Performance of chromID ESBL, a chromogenic medium for detection of *Enterobacteriaceae* producing extended-spectrum β -lactamases

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The chromogenic agar medium chromID ESBL (bioMérieux) was compared with BLSE agar medium (AES) for selective isolation and presumptive identification of extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* from clinical samples. A total of 765 samples (468 rectal swabs, 255 urine samples and 42 pulmonary aspirations) obtained from 547 patients was processed. All bacterial strains isolated on either medium were further characterized using biochemical tests, and ESBL producers were confirmed by synergy testing. Genetic characterization of ESBL genes was determined by PCR. A total of 33 ESBL-producing *Enterobacteriaceae* strains [*Escherichia coli* ($n=16$), *Klebsiella pneumoniae* ($n=8$), *Enterobacter* spp. ($n=3$), *Citrobacter* spp. ($n=5$) and *Proteus mirabilis* ($n=1$)] was recovered. The sensitivity after 24 h incubation was 88 % for chromID ESBL and 85 % for BLSE agar. At 48 h, the sensitivity of chromID ESBL increased to 94 % and was higher than that obtained with BLSE agar. The positive predictive value at 24 h for chromID ESBL was 38.7 % [95 % confidence interval (95 % CI) 28.3–50.2 %], which was significantly higher than that for BLSE agar [15.4 %, 95 % CI 10.1–21.5 %]. On both media, false-positive results were mostly due to *Pseudomonas aeruginosa* and to *Enterobacteriaceae* overproducing chromosomal cephalosporinase (*Enterobacter* spp.) or a chromosomal penicillinase (*Klebsiella oxytoca*). This study showed that chromID ESBL, a ready-to-use chromogenic selective medium, is sensitive and specific for rapid, presumptive identification of ESBL-producing *Enterobacteriaceae*. Its chromogenic properties and its selectivity are particularly useful in specimens containing resident associated flora.

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INTRODUCTION

Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs) are of growing concern among nosocomial and also community-acquired infections (Canton & Coque, 2006; Paterson, 2006; Pena *et al.*, 1997; Pitout *et al.*, 2005; Ramphal & Ambrose, 2006). Targeted surveillance involving high-risk patients and their screening is mandatory to prevent outbreaks of nosocomial infections by these organisms (Lucet *et al.*, 1999; Pfaller & Segreti, 2006; Valverde *et al.*, 2004).

Several selective culture media such as MacConkey (Pena *et al.*, 1997; Pfaller & Segreti, 2006; Valverde *et al.*, 2004) and Drigalski (Lucet *et al.*, 1999; Pfaller & Segreti, 2006)

agar supplemented with cefotaxime and/or ceftazidime at various concentrations have been proposed for the detection of ESBL producers. A commercially available medium (BLSE agar; AES) devoted to the detection of ESBL producers has existed since 2001. However, this medium does not specifically detect ESBL producers, but rather all Gram-negative bacteria resistant to expanded-spectrum cephalosporins. Media using chromogenic enzyme substrates and selective antibiotics have been developed recently for the detection of meticillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (Diederer *et al.*, 2006; Ledeboer *et al.*, 2007).

The aim of this study was to compare and evaluate the sensitivity and specificity of two selective media, BLSE agar and the chromogenic medium chromID ESBL (bioMérieux), which enable the detection and presumptive

Abbreviations: 95 % CI, 95 % confidence interval; ESBL, extended-spectrum β -lactamase; PPV, positive predictive value.

identification of ESBL-producing *Enterobacteriaceae* directly from clinical specimens.

METHODS

Hospital setting and specimen collection. The study was performed simultaneously in two university hospitals in Paris from May 2006 to July 2006 [Cochin Saint Vincent de Paul Hospital (1300 beds) and Bicêtre Hospital (1000 beds)]. A total of 765 samples obtained from 547 patients was processed comprising 468 rectal swabs, 255 urine samples and 42 pulmonary aspirates. Patients were hospitalized in intensive care, surgery and geriatric units.

