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Evaluation of a chromogenic medium for extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae.

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ABSTRACT

Objectives: ESBL-producers are increasingly considered an infection control problem worldwide. Simple and specific means of screening for ESBL-producing *Escherichia coli* and *Klebsiella* sp. are complicated by the fact that cephalosporin resistance in Enterobacteriaceae may be mediated by other mechanisms. The purpose of this study is to evaluate the performance of a differential chromogenic screening medium selective for ESBL-producers using a large library of genetically characterised cephalosporin -resistant *E. coli* and *K. pneumoniae*.

Methods: Isolates were from 12 medical centers across Canada. 213 *E. coli* and 17 *K. pneumoniae* with ESBL or AmpC mediated cephalosporin resistance were studied. *E. coli* and *K. pneumoniae* were screened using ceftazidime and/or ceftriaxone MIC >/= 1µg/ml. ESBL genes (CTX-M, SHV, TEM, OXA), AmpC promoter/attenuator alterations and presence of acquired AmpC-type genes (ACT-1/MIR-1 -related, DHA-related, FOX-related and CMY-2-related) were detected by PCR and, where needed, sequenced. Colorex ESBL (CHROMagar, Paris, France) was inoculated with 150 cfu of each test organism. Growth characteristics, including colour and colony counts were obtained.

Results: 114 ESBL-*E. coli* (1 each of CTX-M-1,9,24 and 65, 2 CTX-M-2, 24 CTX-M-14, 77 CTX-M-15, 4 CTX-M-27, and 3 SHV-2a), 91 AmpC-*E. coli* (46 CMY-2 and 45 promoter/attenuator mutants), 8 co-expressing ESBL and AmpC-*E. coli* (4 CTX-M-15, 2 CTX-M-14, 1 each of CTX-M-3 and TEM-12, all with promoter/attenuator mutations), 14 ESBL-*K. pneumoniae* (co-expression of CTX-M and SHV-type ESBLs) and 50 wild-type *E. coli* were studied. 2.5% of *E. coli* had an atypical chomogenic reaction with no colour produced. All *K. pneumoniae* had a typical chromogenic reaction. 9/10 uninhibited AmpC hyperproducing organisms had >100 colonies/plate and for 7/10, the mechanism of cephalosporin resistance was the CMY-2 gene. One ESBL-*E. coli* was inhibited by the medium. This isolate expressed the TEM-12 gene and had a ceftriaxone MIC of 0.12 µg/ml and a ceftazidime MIC of 8 µg/ml.

Conclusions: For *E. coli* with ceftriaxone and/or ceftazidime MIC >/= 1µg/mL, sensitivity for ESBL-producers is 99.2%, specificity is 89.0%. Sensitivity for *K. pneumoniae* was 100%, but numbers were small. This medium may facilitate the screening of ESBL-carriers, particularly in areas where prevalence of ESBL producers is relatively low compared to AmpC or other class C cephalosporinase-producing organisms.

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INTRODUCTION

Antibiotic resistance in *Escherichia coli* continues to rise and isolates producing extended-spectrum beta lactamases (ESBLs) are increasingly prevalent in hospitals and community settings worldwide (1). In Canada, national surveillance has shown resistance to ceftriaxone increased from 3.7% in a 2006 study of Canadian ICUs to 8% in ICU isolates from the CANWARD 2008 study (p = 0.043) (2). Our evaluation of *E. coli* isolates with reduced susceptibility to third generation cephalosporins has shown that nearly half are ESBL-producers and half are AmpC producers (2). Some evidence supports screening patients for carriage of ESBL-producing organisms and contact isolation of colonized individuals in healthcare settings. CHROMagar (Paris, France) has produced a selective/differential medium for screening specimens for ESBL-producers (marketed as Colorex ESBL in North America), which is selective for ESBL-producers and differentiates species of Enterobacteriaceae. In healthcare settings where ESBL-screening is considered, the medium may reduce workload associated with differentiating ESBL-producers, from other cephalosporin resistance organisms. The purpose of this study was to determine the growth characteristics of ESBL-producing *E. coli* and *K. pneumoniae* with defined ESBL genotypes (SHV, TEM and CTX-M) and AmpC-producing *E. coli* from Canadian hospitals on Colorex ESBL.

MATERIALS AND METHODS

Bacterial Isolates:

Isolates of were obtained from the 2007 and 2008 segments of the ongoing CANWARD study (2) (also see www.can-r.ca) and 2006 CANICU study. These isolates are obtained from 12 medical centers across Canada representing 7 of the 10 provinces. 213 *E. coli* and 14 *K. pneumoniae* resistant to at least one third generation cephalosporin as well as 50 clinical wild-type (no phenotypic resistance to cephalosporins) were studied. Putative ESBL/AmpC *E. coli* and *K. pneumoniae* were screened using ceftazidime and/or ceftriaxone MIC >/= 1µg/ml. We used PCR for ESBL genes (CTX-M, SHV, TEM, OXA) and sequencing of products to identify ESBL genotypes and PCR and sequencing of AmpC gene and promoter/attenuator regions to identify polymorphisms responsible for AmpC expression in *E. coli* (2). We also performed PCR for known acquired AmpC resistance (EBC (ACT-1/MIR-1-related genes), DHA (DHA-92 related genes), FOX-93 (FOX-related genes) and CIT (CMY-2-related genes)) (2).

Plating and Culture:

Colorex ESBL, provided by CHROMagar (Paris, France), was inoculated with each organism from the study. Using turbidometry and volumetric loops, ~150 cfu of each test organism was inoculated to each plate. Plates were incubated at 35°C in ambient air for 18-24 hours in the dark. Growth characteristics, including colour and colony counts and photographs were be obtained.

