JOURNAL OF CLINICAL MICROBIOLOGY, June 2010, p. 2091–2096 Vol. 48, No. 6
0095-1137/10/$12.00 doi:10.1128/JCM.02342-09

Evaluation of Brilliance ESBL Agar, a Novel Chromogenic Medium for Detection of Extended-Spectrum-Beta-Lactamase-Producing *Enterobacteriaceae*▽

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Received 30 November 2009/Returned for modification 20 January 2010/Accepted 12 April 2010

The aim of this study was to evaluate the performance of Brilliance ESBL agar (OX; Oxoid, Basingstoke, United Kingdom), a novel chromogenic agar for the selective isolation and the presumptive identification of extended-spectrum-beta-lactamase (ESBL)-producing *Enterobacteriaceae*. A panel of 200 clinical Gram-negative *Enterobacteriaceae* and nonfermenting isolates with defined resistance mechanisms was inoculated onto OX and onto ChromID ESBL agar (BM; bioMérieux, Marcy l'Etoile, France) chromogenic medium in the first part of the study to evaluate the growth selectivity and chromogenic features of these two media. Of the 156 *Enterobacteriaceae* challenge isolates, 8 fully susceptible isolates were inhibited, all 98 ESBL producers were detected, and 50 isolates harboring other resistance mechanisms were recovered on both chromogenic agars. In the second phase, 528 clinical samples (including 344 fecal specimens) were plated onto OX, BM, and MacConkey agar with a ceftazidime disk (MCC) for the screening of ESBL-producing *Enterobacteriaceae*. Growth on at least one medium was observed with 144 (27%) of the clinical samples screened. A total of 182 isolates, including 109 (60%) of *Enterobacteriaceae*, were recovered and 70 of these (from 59 specimens) were confirmed as ESBL-producing isolates. The sensitivities of MCC, BM, and OX were 74.6%, 94.9%, and 94.9%, respectively. The specificities of MCC, BM, and OX by specimens reached 94.9%, 95.5%, and 95.7%, respectively, when only colored colonies were considered on the two selective chromogenic media. The high negative predictive value (99.3%) found for OX suggests that this medium may constitute an excellent screening tool for the rapid exclusion of patients not carrying ESBL producers.

Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) are of growing importance in the field of medical bacteriology following their epidemiological success (1, 2), remarkably illustrated by the worldwide dissemination of CTX-M-type ESBL enzymes (2). The spread of ESBLs in Gram-negative bacteria represents a major challenge to the antimicrobial therapy of infections caused by these organisms either in hospitals or in a community setting (12). While definitive guidelines for the management of patients infected with ESBL-producing bacteria are still awaited, there is strong evidence that failure to detect ESBL-mediated resistance can lead to treatment failure (13). The use of surveillance cultures or of targeted screening for ESBL producers in high-risk patients or in high-risk units such as intensive care units has been advocated to prevent or control outbreaks of nosocomial infections with these organisms (10, 11).

A range of screening and confirmation tests have been developed to detect isolated colonies of ESBL producers following culture, and these tests have been validated mostly for *Escherichia coli* and *Klebsiella* spp. However, the increasing diversity of ESBL enzymes, together with the expression of non-ESBL resistance mechanisms such as overproduction of cephalosporinases, has led to an overlap of different resistance phenotypes, making the identification of ESBL producers by conventional phenotypic techniques a constant challenge (6).

Selective culture media such as MacConkey (14) and Drigalski (18) agars supplemented with cefotaxime and/or ceftazidime at various concentrations have been proposed for the detection of ESBL producers. BLSE agar (AES Chemunex, Bruz, France) was the first commercially available selective medium dedicated to the recovery Gram-negative bacteria resistant to expanded-spectrum cephalosporins. Recently, breakthroughs in the developments of selective chromogenic media have improved the detection of methicillin-resistant *Staphylococcus aureus* (5) and vancomycin-resistant enterococci (9) directly from clinical specimens. The selectivity provided by the mixture of antibiotics and the presumptive identification of microorganisms based on colony coloration by chromogenic media have made their use possible for rapid and accurate screening of specific resistant pathogens in highly contaminated samples such as stool samples or rectal swabs. This is of particular interest since fecal colonization is a known factor in infection due to ESBL-producing *Enterobacteriaceae* (16).