Inoculation of media and incubation. Swab samples were placed in 1 ml sterile 0.9% saline and then vortexed. From this suspension, 100 µl was inoculated on two culture media: chromID ESBL (bioMérieux) and BLSE agar (AES). chromID ESBL is a medium designed for the isolation and detection of ESBL, based on a rich nutrient capacity with a mixture of antibiotics (for Gram-positive bacteria and yeast inhibition), including cefpodoxime. This antibiotic is recognized as being the marker of choice for the ESBL resistance mechanism. BLSE agar is a commercially available bi-plate made of two selective media, Drigalski and MacConkey, supplemented with cefotaxime (1.5 µg ml⁻¹) and ceftazidime (2 µg ml⁻¹), respectively, enabling the detection of Gram-negative bacteria resistant to these antibiotics. For swab samples treated as above, 100 µl was cultured onto each side of the bi-plate (i.e. 100 µl onto MacConkey agar and 100 µl onto Drigalski agar). For urine and aspiration samples, 50 µl of the sample was plated directly onto both selective media. Plates were incubated at 37 °C under aerobic conditions and assessed after 24 and 48 h incubation.

Identification of ESBL producers. Two staff members assessed the plates independently. For both culture media, the intensity of the growth of each type of colony was noted. For chromID ESBL, the colour and intensity of the colonies was recorded according to the colour chart provided by the manufacturer (*Escherichia coli* pink/burgundy; *Klebsiella/Enterobacter/Serratia* group blue/green; the *Proteae* tribe light to dark brown; Fig. 1). All isolates growing on BLSE agar and/or chromID ESBL were identified by the VITEK 2 system using GN cards or by the API 20E system (bioMérieux). For ESBL-positive isolates, an antibiogram was performed using a disc diffusion assay on Mueller–Hinton agar plates and interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2005). The following β-lactam antibiotics were tested: amoxicillin ± clavulanic acid, ticarcillin ± clavulanic acid, piperacillin ± tazobactam, cefoxitin, moxalactam, cefotaxime, ceftazidime, aztreonam, cefepime and imipenem. Confirmation of ESBL producers was performed by double disc synergy testing between ticarcillin/clavulanate and aztreonam and/or ceftazidime and/or cefepime (Jarlier *et al.*, 1988).

Molecular characterization of resistance mechanisms. The genotypic characterization of ESBL resistance mechanisms was determined by PCR assays targeting the *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{GES}, *bla*_{PER}, *bla*_{BEL-1}, *bla*_{VEB}, *bla*_{TLA-1}, *bla*_{TLA-2}, *bla*_{BES} and *bla*_{OXA-18} genes and subsequent amplicon sequencing according to published methods (Bonnet *et al.*, 2000; Girlich *et al.*, 2005; Naas *et al.*, 2006, 2007; Philippon *et al.*, 1997; Poirel *et al.*, 2005; Silva *et al.*, 2000). Isolates were considered to be ESBL-positive when the sequence of either the *bla*_{TEM} and/or the *bla*_{SHV} gene amplification products matched previously identified ESBLs (<http://www.lahey.org/Studies/>) and/or when the PCR for the other genes was positive.

Screening of reference clavulanic acid-inhibited ESBL producers. Strains from our collection of genetically characterized

Fig. 1. chromID ESBL medium plated with *E. coli* (a), and a mixture of *K. pneumoniae* and *P. mirabilis* (b). On chromID ESBL, colonies of *E. coli* appear pink/burgundy, colonies of the *Klebsiella/Enterobacter/Serratia* group appear blue/green and colonies of the *Proteae* tribe appear light to dark brown. The plates were observed after 24 h of growth.

enterobacterial species producing common ESBLs (TEM, SHV and CTX-M derivatives) or less common ESBLs (GES, VEB, PER, BES, BEL, TLA-1 and TLA-2), carbapenem-hydrolysing class A enzymes (GES-5, KPC, NMCA, Sme-1/2 and IMI-1) or class D ESBL (OXA-18) were streaked onto chromID ESBL and BLSE agar media.