RESULTS

TABLE 1: Growth characteristics of *E. coli* and K. pneumoniae on Colorex ESBL medium. Approximately 150 cfu of each test organism was plated to the medium in all cases.

Mechanism of resistance (organism) (n)	Any growth	>100 colonies (%)	50-100 colonies (%)	1- 50 colonies (%)	No growth
ESBL (*E. coli*) (114)	114 (100)	111 (97.4)	3 (2.6)	0 (0)	0(0)
ESBL (*K.pneumoniae*)	14 (100)	14 (100)	0 (0)	0 (0)	0(0)
AmpC (*E. coli*) (91)	10 (11.0)	8 (8.8)	1 (1.1)	1 (1.1)	81 (89.0)
ESBL + AmpC (*E. coli*) (8)	7 (87.5)	7 (87.5)	0 (0)	0 (0)	1 (12.5)
Wild-type (*E. coli*) (50)	0 (0)	0 (0)	0 (0)	0 (0)	50 (100)

TABLE 2: ESBL-type β-lactamases identified in *E. coli* by PCR and sequencing of blaSHV, TEM and CTX-M. 54 (44.3%) also had TEM-1 and 47 (38.5%) had OXA-1.

β-lactamase	N (% of *E. coli* tested)
CTX-M-1	1 (0.8)
CTX-M-2	2 (1.6)
CTX-M-9	1 (0.8)
CTX-M-14	24 (17.7)
CTX-M-15	77 (63.1)
CTX-M-24	1 (0.8)
CTX-M-27	4 (3.3)
CTX-M-65	1 (0.8)
SHV-2a	3 (2.5)
AmpC + CTX-M-3	1 (0.8)
AmpC + TEM-12	1 (0.8)
AmpC + CTX-M-14	2 (0.8)
AmpC + CTX-M-15	4 (0.8)

TABLE 3: ESBL-type β-lactamases identified in *K. pneumoniae* by PCR and sequencing of blaSHV, TEM and CTX-M. 9 (64.3%) also had TEM-1 1 (7.1%) had SHV-1 and 6 (42.9%) had OXA-1.

β-lactamase	N (% of *K. pneumoniae* tested)
CTX-M-15 + SHV-11	4 (28.6)
CTX-M-15	1 (7.1)
CTX-M-14 + SHV-11	1 (7.1)
SHV-2a	3 (21.4)
CTX-M-15 + SHV-28	1 (7.1)
CTX-M-2 + SHV-11	1 (7.1)
CTX-M-15 + SHV-12	2 (14.3)
SHV-12	1 (7.1)

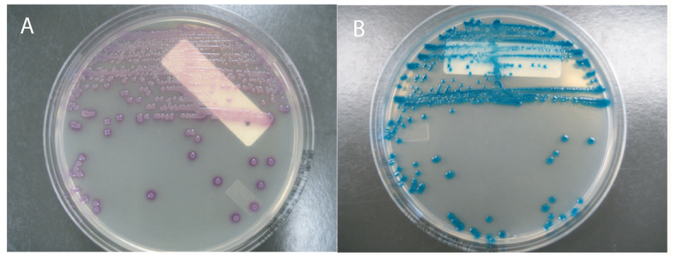

FIGURE 1: Appearance of colonies on Colorex ESBL.
A: Appearance of ESBL-producing *E. coli*. 119/122 (97.5%) of *E. coli* studied displayed the typial pink colouration and 3/122 (2.5%) were colourless.
B: Appearance of ESBL-producing *K. pneumoniae*. All *K. pneumoniae* displayed the typical metallic blue colonies.

The single ESBL inhibited by the medium harboured TEM-12, a β-lactamase known to have weak ceftriaxone hydrolysis and strong ceftazidime hydrolysis (3). The isolate had a ceftriaxone MIC of 0.12 µg/ml, a ceftazidime MIC of 8 µg/ml and a cefepime MIC of 0.5 µg/ml.

Nine of ten (90%) of the AmpC producing *E. coli* that showed growth on the medium showed good growth (>50 colonies). In 70% (7/10), the mechanism of AmpC production was the presence of the CMY-2 gene and in 3/10, the mechanism was a promoter alteration leading to production of AmpC. In all cases, no SHV, TEM or CTX-M β-lactamases with activity against third generation cephalosporins were identified. However, "non-ESBL" β-lactamases (TEM-1) were identified in 4/10 strains.

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CONCLUSIONS

For all resistant *E. coli* (AmpC and ESBL), sensitivity for identification of ESBLs is 99.2%, specificity for ESBL producers compared to AmpC *E. coli* was 89% and specificity compared to wild-type *E. coli* was 100%.

The medium is more specific for ESBL-producers than published references for other media (e.g. MacConkey with ceftriaxone - 54.2% and other chromogenic media - 60.7% in one study (4).

ESBL-producers with low MICs to ceftriaxone (e.g. TEM-12) may not be identified by this medium.

False positive results from AmpC producing *E. coli* may be due to non-ESBL β-lactamases (e.g. TEM-1/2, SHV1) expressed in large quantities by the organism or porin alterations, or both. Such organisms may express an ESBL-phenotype as has been observed by our group and others (3).

In Canada, where AmpC-producing *E. coli* constitute ~50% of ceftriaxone resistant isolates, a substantial reduction in workload would be expected, particularly in ESBL confirmation testing, if this medium was used to screen for ESBL-producers when compared to the less specific media.