In this study, we evaluated the sensitivity and specificity of the prototype chromogenic selective medium Brilliance ESBL agar (OX; Oxoid, Basingstoke, United Kingdom) in comparison with those of another commercial medium, ChromID ESBL agar (BM; bioMérieux, Marcy l'Etoile, France), for the detection and presumptive identification of ESBL-producing *Enterobacteriaceae*. The study was split into two phases; in the first part, we challenged a collection of microorganisms with

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▽ Published ahead of print on 21 April 2010.

well-characterized resistance mechanisms, and in the second part, we tested clinical samples directly.

MATERIALS AND METHODS

Interpretation of isolates on chromogenic media. The density of growth was scored semiquantitatively (+1, +2, +3, or +4, based on the number of quadrants in which growth was observed). Colony size was measured in millimeters, and the type or absence of coloration of each isolate on each medium was also recorded. According to the expected chromogenic features provided in the manufacturers' specifications, *E. coli* isolates should produce red-burgundy colonies on BM and blue-violet (or pink if beta-galactosidase negative) colonies on OX; the organisms of the KESC (*Klebsiella-Enterobacter-Serratia-Citrobacter*) group should grow green colonies on both BM and OX; the species of the PMP (*Proteus-Morganella-Providencia*) group should display tan or light brown colonies on both chromogenic agars; *Pseudomonas* spp. should be suspected on the basis of naturally pigmented irregular spreading colonies and a positive oxidase character on both media. According to the instructions of both manufacturers, any colored and oxidase-negative colonies growing on either of the two chromogenic media were regarded as presumptive ESBL-producing *Enterobacteriaceae* isolates and should be confirmed using additional phenotypic or genotypic methods while colorless colonies were assumed to be nonenterobacterial isolates. In our study, for the purpose of evaluation, oxidase-positive colonies and colorless colonies were identified in order to assess the specificity and sensitivity of the media.

Challenge strain study. (i) Bacterial isolates. The isolates tested were selected from the collection of clinical bacterial isolates of the bacteriology laboratory of the Cliniques UCL de Mont-Godinne. All isolates had been identified and tested for susceptibility by the Vitek 2 system using GN and AST-N046 cards (bioMérieux, Marcy l'Etoile, France). The confirmation of ESBL-producing isolates was performed by combined double disks (30 μg ceftazidime and 30 μg cefotaxime with and without 10 μg clavulanic acid) according to CLSI guidelines (3). Genotypic characterization of ESBL resistance mechanisms in *Enterobacteriaceae* isolates was done by molecular testing using endpoint PCR assays targeting the bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ bla_{OXA}, and bla_{AmpC} genes with amplicon sequencing according to previously published methods (4, 15).

The challenge set of organisms tested for the ability to grow on the two chromogenic media comprised 200 isolates, including 156 *Enterobacteriaceae* and 44 nonfermenting Gram-negative bacteria (Table 1). The 98 ESBL-positive *Enterobacteriaceae* isolates carried 41 CTX-M-type, 32 SHV-type, and 28 TEM-type ESBL enzymes (including 4 strains harboring two or more ESBL resistance genes). Two carbapenemase-producing *Klebsiella pneumoniae* isolates (one VIM-1 and one KPC-2) were plated on the media as well.

(ii) Culture conditions. All strains had been stored at −70°C in cryobeads with glycerol and were cultured after one passage on tryptic soy agar containing 5% sheep blood before testing. Subcultured isolates were suspended in 1 ml sterile 0.9% saline, and the inoculum was adjusted to a density corresponding to a 0.5 McFarland standard. A 10-μl inoculum of this suspension (i.e., about 10^6 CFU) was streaked onto OX, BM, and MacConkey agar plates (used as growth controls). All plates were incubated at 35°C in ambient air for 24 h before being read.