Statistical analysis. To determine whether the results from each medium were significantly different, a 95 % confidence interval (95 % CI) on the proportion was calculated; if the intervals overlapped, then the compared performances were not different statistically.

RESULTS AND DISCUSSION

Detection of ESBL-mediated resistance in Gram-negative bacilli is crucial because of its clinical implications for treating infected patients and to limit the spread of these multidrug-resistant organisms in hospital settings (Canton & Coque, 2006; Pfaller & Segreti, 2006; Ramphal & Ambrose, 2006). Laboratory-based detection of ESBL producers from clinical specimens must be highly sensitive and specific, combined with a short reporting time for results, thus decreasing the workload and reducing the need for unnecessary confirmatory tests (Pfaller & Segreti, 2006). BLSE agar from AES, the only commercially

available test, made of two selective media (Drigalski and MacConkey agar) each supplemented with one antibiotic enabling the detection of Gram-negative bacteria resistant to cefotaxime and/or ceftazidime, was used to evaluate the chromogenic selective medium chromID ESBL (bioMérieux) intended to screen for carriage of *Enterobacteriaceae* ESBL producers.

Isolation of ESBL producers

Among the 765 samples analysed, 32 (4 %) were positive on chromID ESBL corresponding to 33 isolates, as 1 sample contained 2 different ESBL-producing enterobacterial species. A total of 18 isolates was recovered from rectal swabs, 13 from urine samples and 1 from a tracheal aspiration. The distribution of the enterobacterial species was in agreement with the current distribution of ESBL producers reported from clinical laboratories in Paris (Naas *et al.*, 2007). Accordingly, *E. coli* ($n=16$; 49 %) and *Klebsiella pneumoniae* ($n=8$; 24 %) were the most frequent *Enterobacteriaceae* ESBL producers recovered, followed by *Citrobacter* spp. ($n=5$; 15 %), *Enterobacter* spp. ($n=3$; 9 %) and *Proteus mirabilis* ($n=1$; 3 %).

Due to its chromogenic behaviour, chromID ESBL allowed easier detection of ESBL producers in mixed cultures, particularly when present at low colony counts. Moreover, a presumptive identification led to a one-step identification of bacterial species such as *E. coli*. This result is clinically relevant, as *E. coli* is currently the predominant ESBL producer in many countries (Canton & Coque, 2006; Naas *et al.*, 2007; Paterson, 2006). In this study, *E. coli* accounted for 49 % of the ESBL producers.

Specificities of chromID ESBL and BLSE agar

Specificity was assessed for 733 samples that were found to be negative for ESBL-producing isolates by both media. The specificity of chromID ESBL was 94.4 % (95 % CI 92.5–95.9 %) and 90.5 % (95 % CI 88.0–92.4 %) at 24 and 48 h, respectively. These results were significantly higher than those observed on BLSE agar of 82.0 % (95 % CI 79.1–84.7 %) and 74.8 % (95 % CI 71.5–77.9 %) at 24 and 48 h, respectively. As shown in Table 1, the positive predictive

value (PPV) at 24 h was also significantly higher for chromID ESBL (38.7 %; 95 % CI 28.3–50.2 %) than for BLSE agar (15.4 %; 95 % CI 10.1 %; 21.5 %).

Sensitivity of chromID ESBL and BLSE agar

The sensitivity was based on the 33 strains isolated. The sensitivity at 24 h of growth was 88 % (95 % CI 72.3–95.3 %) for chromID ESBL and 85 % (95 % CI 68.7–93.5 %) for BLSE agar (Table 1). Four strains failed to be detected on chromID ESBL because they did not produce the expected-coloured colony (two non-coloured *E. coli*, one non-coloured *P. mirabilis* and one iridescent *Citrobacter freundii*). The sensitivity increased after 48 h incubation on chromID ESBL to 94 % (95 % CI 80.0–98.4 %) (one of the *E. coli* strains and the *P. mirabilis* strain that were non-coloured at 24 h became coloured at 48 h). On BLSE agar, five strains failed to grow (two *E. coli*, two *K. pneumoniae* and one *Enterobacter cloacae*) at 24 and at 48 h.

