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NOTE TO THE EDITOR

Evaluation of a chromogenic agar medium for the detection of extended-spectrum β-lactamase-producing *Enterobacteriaceae*

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Abstract

Aim: To compare the performance of a new chromogenic agar medium CHROMagar ESBL (KC-ESBL) to chromID ESBL (SB-ESBL) for the detection and presumptive identification of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* directly from clinical specimens.
Methods and Results: A total of 256 specimens were screened for ESBL producers. Also, the genotypes of the ESBLs and plasmid-mediated AmpC β-lactamases (pAmpCBLs) were characterized by PCR and sequencing. Among the 256 specimens, 17 (6·6%) ESBL producers were isolated on both media. The sensitivity, specificity, positive predictive value and negative predictive value were higher for KC-ESBL (100, 93·3, 51·5 and 100%, respectively) than for SB-ESBL (88·2, 92·9, 46·9 and 99·1%, respectively) ($P = 0·72$). *Enterobacteriaceae* harbouring pAmpCBL genes as well as chromosomal cephalosporinase- and penicillinase-hyperproducing *Enterobacteriaceae* and *Pseudomonas aeruginosa* accounted for the false-positive results.
Conclusion: KC-ESBL can detect ESBL producers from clinical specimens with good selectivity and rapid presumptive identification by means of colony colour at 24 h.
Significance and Impact of the Study: This is the first study that has evaluated the performance of KC-ESBL that enables the detection and presumptive identification of ESBL producers from clinical specimens.

The proliferation of clinical isolates producing β-lactam-hydrolysing enzymes, such as extended-spectrum β-lactamases (ESBLs), has become a great concern. Because infectious diseases caused by strains producing these enzymes are associated with severe adverse clinical outcomes, it is essential to have rapid diagnostic methods for the detection of ESBL-producing organisms from clinical specimens. Recently, a chromogenic agar medium chromID ESBL (SB-ESBL; Sysmex-bioMerieux, Tokyo, Japan) has become commercially available for the rapid detection of ESBL producers (Glupczynski *et al.* 2007; Reglier-Poupet *et al.* 2008). This study compared the performance of a new chromogenic agar medium CHROMagar ESBL (KC-ESBL; Kanto Chemical, Tokyo, Japan) to SB-ESBL in the detection and presumptive identification of ESBL-producing *Enterobacteriaceae* from clinical specimens.

Our samples were a total of 256 nonduplicate specimens referred to our laboratory for the screening of ESBL-producing organisms. These specimens were collected from hospitalized patients between June 2008 and August 2008 and came from the following sites: 186 stool, 48 urine, 12 sputum, and 10 wound swabs. Each stool, sputum and wound swab specimen was homogenized in 0·5 ml of sterile physiological saline. Then, using calibrated loops, 10 μl of the resulting suspension was inoculated onto SB-ESBL and KC-ESBL. For urine samples, 10 μl of the sample was plated directly onto both media. SB-ESBL was obtained from the manufacturer as a prepared plate medium, and

KC-ESBL was prepared from a dehydrated medium according to manufacturer's instructions. All media were incubated at 35°C under aerobic conditions and assessed after 24 h of incubation. Reference strains of *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the negative and positive control for ESBL production, respectively. Ten clinical isolates of ESBL-producing *E. coli* (three CTX-M-type and one SHV-type), *Kl. pneumoniae* (one CTX-M-type and two SHV-types) and *Proteus mirabilis* (three CTX-M-type) were also tested for their ability to grow on both media using the modified Miles–Misra method (Miles *et al.* 1938). The colour and intensity of the colonies on both media was recorded according to the colouration types provided by the manufacturer's instructions. All isolates growing on SB-ESBL and/or KC-ESBL were regarded as presumptive ESBL producers and were identified by the Microscan Walkaway 96 system (Siemens Healthcare Diagnostics Inc., Tokyo, Japan). For the confirmation of ESBL-positive isolates, a synergy test was performed and interpreted according to CLSI guidelines (CLSI 2008). Furthermore, ESBL producers were confirmed by using a double-disc diffusion (DDD) method described by Wiegand *et al.* (2007). Genotypes of bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ and plasmid-mediated AmpC β-lactamases (pAmpCBLs) were characterized according to published methods (Yagi *et al.* 2000; Perez-Perez and Hanson 2002; Shibata *et al.* 2006). The constitutive overexpression of chromosomal cephalosporinases and penicillinases was determined as described by Livermore *et al.* (2007). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were assessed by the chi-square test using JMP software, version 6.0 (SAS Institute, Tokyo, Japan). A two-tailed P value of <0.05 was taken as significant.