Clinical specimen study. (i) Hospital setting and specimen collection. This study was performed at the Cliniques Universitaires St-Luc (a 1,000-bed tertiary-care university teaching hospital) in Brussels, Belgium, from February 2009 to April 2009. A total of 528 samples were processed, including 344 fecal samples (296 stool samples and 48 rectal swabs), 134 respiratory tract samples (117 lower respiratory tract aspirates, 17 throat swabs), and 50 miscellaneous samples (33 wound swabs, 14 urine samples, 2 blood cultures, and 1 vaginal swab). The 528 specimens were obtained from 424 patients in either an ambulatory-care setting (25% of the samples were from the emergency department and polyclinics) or a hospital setting (75%), including 187 samples collected in intensive care units, 61 from oncology-hematology units, 65 from general medicine units, 31 from general surgery units, and 51 from pediatric and obstetric units.

(ii) Inoculation and incubation of the media. Each specimen was homogenized in 1 ml sterile 0.85% saline, and 50-μl aliquots of the resulting suspension were inoculated onto OX, BM, and MacConkey agar onto which a 30-μg ceftazidime Neosensitabs tablet (Rosco, Taastrup, Denmark) was placed. All media were incubated at 35°C in ambient air for a full 24 h before reading.

(iii) Identification and susceptibility testing. All colonies developing on one of the two chromogenic media or within a 20-mm inhibition zone diameter of a ceftazidime disk on MacConkey agar (all of these isolates are referred to in this report as isolates growing on MCC medium) were subcultured to Columbia 5% sheep blood agar. After overnight incubation at 35°C, Gram-negative isolates were submitted for complete identification and for susceptibility testing using the Phoenix system panels (NMIC/ID-51; BD Diagnostic Systems, Sparks, MD). The presence of an ESBL (based on synergy between clavulanic acid and one or several cephalosporin substrate indicators) was suggested by double-combination disks using Neosensitabs tablets (30-μg ceftazidime and 30-μg cefotaxime disks with or without 10 μg clavulanic acid) according to CLSI guidelines (3).

All isolates belonging to the *Enterobacteriaceae* group were referred to the bacteriology laboratory of the Mont-Godinne hospital for genotypic characterization of resistance mechanisms by multiplex PCR targeting the most common ESBL-encoding genes (as described above).

Statistical analysis of results. An isolate was categorized as ESBL positive when the ESBL phenotype displayed in the double-disk test was confirmed by genotypic characterization with the multiplex PCR. The evaluation of the performance of the different media (sensitivities, specificities, and positive predictive value [PPV] and negative predictive value [NPV]) based on the total number of samples was assessed by statistical analysis using nonparametric McNemar tests and 95% confidence intervals (CI) calculated for each performance attribute compared. A sample was considered ESBL positive when at least one ESBL-positive isolate was recovered from it. A sample was considered ESBL negative when there was no growth on any medium or when no isolate recovered was confirmed to be an ESBL producer.

TABLE 1. Species and resistance mechanism distribution of the 200 challenge strains tested on the two chromogenic media

Group and resistance mechanism	Species	No. of isolates growing on:		No. of isolates tested
		BM	OX	
Enterobacteria				
ESBL	*E. coli*	45	45	45
	K. pneumoniae	22	22	22
	E. aerogenes	20	20	20
	E. cloacae	5	5	5
	C. freundii	3	3	3
	K. oxytoca	3	3	3
Carbapenemase	*K. pneumoniae*	2	2	2
AmpC cephalosporinase	*E. coli*	9	9	12
	E. aerogenes	11	11	11
	C. freundii	6	5	6
	M. morganii	2	3	3
	K. pneumoniae	2	2	2
K1-OXY penicillinase	*K. oxytoca*	8	8	8
OXA-30 penicillinase	*E. coli*	3	3	6
Susceptible (wild type)	*E. coli*	0	0	4
	K. pneumoniae	0	0	2
	E. aerogenes	0	0	1
	K. oxytoca	0	0	1
Nonfermenters	*P. aeruginosa*	21	20	21
	S. maltophilia	13	2	15
	A. baumannii	8	7	8

RESULTS

Challenge strain study. Among the 156 strains belonging to the *Enterobacteriaceae* group, 8 isolates (4 *E. coli*, 2 *K. pneumoniae*, 1 *K. oxytoca*, and 1 *Enterobacter aerogenes*) showed no growth on either of the two chromogenic selective media. These 8 strains were known to be highly susceptible to most antimicrobials (data not shown) and had been selected as control organisms to test the abilities of the two selective chromogenic media to inhibit bacterial growth.