The percentage of recovered ESBL producers (88 %) from chromID ESBL medium after 24 h of incubation was lower than that reported by Glupczynski *et al.* (2007), as several enterobacterial ESBL producers did not develop the predicted colour on this medium. As described above, an extended incubation increased the recovery of ESBL producers; however, this also increased the growth of mixed flora, thus decreasing the PPV of this selective culture medium (Table 1). Nevertheless, the use of an oxidase test on colourless colonies increased the sensitivity of detection of enterobacterial ESBL producers (data not shown). The high negative predictive value allowed a quick and easy confirmation of the absence of ESBL-producing *Enterobacteriaceae* in a clinical sample.

The presence of cefpodoxime in chromID ESBL rather than cefotaxime or ceftazidime (as in BLSE agar) probably explains the higher sensitivity of this medium over BLSE agar. This result is in agreement with the fact that cefpodoxime has been shown to be the best molecule for screening all types of ESBL producer in clinical specimens (Pfaller & Segreti, 2006). In addition, ceftazidime alone can no longer be recommended as a selection antibiotic in medium used for ESBL screening. This is due to the

Table 1. Sensitivity and PPV for both media after 24 and 48 h of incubation tested with 765 specimens

Incubation time (h)	Medium	No. of isolates			Sensitivity (95% CI)*	PPV (95 % CI)†
		True positive (TP)	False positive (FP)	False negative (FN)		
24	chromID ESBL	29	46	4	88 % (72.3–95.3 %)	38.7 % (28.3–50.2 %)
	BLSE agar	28	154	5	85 % (68.7–93.5 %)	15.4 % (10.1–21.5 %)
48	chromID ESBL	31	78	2	94 % (80.0–98.4 %)	28.4 % (20.7–37.7 %)
	BLSE agar	28	220	5	85 % (68.7–93.5 %)	11.3 % (7.9–15.9 %)

*Sensitivity: TP/(TP + FN).

†PPV: TP/(TP + FP).

increasing spread of CTX-M ESBL enzymes among clinical isolates, as many of these enzymes do not confer resistance to ceftazidime (Canton & Coque, 2006).

False positives

False-positive strains were those growing on either media with the correct colour but negative for ESBL production; these required additional testing. A total of 46 strains gave false-positive results on chromID ESBL: 13 *Pseudomonas aeruginosa* (with a green or brownish colour), and *Enterobacteriaceae* overproducing a chromosomal cephalosporinase (18 *Enterobacter* spp., 8 *E. coli*, 1 *Hafnia alvei*) or a chromosomal penicillinase (6 *Klebsiella oxytoca*). A significantly higher number of false-positive colonies (154) was detected on BLSE agar: 73 *Pseudomonas* spp., 34 *Klebsiella/Enterobacter/Serratia* group, 13 *E. coli*, 10 *Morganella morganii*, 8 *H. alvei*, 4 *Acinetobacter* spp., 4 *Stenotrophomonas maltophilia*, 3 *Enterococcus faecalis* and 5 other bacteria. Non-significant differences in negative predictive value at 24 and 48 h for chromID ESBL and BLSE agar were observed (24 h 99.6 and 99.2%; 48 h 99.9 and 99.1%, respectively). Thus, these results showed that in 99.6% of samples (95% CI 98.7–99.9%), the absence of coloured colonies on chromID ESBL indicated that no ESBL producer was present in the sample.