The two media gave a similar isolation rate in ten clinical isolates of ESBL producers, and all three methods (a synergy test, a DDD method and the molecular techniques) showed similar sensitivities for the detection of ESBL producers in all strains growing on the both media (data not shown). From the 256 specimens, 17 (6·6%) ESBL-producing *Enterobacteriaceae* were isolated on KC-ESBL (Table 1). Although, for SB-ESBL, among the 17 isolates, two strains of ESBL-producing *E. coli* failed to produce a chromogenic reaction, with no colour evident at 24 h, the use of a spot indole test and oxidase test on the colourless colonies allowed a rapid presumptive identification of *E. coli*. This result was similar to the previous finding (Reglier-Poupet *et al.* 2008) and was thought to be because of the use of different chromogenic substrates in each of the two media to detect specific bacterial enzymes, such as β-glucuronidase and β-galactosidase.

The sensitivity, specificity, PPV and NPV at 24 h were higher for KC-ESBL (100, 93·3, 51·5 and 100%, respectively) than for SB-ESBL (88·2, 92·9, 46·9 and 99·1%, respectively). However, these respective differences were not significant ($P = 0·72$). While the sensitivity of KC-ESBL was higher than that reported by Reglier-Poupet *et al.* (2008) and Glupczynski *et al.* (2007), the specificity of KC-ESBL was lower than that reported by Reglier-Poupet *et al.* (2008) but higher than that reported by Glupczynski *et al.* (2007). The PPV of KC-ESBL was higher than that of SB-ESBL and that reported by Reglier-Poupet *et al.* (2008). The high NPV on both media also permitted a quick and easy confirmation of the absence of ESBL-producing *Enterobacteriaceae* in a clinical sample as described previously (Reglier-Poupet *et al.* 2008).

Regarding false-positive strains (ESBL-negative organisms on the disc synergy testing, the DDD method and PCR) growing on each medium with the correct enterobacterial species chromogenic character, 22 were observed on SB-ESBL and 21 on KC-ESBL. In these strains, pAmpCBL genes were detected in seven *Enterobacter cloacae*,

Table 1 Characterization of extended-spectrum β-lactamase producers detected on either of two media

Species (no. of isolates)	Molecular typing of β-lactamases			
	CTX-M group	SHV	TEM	pAmpCBL
Escherichia coli (1)	CTX-M-1	–	–	–
E. coli (2)	CTX-M-2	–	–	–
E. coli (3)	CTX-M-9	–	–	–
E. coli (2)	CTX-M-1	–	TEM-1	–
E. coli (1)	CTX-M-1	–	TEM-1	MOX
E. coli (1)	CTX-M-2	–	TEM-1	MOX
E. coli (1)	–	SHV-12	–	–
E. coli (1)	–	SHV-12	TEM-1	–
Klebsiella pneumoniae (2)	–	SHV-12	–	–
Klebsiella oxytoca (1)	CTX-M-9	–	–	–
Enterobacter cloacae (1)	CTX-M-9	SHV-12	–	EBC
Citrobacter freundii (1)	CTX-M-9	–	–	–

pAmpCBL, plasmid-mediated AmpC β-lactamase.

one *Enterobacter aerogenes* and three *Citrobacter freundii*. Chromosomal cephalosporinase-hyperproducing isolates of three *Ent. cloacae* and two *Cit. freundii*, and one chromosomal penicillinase-hyperproducing *Klebsiella oxytoca* were also observed. This result was in agreement with previous studies (Glupczynski *et al.* 2007; Reglier-Poupet *et al.* 2008) and might be accomplished by the incorporation of improved inhibitors of AmpC β-lactamase, such as phenylboronic acid. Five non-*Enterobacteriaceae* isolates of *Pseudomonas aeruginosa* grown on SB-ESBL (four *Ps. aeruginosa* on KC-ESBL), which produced a green or brown colour, also accounted for false-positive results as described previously (Reglier-Poupet *et al.* 2008). However, these isolates were easily identified on the both media by the pattern of the colonies and/or by a rapid simple test (positive oxidase reaction) which could be performed directly on the colonies.

In conclusion, the present study has a concern limitation, in that the numbers of ESBL producer detected were small. However, KC-ESBL can detect most ESBL producers with good selectivity and reduce workload for the screening of those as well as SB-ESBL.

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