TABLE 2. Identification and distribution of microorganisms recovered from 528 clinical samples on the three selective media tested

Group and species	No. of isolates (ESBL producers) growing on:			
	MCC	BM	OX	All media
Enterobacteria				
E. coli	36 (32)	52 (45)	54 (47)	62 (50)
E. cloacae	10 (5)	10 (4)	9 (4)	11 (5)
K. pneumoniae	7 (6)	8 (7)	7 (7)	9 (7)
E. aerogenes	7 (3)	7 (3)	6 (3)	7 (3)
K. oxytoca	0	5	3	5
M. morganii	3 (1)	2 (1)	5 (1)	5 (1)
C. freundii	3 (1)	3 (2)	3 (1)	4 (2)
S. marcescens	0	1 (1)	2 (2)	2 (2)
Citrobacter farmeri	0	2	2	2
Hafnia alvei	0	2	0	2
Nonfermenters and others				
P. aeruginosa	5	23	41	43
Pseudomonas putida	0	6	9	9
S. maltophilia	2	9	0	9
A. baumannii	1	6	1	6
Acinetobacter lwoffii	1	1	0	1
Yeast	2	0	0	2
E. faecium	0	2	0	2
E. faecalis	0	1	0	1

TABLE 3. Characterization of ESBLs from *Enterobacteriaceae* isolates from clinical samples growing on any of the three selective media tested

Species	No. of isolates carrying ESBL enzymes:				
	TEM type	SHV type	CTX-M group 1	CTX-M group 2	CTX-M group 9
E. coli	9	2	34	2	3
K. pneumoniae		1	6		
E. cloacae[a]		4	1		3
E. aerogenes	2		1		
S. marcescens		1	1		
C. freundii		1	1		
M. morganii				1	

[a] Two ESBLs (SHV-type and CTX-M group 9) were detected in three *E. cloacae* isolates.

Of the 148 *Enterobacteriaceae* strains with defined mechanisms of resistance to β-lactam agents, 140 grew on both BM and OX; 6 *E. coli* strains did not grow on any of the chromogenic media, while 1 *Citrobacter freundii* strain was inhibited on BM and 1 *Moraxella morganii* strain was inhibited on OX (Table 1). Overall, from a mechanistic perspective, all 98 ESBL-producing isolates, as well as the 2 carbapenemase-producing isolates, were recovered on both selective chromogenic media whereas 30 AmpC-overproducing isolates and all 8 *K. oxytoca* K1-OXY penicillinase hyperproducers grew on both media; 3 out of 6 OXA-30 penicillinase-producing *E. coli* isolates were inhibited and did not grow on either medium.

No significant differences in growth density on the two media were observed (growth up to the third or fourth streaking quadrant was obtained for 133 isolates on both media). Likewise, the size of the colonies (2 mm, on average, after 24 h of incubation) was found acceptable and comparable on both media.

Regardless of the resistance mechanisms, all *Klebsiella* sp. and *Enterobacter* sp. isolates produced the expected green colony color on both BM and OX. A major difference was that on BM, all strains of *C. freundii* grew as colorless colonies while they were green on OX. Another interesting chromogenic characteristic was that 7 of the 11 *K. oxytoca* isolates grew as turquoise colonies on OX (but not on BM); these were slightly different from the green colonies of the other species of the KESC group. For the 67 *E. coli* isolates tested, all but 3 strains that remained colorless appeared with a uniform pink/burgundy color on BM while there was some variability in the color of the colonies on OX, which ranged from the expected blue/purple ($n = 49$) to green/turquoise ($n = 18$).

