhanced on chromID CARBA, ESBL-BH, and chromID ESBL (85/92 CPE strains; sensitivity, 92.4%) compared with that on CDC-TS and MCM (82/92 CPE strains; sensitivity, 89.1%) ($P = 0.631$; Table 3). The specificity was greater for chromID CARBA (96.9%) and ESBL-BH (93.3%) than for CDC-TS (86.4%), MCM (85.2%), and chromID ESBL (84.7%) ($P = 0.014$; Table 3), largely due to the recovery of ESBL-producing *Enterobacteriaceae* on ESBL chromogenic medium and nonfermenting bacteria with the CDC-TS and MCM methods. PPVs and NPVs of the tested methods for CPE detection were, respectively, 93.4% and 94.8% for chromID CARBA, 90.4% and 94.8% for ESBL-BH, 80.4% and 92.7% for CDC-TS, 78.8% and 92.7% for MCM, and 73.9% and 94.8% for chromID ESBL. chromID CARBA and ESBL-BH were the most accurate screening methods for CPE detection in rectal swabs within 24 h upon receipt (overall accuracies, 95.1% and 93%, respectively; Table 3).

TABLE 3 Performance of the five culture screening methods for detection of CPE in active surveillance

Method[a]	Sensitivity (%)[b]	Specificity (%)[c]	PPV (%)	NPV (%)	Accuracy (%)
CDC-TS	89.1	86.4	80.4	92.7	87.4
ESBL-BH	92.4	93.3	90.4	94.8	93.0
chromID ESBL	92.4	84.7	73.9	94.8	85.1
chromID CARBA	92.4	96.9	93.4	94.8	95.1
MCM	89.1	85.2	78.8	92.7	86.7

[a] CDC-TS, inoculation of swabs in tryptic soy broth with a 10-μg ertapenem disk followed by plating to MacConkey agar (100 μl of the selective tryptic soy broth after overnight enrichment); ESBL-BH, inoculation of swabs in brain heart infusion broth with a 10-μg ertapenem disk followed by plating to a chromID ESBL plate (100 μl of the selective brain heart infusion broth after 4 to 6 h of enrichment); MCM, supplementation of MacConkey agar plates with meropenem (1 μg/ml).
[b] Differences in sensitivities among the five screening methods were not significantly different ($P = 0.631$).
[c] Differences in specificities among the five screening methods were significantly different ($P = 0.014$).

DISCUSSION

Spread of CPE is rising in several parts of the world (11, 22). CPE have the ability to transfer their resistance genes to other pathogens in the hospital environment as well as to cause large mono- or multiclonal hospital outbreaks (11, 12, 30, 32, 35). Thus, the control of these pathogens is imperative and clinical laboratories are facing the challenge of screening surveillance specimens for CPE. Moreover, detection of these pathogens should be accomplished in a short time interval from hospital admission in order to prevent further dissemination in the hospital environment.

In this surveillance study, we evaluated a new chromogenic medium, chromID CARBA, and compared its performance to that of four other culture-based techniques for the detection of CPE from rectal swabs. As comparators we used the screening technique proposed by CDC (8), a CDC-like bioMérieux protocol using chromID ESBL medium, the chromID ESBL screening medium designed for the detection of ESBL producers (28), and an in-house selective MacConkey plate supplemented with meropenem (1 μg/ml). In the last medium we preferred to use mero-

penem instead of imipenem, because the stability of imipenem in culture media is not sufficient (36).