Three times more false-positive bacteria were detected after 24 h of incubation on BLSE agar than on chromID ESBL medium. The main difficulty in these screening media was to differentiate between ESBL producers and resistant isolates related to other resistance mechanisms, in particular plasmid- or chromosomally mediated AmpC overproducers. *Enterobacter* spp. accounted for the most frequent cause of false positives on both culture media, although to a lower extent on chromID ESBL. *K. oxytoca* overproducing chromosomal β -lactamase was also frequently recovered on chromID ESBL (but not on BLSE agar). However, the direct detection of indole production by green/blue-pigmented colonies allowed a rapid presumptive identification of this species among microorganisms of the *Klebsiella/Enterobacter/Serratia* group.

Interfering growth

On each medium, the growth of non ESBL-producing bacteria was also evaluated. This selectivity was studied on the 733 samples in which no ESBL-producing bacteria were detected. After 24 h incubation, no competing flora was found in 88.7 and 82.5% for chromID ESBL and BLSE agar, respectively, suggesting a better selectivity for chromID ESBL. Moreover, when a competing flora was present, growth intensity was lower on chromID ESBL than on BLSE agar in 47.5% of cases.

Besides enterobacterial isolates, non-fermentative Gram-negative bacilli, mainly *Pseudomonas aeruginosa*, were able to grow on both media. However, a higher proportion of *Pseudomonas aeruginosa*, *S. maltophilia* and *Acinetobacter* spp. were recovered on BLSE agar compared with chromID ESBL. These isolates grew mostly as colourless colonies on chromID ESBL medium and did not spread on the agar; thus, they did not interfere with the detection of ESBLs when they were present in mixed flora. Furthermore, these non-fermenter bacilli could rapidly be identified by simple tests performed directly on the colonies (i.e. positive oxidase test for *Pseudomonas aeruginosa*).

ESBLs and testing of reference ESBL producers

Overall, CTX-M enzymes (17 isolates) were the most predominant enzyme encountered and more rarely the TEM-type (10 isolates), whereas SHV-type ESBLs (7 isolates) were less commonly recovered (Table 2). There was no difference in the rate of recovery according to the type of ESBL enzyme produced between the two tested media. As only CTX-M, TEM-type or SHV-type ESBLs were detected from the positive specimens, we also tested other enterobacterial strains producing other genetically characterized types of ESBL on these media. chromID ESBL was able to identify all of the tested reference ESBL producers, whether with frequently encountered enzymes (TEM, SHV and CTX-M derivatives) or with less frequently isolated class A ESBLs (VEB, GES, BEL, PER, TLA-1, TLA-2 and BES), carbapenem-hydrolysing class A enzymes (GES-2, KPC,

Table 2. Characterization of ESBLs from *Enterobacteriaceae* isolates growing on either of the two selective media

Species (no. of isolates)	Classes of ESBL enzymes (no. of isolates)		
	CTX-M (17)	TEM (10)	SHV (7)
<i>Escherichia coli</i> (16)	CTX-M-1 (4), CTX-M-15 (9)	TEM-3 (2)	SHV-2 (1)
<i>Klebsiella pneumoniae</i> (8)*	CTX-M-15 (3)	TEM-24 (2)	SHV-5 (1), SHV-12 (3)
<i>Citrobacter koseri</i> (3)		TEM-3 (3)	
<i>Enterobacter cloacae</i> (2)			SHV-12 (2)
<i>Citrobacter freundii</i> (2)		TEM-21 (2)	
<i>Proteus mirabilis</i> (1)	CTX-M-1 (1)		
<i>Enterobacter aerogenes</i> (1)		TEM-24 (1)	

*One *K. pneumoniae* isolate harboured two ESBLs.