Among the 44 nonfermenters tested, all but 1 *Pseudomonas aeruginosa* isolate and 1 *Acinetobacter baumannii* isolate grew on both chromogenic media; only 1 isolate each of these two species was inhibited on OX (Table 1). A major difference between the two chromogenic media was that all of the 15 *Stenotrophomonas maltophilia* isolates tested (all being intrinsically resistant to most β-lactams, including carbapenems) did grow on BM while 13 of these were completely inhibited on OX (two yielded growth with a low density score). Overall, the aspect and color of the different nonfermenter colonies observed on OX matched well those of colonies observed on BM (white for *A. baumannii* and reddish brown for most *P. aeruginosa* strains, reflecting the presence of the species-related pyocyanin pigmentation).

Clinical specimen study. Of the 528 samples analyzed, 384 (73%) showed no growth on any of the three media and 144 (27%) yielded growth on at least one of the three media. A total of 182 isolates, including 109 of *Enterobacteriaceae* (60%) were recovered, and 70 of these were confirmed as ESBL-producing isolates. The 70 ESBL-producing isolates were recovered from 59 specimens (11% of the collected specimens), mostly from fecal samples ($n = 44$; 75% of the ESBL-positive samples). The species distribution of *Enterobacteriaceae* isolates was similar to the distribution of ESBL producers seen at our hospital, with *E. coli* representing a large majority (71%) of the isolates recovered (Table 2).

Overall, CTX-M-type ESBLs (53 isolates) were the most frequently encountered, whereas isolates producing TEM-type ($n = 11$) and SHV-type ($n = 9$) ESBLs were less commonly recovered (Table 3). Three *E. cloacae* isolates harbored two different types of ESBLs (CTX-M group 9 and SHV enzymes).

Of the 70 ESBL-producing *Enterobacteriaceae* isolates recovered on either of the media, only 1 isolate (*Serratia marcescens*) appeared colorless on OX while 6 isolates (4 of *E. coli* and 2 of *C. freundii*) would have been missed on BM had the selection been based only on the color of the colonies. However, of the 50 *E. coli* isolates, 41 displayed a homogeneous burgundy color on BM whereas on OX, 34 had the expected blue-violet colonies, 1 had pink colonies lacking β-galactosidase, and 12 showed a variable turquoise color. The 16 KES group isolates (7 of *K. pneumoniae*, 5 of *Enterobacter cloacae*, 3 of *E. aerogenes*, 1 of *S. marcescens*) and 1 strain of *M. morganii* displayed the predicted green and tan-brown colors, respectively, on both media.

TABLE 4. Sensitivities, specificities, PPVs, and NPVs of the three media tested on 528 clinical specimens

Samples considered and medium	No. of samples			% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
	TP[a]	FP[b]	FN[d]				
All samples							
MCC	44	24	15	74.6 (68.9–80.2)	96.8 (96.0–97.6)	64.7 (58.9–70.5)	96.8 (96.0–97.6)
BM	56	59	3	94.9 (92.1–97.8)	94.9 (93.9–95.9)	48.7 (44.0–53.4)	99.3 (98.9–99.7)
OX	56	64	3	94.9 (92.1–97.8)	95.1 (94.1–96.1)	46.7 (42.1–51.2)	99.3 (98.9–99.7)
Selected samples[c]							
BM	51	21	8	86.4 (82.0–90.9)	95.5 (94.6–96.5)	70.8 (65.5–76.2)	98.2 (97.6–98.9)
OX	56	20	3	94.9 (92.1–97.8)	95.7 (94.8–96.7)	73.7 (68.6–78.7)	99.3 (99.0–99.7)

[a] TP, true positive. A result was considered TP when at least one ESBL-positive isolate was recovered from the sample.
[b] FP, false positive. A result was considered FP when the isolate(s) recovered was not confirmed to be an ESBL producer.
[c] When selection criteria (oxidase-negative and colored colonies) were applied.
[d] FN, false negative.

