JCM Accepts, published online ahead of print on 22 April 2009
J. Clin. Microbiol. doi:10.1128/JCM.01406-08

Enrichment broth improves detection of extended-spectrum beta-lactamase-producing bacteria in throat and rectal surveillance cultures of ICU patients

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Running Title: Enrichment broth improves ESBL detection

Keywords: extended-spectrum beta-lactamase detection, enrichment broth, bacterial culture, selective decontamination of the digestive tract, screening

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Abstract:

We evaluated the use of a trypticase soy broth (TSB) to improve detection of extended-spectrum beta-lactamase producing (ESBL +) bacteria. Pre-enrichment of throat and rectal swabs in TSB prior to inoculation on solid media doubled the number of ESBL + bacteria detected in samples obtained from patients in our intensive care unit.

ESBLs are enzymes in Gram-negative bacilli that confer resistance to the majority of β-lactam antibiotics up to the third generation cephalosporins. Their worldwide dissemination concerns clinicians, because infections with ESBL + micro-organisms are often not adequately covered with empirically started antibiotics. The proper choice of antibiotic therapy and infection control measures depend upon early and accurate ESBL detection; it is therefore pivotal to have a rapid and sensitive laboratory assay (4).

The sensitivity of methicillin-resistant *Staphylococcus aureus* (MRSA) detection by culture is increased with 9-25% by the overnight enrichment of culture swabs in broth before inoculation on solid media (2, 6). To the best of our knowledge the effect of pre-enrichment on the sensitivity of the detection of ESBL + bacteria has not yet been determined. We have evaluated the effect of overnight enrichment in broth by culturing fecal samples that were spiked with genotypically characterized ESBL + strains to see if normal flora of a fecal sample would interfere with detection of low numbers of ESBL positive strains. The enrichment broth was also evaluated with clinical samples obtained from adult patients in two ICUs of our hospital.

For the spiking experiments we used the *Klebsiella pneumoniae* K6 ATCC 700603 strain, which produces an SHV-18 ESBL (5), and two clinical isolates of *Escherichia coli* with a CTX-M type ESBL. Bacterial suspensions of these strains with an optical density of 0.5 McFarland were serially diluted in phosphate-buffered saline (PBS); 9 ten-fold dilutions were made. To quantify the viable bacteria in each dilution step a MacConkey agar was inoculated with 100 µl suspension and incubated overnight at 37^0 C; the number of grown colonies was counted the following day. Spiked samples were made by adding 100 µl of each dilution in PBS to 900 µl of a fecal suspension that was obtained by suspending six grams of fresh feces from healthy volunteers in 60 ml of antibiotic

free TSB with 0.5 % sodium chloride (Becton Dickinson, Breda, Netherlands). Fecal suspension without addition of ESBL + strain was used as negative control. Aliquots of 100 µl of the spiked samples were subcultured in 900 µl of TSB and onto beta-lactamase screening agar (BLSE, AES CHEMUNEX, Bruz cedex, France). The BLSE agar is a commercially available double plate containing Drigalski medium supplemented with 1.5 µg per ml cefotaxime and MacConkey with 2 µg per ml ceftazidime. Gram-negative bacteria that are resistant to cephalosporins (including *AmpC*-producers) can grow on this selective agar. Colonies of *Pseudomonas aeruginosa* can be discriminated from those of *Enterobacteriaceae* by colony morphology and colour, and by oxidase test. The samples in enrichment broth and BLSE plates were incubated for one night at 37°C. The following day 100 µl of the enriched samples were subcultured onto BLSE as described above. Colonies on BLSE were counted after one night incubation and the recovery of the spiked strains was confirmed with the VITEK 2 system (VITEK ID and VITEK AST, BioMérieux, Marcy l'Etoile, France). All experiments were performed in triplicate.

Surveillance cultures (throat, rectum) of mechanically ventilated patients on the ICU of our hospital were performed one to two times per week and collected from March 16 to May 17, 2007. Specimens were obtained with an Amies swab (Copan, Brescia, Italy). On the day that the surveillance cultures were obtained, the patient's swabs were first streaked on BLSE agar and then inserted into 5 ml of antibiotic free TSB for overnight incubation at 37°C. The next day, the swabs in the TSB enriched cultures were streaked on BLSE plates. The BLSE plates, both those inoculated with swabs before enrichment and those inoculated with swabs after overnight enrichtment in TSB, were incubated for two days at 37°C. Gram-negative isolates growing on BLSE agars were identified by the VITEK 2 system and tested for ESBL production with three methods: the double disc synergy test with an amoxicillin clavulanate tablet in the center surrounded by cefpodoxime,

ceftazidime and cefotaxime tablets, by the combined disc diffusion test with cefepime and cefepime clavulanate tablets (all tablets from Rosco Diagnostica, Neo-Sensitabs, Taastrup, Denmark), and by E-test with both cefepime and cefepime clavulanate (AB Biodisk, Solna, Sweden) (3). Patient characteristics and culture results were recorded; data were analyzed with SPSS (version 14.0).