Our comparative study showed that chromID CARBA exhibits the greatest specificity and PPV (96.9% and 93.4%, respectively) for CPE detection. However, it should be mentioned that the study was performed in a region where hospitalized patients are frequently colonized with KPC and/or VIM producers, and in countries with scarcely detected CPE, the screening method may exhibit considerably lower PPVs. chromID CARBA also demonstrated equal sensitivity (92.4%) with direct plating onto chromID ESBL medium, as well as the short selective enrichment method followed by plating onto chromID ESBL medium. All three methods using chromogenic media exhibited higher sensitivities and NPVs than the CDC-TS and MCM screening methods. However, a conclusion regarding the sensitivity of the tested methods to detect other types of CPE, especially OXA-48 producers, which could show very low carbapenem MICs, cannot be drawn. It is also worth mentioning that the CDC-TS method gave results in one additional day compared to the direct plating methods, which may delay the implementation of appropriate infection control measures (19, 20, 23).

In previous active surveillance studies, the performance of another chromogenic medium, CHROMagar KPC, was compared with that of other screening methods for the detection of carbapenem-resistant *Enterobacteriaceae* (23, 29). CHROMagar KPC was found to exhibit greater sensitivity and specificity than in-house-prepared MacConkey agar supplemented with imipenem (23) or MacConkey agar with carbapenem disks (29). Also, Adler et al. (1) compared the performance of CHROMagar KPC with the performances of MacConkey agar with imipenem at 1 μg/ml (MacI) and MacConkey plates with carbapenem disks. In that study, MacI demonstrated the greater overall accuracy for the detection of carbapenem-resistant *Enterobacteriaceae*, even though MacI and CHROMagar KPC had similar sensitivities and NPVs (1). Finally, Carrër et al. (5) compared the performance of chromID ESBL with that of CHROMagar KPC and concluded that the former medium is more sensitive for detecting isolates with low-level carbapenem resistance.

This is the first study describing the use of the prototype chromID CARBA medium for rapid and direct detection of CPE from clinical specimens. This chromogenic medium, especially designed for CPE detection, demonstrated an excellent ability to detect CPE, and its LOD performance was comparable to the LOD performances of other screening methods. chromID CARBA was able to detect CPE strains with variable carbapenem MICs (2 to >32 μg/ml), different from the findings for CHROMagar KPC (5). Moreover, unlike chromID ESBL and ESBL-BH, chromID CARBA was able to recover the single OXA-48 reference strain in LOD experiments, but this was achieved only at a high inoculum (10^7 CFU/ml). This is of particular interest, since detection of OXA-48-producing pathogens remains problematic, especially in countries where such isolates may have disseminated (13, 22).

The color characteristics on chromID CARBA permit easy differentiation of the bacterial colonies (*E. coli* appears pink to burgundy, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Citrobacter* spp. appear green-blue to browny green, and *Proteeae* have dark to light brown colonies). Thus, with chromID CARBA it was possible to distinguish a variety of CPE growing simultaneously on the plate owing to the different colony colorations. Moreover, this medium inhibited all ESBL isolates that were recovered on chromID ESBL or by the ESBL-BH protocol. Recently, chromID CARBA was compared with Colorex KPC (the only preprepared chromogenic medium for CPE isolation marketed in the United Kingdom) for their ability to support the growth of CPE (24). The evaluation was not performed directly from rectal swabs but was performed with bacterial suspensions recovered from stool samples, and chromID CARBA was found to be more sensitive than Colorex KPC to detect NDM MBL-producing pathogens (24).

It should be mentioned that different CPE strains may coexist in a fecal sample, particularly in regions of endemicity. These strains could not always be easily differentiated by color or colony morphology in different screening media. In the present study, this may explain why some CPE strains that coexisted in the same sample with other carbapenemase-producing strains were not identified using either the chromogenic media or the CDC-TS and MCM methods (Fig. 1).

In conclusion, chromID CARBA was found to be an easily performed and very accurate screening method for CPE detection in rectal swabs. Taking into account that the time of detection of such pathogens is crucial in infection control policies, this method efficiently identified patients colonized with CPE strains in a much shorter time (24 h upon receipt) than in-house-prepared screening media. This is crucial to promptly report isolates with carbapenemase activity and implement appropriate infection control interventions as well as to identify colonized patients at risk of invasive infection.

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