ESBL-producing organisms were detected in 44 specimens on MCC and in 56 specimens each on BM and OX (sensitivity of 94.9% for BM and OX and 74.6% for MCC; $P < 0.01$). Of the 70 ESBL-positive isolates detected, 48 were recovered on MCC (sensitivity, 68.6%), 63 were recovered on BM (sensitivity, 90.0%), and 65 were recovered on OX (sensitivity, 92.9%). The NPV calculated for all of the samples reached 99.3% for both BM and OX and was higher than that for MCC (96.1%). When only samples yielding colored colonies of *Enterobacteriaceae* isolates were considered, OX displayed a higher sensitivity (94.9%) than BM (86.4%; $P = 0.07$ [not significant]) for ESBL detection (Table 4). However, among ESBL-producing isolates recovered from either of the two chromogenic media, BM showed a higher proportion of isolates (57/63, 90%) displaying the predicted color stated in the manufacturer's instructions than did OX (52/65, 80%), on which 12 ESBL-positive *E. coli* isolates did not express the expected blue or pink color.

The specificities assessed of MCC, BM, and OX for 469 samples that were found to be negative for ESBL-producing isolates by all of the media used were 94.9%, 87.4%, and 86.4%, respectively. The specificity increased for the two chromogenic media when only colored *Enterobacteriaceae* colonies were considered, reaching 95.5% and 95.7% for BM and OX. However, PPVs remained low under these interpretative conditions, reaching 74.6%, 70.8%, and 73.7% for MCC, BM, and OX, respectively (Table 3).

For the 60 ESBL-producing isolates that grew on both chromogenic media, a growth density comparison showed identical scores for 53 isolates, while a higher growth score with a higher number of colonies was found on OX for 5 isolates and on BM for 2 isolates. ESBL-positive colonies showed a comparable average size of 2 mm on both media, although 13 isolates grew larger colonies on OX versus 9 on BM.

A total of 112 non-ESBL-producing isolates from 94 samples grew on any of the three media, including 68 nonfermenting Gram-negative bacilli, 39 *Enterobacteriaceae* isolates, and 5 non-Gram-negative microorganisms. Of the 39 *Enterobacteriaceae* isolates, natural AmpC hyperproducers ($n = 21$) accounted for most of the "false-positive" results on all three media. Of the other 18 non-AmpC- and non-ESBL-producing enterobacteria, 13 and 11 isolates grew on BM and OX, respectively, including 5 *K. oxytoca* hyperproducers of chromosomal K1-OXY penicillinase, 4 non-ESBL TEM- or SHV-producing *E. coli* isolates, and 2 OXA-30 penicillinase-producing *E. coli* isolates (Table 5).

Besides *Enterobacteriaceae* isolates, *Pseudomonas* spp. represented the most frequently recovered isolates on the two chromogenic media (29 on BM and 50 on OX). Colonies of *Pseudomonas* isolates were colorless or variably displayed a straw or brown color (31% on BM and 78% on OX) and could be easily identified by a positive oxidase test. Other nonfermenting isolates (9 *S. maltophilia* and 6 *Acinetobacter* spp.) mostly grew on BM but not on OX, produced colorless colonies, and hence could also be easily distinguished from *Enterobacteriaceae* (Table 2). Organisms other than Gram-negative bacteria were only recovered on MCC (2 yeasts) and on BM (3

TABLE 5. Distribution of resistance mechanisms other than ESBL among enterobacterial isolates from clinical samples growing on the three selective media

Resistance mechanism, species	No. of isolates growing on:			
	MCC	BM	OX	All media
Chromosomal AmpC cephalosporinase				
C. freundii	2	1	2	2
E. aerogenes	4	4	3	4
E. cloacae	5	6	5	6
E. coli	3	1		3
H. alvei		2		2
M. morganii	2	1	4	4
Plasmidic AmpC cephalosporinase (CMY-type), *E. coli*	1	1	1	1
TEM- or SHV-derived penicillinases				
E. coli		1	4	4
K. pneumoniae	1	1		2
K-OXY penicillinase, *K. oxytoca*		5	3	5
OXA group 3 beta-lactamase, *E. coli*		2	2	2
Other chromosomal beta-lactamase, *C. farmeri*		2	2	2
Nonenzymatic, *E. coli*		2		2

Enterococcus spp., including two *vanA*-type vancomycin-resistant *Enterococcus faecium* isolates).