Table 3. Tested reference strains that produce frequently and less frequently isolated ESBLs

Strain	ESBL produced	BLSE agar		chromID ESBL	
		DRIG*	MacC†	Colour	Growth
<i>Escherichia coli</i>	CTX-M-1	+	+	Pink	+
<i>Escherichia coli</i>	CTX-M-2	+	+	Pink	+
<i>Klebsiella pneumoniae</i>	CTX-M-15	+	+	Blue	+
<i>Escherichia coli</i>	CTX-M-15	+	+	Pink	+
<i>Escherichia coli</i>	CTX-M-14	+	+	Pink	+
<i>Klebsiella pneumoniae</i>	CTX-M-26	+	+	Blue	+
<i>Escherichia coli</i>	pCTX-M-8	+	+	Pink	+
<i>Enterobacter cloacae</i>	SHV-12	+	+	Blue	+
<i>Escherichia coli</i>	pSHV-2a	+	+	Pink	+
<i>Klebsiella pneumoniae</i>	SHV-5	+	+	Blue	+
<i>Enterobacter aerogenes</i>	TEM-24	+	+	Blue	+
<i>Escherichia coli</i>	TEM-3	+	+	Pink	+
<i>Citrobacter freundii</i>	TEM-21	+	+	Blue	+
<i>Klebsiella pneumoniae</i>	VEB-1	+	+	Blue	+
<i>Enterobacter cloacae</i>	VEB-1	+	+	Blue	+
<i>Providencia stuartii</i>	VEB-1	+	+	Brown	+
<i>Proteus mirabilis</i>	VEB-1	+	+	Brown	+
<i>Proteus mirabilis</i>	VEB-1	+	+	Brown	+
<i>Escherichia coli</i>	VEB-1	+	+	Pink	+
<i>Serratia marcescens</i>	BES-1	+	+	Blue	+
<i>Salmonella</i>	PER-1	+	+	Colourless	+
<i>Salmonella</i>	PER-2	+	+	Colourless	+
<i>Escherichia coli</i> PpZ-1	PER-1	+	+	Blue	+
<i>Escherichia coli</i> pBEL 32	BEL-1	+	+	Pink	+
<i>Escherichia coli</i>	TLA-1	+	+	Pink	+
<i>Klebsiella pneumoniae</i>	TLA-1	+	+	Blue	+
<i>Escherichia coli</i> pTLA-2	TLA-2	+	+	Pink	+
<i>Klebsiella pneumoniae</i>	GES-1	+	+	Blue	+
<i>Enterobacter cloacae</i> CHE2	GES-5	+	+	Blue	+
<i>Escherichia coli</i> pGES 3	GES-3	+	+	Pink	+
<i>Escherichia coli</i> pGES 4	GES-4	+	+	Pink	+
<i>Escherichia coli</i> pGES 1	GES-1	+	+	Pink	+
<i>Enterobacter aerogenes</i>	TEM-121	+	+	Blue	+
<i>Enterobacter cloacae</i>	NmcA	+	+	Blue	+
<i>Serratia marcescens</i> S6	SME-1	+	+	Blue	+
<i>Serratia marcescens</i> SW	SME-2	+	+	Blue	+
<i>Serratia marcescens</i> CHE4	SME-2	+	+	Blue	+
<i>Serratia marcescens</i> CHE3	SME-2	+	+	Blue	+
<i>Klebsiella pneumoniae</i>	KPC-2	+	+	Blue	+
<i>Enterobacter cloacae</i> CHE	IMI 1	+	+	Blue	+
<i>Escherichia coli</i> pOXA 18	OXA-18	+	+	Pink	+

*Drigalski (cefotaxime).

†MacConkey (ceftazidime).

NMCA, Sme-1/2 and IMI-1) or class D ESBL (OXA-18) (Table 3). Therefore, this culture medium can be used in regions where these enzymes are more prevalent.

Conclusions

Our results show that chromID ESBL is a reliable culture medium for screening and presumptive identification of ESBL-producing *Enterobacteriaceae* directly from clinical

samples. This ready-to-use chromogenic selective medium offers good sensitivity and high specificity, and has the advantages of allowing easy discrimination of different colonies simply according to their colour, which is particularly useful in specimens containing a resident associated flora. Furthermore, although it is slightly more expensive, by significantly reducing the need for unnecessary confirmation tests, chromID ESBL allows significant time and cost reductions for ESBL screening.

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