The suspensions of ESBL + strains in PBS that were used to spike fecal samples yielded growth on MacConkey agars up to the seventh *(E. coli*, isolate 1*)* and eighth log dilutions (*K. pneumonia; E. coli*, isolate 2). When cultured without TSB enrichment, spiked fecal suspensions showed numbers of colonies on BLSE agars that were similar to growth of corresponding PBS dilutions of ESBL + strains on MacConkey agars. After TSB enrichment the cultures produced significantly more colonies on BLSE agars than without enrichment ($p < 0.05$ by Wilcoxon signed-rank test). TSB enrichment of the *K. pneumoniae* and *E. coli* (isolate 1) also yielded growth one log dilution further than without enrichment. Thus, for these strains and conditions, the spiking experiments demonstrate that the growth of ESBL + strains in enrichment broth is not inhibited by fecal flora; enrichment in TSB can even improve the detection of ESBL + bacilli.

We also compared the yields of the clinical samples cultured with and without enrichment. During a two-month period we collected 500 surveillance specimens (throat and rectal swabs) from 88 mechanically ventilated ICU patients. The ICU patients in our hospital receive selective decontamination of the digestive tract (SDD), an antibiotic cocktail containing polymyxin E, tobramycin and amphotericin B and an initial three days of cefotaxime intravenously administrated, to reduce ventilator-associated infections (1). Surveillance cultures are routine in our ICUs and are performed to detect pathogens that are resistant to the SDD. With enrichment, twice the number of cultures yielded ESBL + bacteria compared to cultures without enrichment; this corresponded to 9

patients detected as carriers of ESBL + strains when culture with pre-enrichment was used, compared to 5 patients detected by conventional culture (see table 1). On the premise that differences in culture outcome were not affected by patient characteristics (null hypothesis not rejected by goodness-of-fit test), we analyzed the two culture methods at the sample level with McNemar's test, hypothesizing that both methods detect ESBL + species equally well. The difference in detection between the two methods was statistically significant (p=0.006), hence we concluded that the enrichment step improved ESBL detection.

With one exception, all samples that were ESBL-positive without enrichment were also positive with enrichment. It should be noted that six of the nine patients already carried ESBL + species upon admission to the ICU. All six were detected by culture with enrichment step. Colonization with ESBL + strains at admission to the ICU was, however, detected only in three of these six patients by culture without enrichment (see table 1). In two of the patients that were positive with both culture methods, ESBL + strains were detected approximately one week earlier by culture with enrichment broth. This may be due to low numbers of ESBL + bacteria in the gut upon admission to the ICU, and selection of these strains in the course of the ICU stay by the SDD prophylaxis. Although an overnight enrichment step may delay individual culture results by one day, the results presented here show that at the patient level detection can be accelerated. For optimal rapidity, culture with and without enrichment could be used side by side, as we have done in this study.

In conclusion, a simple overnight pre-enrichment step in TSB improves the detection of ESBL + strains and permits earlier recognition and isolation of patients that carry these strains.

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140 **Table 1 ESBL positive clinical samples with and without enrichment**

Patient with ESBL + strain	No. of cultured swabs of patient	**culture without enrichment in TSB**			**culture with enrichment in TSB**		
		ESBL + swabs (cultured on BLSE agar)	Days on ICU until first positive swab	Identified ESBL + micro-organism	ESBL + swabs (cultured on BLSE agar)	Days on ICU until first positive swab	Identified ESBL + micro-organism
1	4	1	0	*E. coli*	2	0	*E. coli*
2	14	5	8	*E. coli*	7	1	*E. coli* & *Raoultella ornithinolytica*
3	19	1	7	*E. coli*	3	1	*E. coli* & *Proteus mirabilis*
4	4	2	0	*Enterobacter cloacae*	1	0	*E. cloacae*
5	2	1	1	*Citrobacter freundii*	1 §	1	*C. freundii*
6	23	0			2	16	*E. coli*
7	6	0			2	0	*P. mirabilis*
8	9	0			1 §	18	*Achromobacter xylosoxidans*
9	8	0			1	14	*E. coli*
Total: 10 swabs with ESBL + strains from 5 patients; 490 swabs without ESBL + strain from 83 patients					**Total:** 20 swabs with ESBL + strains from 9 patients; 480 swabs without ESBL + strain from 79 patients		

141 Time to the first positive swab was on average 3.2 days when cultured without enrichment, and 0.6

142 days with enrichment. § The only 2 swabs with ESBL producers from the throat; all other 18

143 positive cultures were from fecal swabs.