DISCUSSION

In this study, we decided not to increase the incubation time beyond 24 h based on previous studies, which all indicated little gain in ESBL recovery rates but rather an increase in interfering growth of associated flora (7, 8, 17).

From this evaluation, it appears that OX performs very well and is similar to BM in both supporting the growth of ESBL-producing *Enterobacteriaceae* isolates and inhibiting susceptible organisms. The two chromogenic media showed equal performance in terms of sensitivity when all ESBL-producing isolates were selected. However, when considering the color of the colonies of the *Enterobacteriaceae* isolates, OX yielded a higher sensitivity than BM, although the difference did not reach statistical significance. Chromogenic media definitely proved superior to MCC, with which a substantial number of CTX-M type ESBL producers (20 out of 22 ESBL-producing isolates not recovered on MCC in our study) were missed.

Our findings regarding the performance of the BM were similar to those obtained from previous studies. Glupczynski et al. reported a sensitivity of 97.7% and a specificity of 89% for BM assessed on ESBL-negative specimens (8). Réglier-Poupet et al. found a lower sensitivity of 88% because 4 out of 33 ESBL-producing strains failed to produce colored colonies (17). In the present study, we also found that 4 ESBL-producing *E. coli* isolates produced colorless colonies on BM, explaining the decrease in sensitivity achieved by this medium when the chromogenic criterion was taken into account. We could confirm the lack of ability of the two selective media to differentiate ESBL-mediated resistance from other very similar resistance mechanism phenotypes (e.g., AmpC and K1-OXY penicillinase overproduction). The same conclusions were suggested by the low PPV of BM (38.7%) found by Réglier-Poupet et al. (17) and by the low specificity of BM (10.5% of the total ESBL-negative strains) reported in the collection strain study conducted by Färber et al. (7).

The chromogenic feature of the medium was fully exploited by OX, which yielded the lowest number of colorless *Enterobacteriaceae* isolates. However, some drawbacks should be mentioned regarding the large variability in the color of the colonies displayed by *E. coli* isolates (gradation and variations from green/turquoise blue to blue/purple), which in some cases led to difficulties in differentiating *E. coli* strains from those of other species belonging to the KESC group. Since it is anticipated that such chromogenic selective media will mainly be used to screen for ESBL carriage, it may be important for infection control purposes (i.e., in hospital outbreaks) to allow both early recognition and easy species differentiation. In this respect, the colors of the colonies were more uniform for *E. coli* (although some *E. coli* strains were colorless) and differed more between different *Enterobacteriaceae* species on BM than on OX. On the other hand, OX proved superior to BM for the detection of *C. freundii*, which was always green on the former medium but colorless on BM.

Regarding the nonfermenters, no significant differences could be observed between the two chromogenic media (colorless *Acinetobacter* spp. or naturally pigmented brownish *P. aeruginosa* on both media), except the marked inhibitory activity of OX against *S. maltophilia*, which grew on BM. Although the PPV of OX was low, the chromogenic features and enhanced selectivity of the medium minimized the number of non-*Enterobacteriaceae* oxidase-negative isolates recovered. The selectivity of OX would have avoided 36% of the unnecessary identification and ESBL confirmatory tests.

Overall, the new Brilliance ESBL agar proved valuable and showed performance as high as that of ChromID ESBL agar for the detection of ESBL-producing *Enterobacteriaceae*, yielding an excellent NPV of 99.3% at 24 h, and appeared to be a promising screening method which could enable rapid exclusion of patients not carrying ESBL producers.

ACKNOWLEDGMENTS

This study was sponsored in part by Oxoid Ltd., part of Thermo Fisher Scientific.

We thank Emma Scopes for her dedicated assistance in this study.

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