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NOTES

Enrichment Broth Improved Detection of Extended-Spectrum-Beta-Lactamase-Producing Bacteria in Throat and Rectal Surveillance Cultures of Samples from Patients in Intensive Care Units▽

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

Extended-spectrum beta-lactamases (ESBLs) are enzymes in gram-negative bacilli that confer resistance to the majority of β-lactam antibiotics up to the extended-spectrum cephalosporins. Their worldwide dissemination concerns clinicians, because infections with ESBL-producing (ESBL$^+$) microorganisms are often not adequately covered with empirically started antibiotics. The proper choices 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 (meticillin)-resistant *Staphylococcus aureus* detection by culture is increased 9 to 25% by overnight enrichment of culture swabs in broth before inoculation on solid medium (2, 6). To the best of our knowledge, the effect of preenrichment on the sensitivity of 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 intensive care units (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 at a 0.5 McFarland standard were serially diluted in phosphate-buffered saline (PBS); nine 10-fold dilutions were made. To quantify the viable bacteria in each dilution step, a MacConkey agar was inoculated with 100 μl of a suspension and incubated overnight at 37°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 6 grams of fresh feces from healthy volunteers in 60 ml of antibiotic-free trypticase soy broth (TSB) with 0.5% sodium chloride (Becton Dickinson, Breda, The Netherlands). A fecal suspension without the addition of an ESBL$^+$ strain was used as a negative control. Aliquots of 100 μl of the spiked samples were subcultured in 900 μl of TSB and onto beta-lactamase screening (BLSE) agar (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 agar 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 observing colony morphology and color and by using an 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 was subcultured onto BLSE as described above. Colonies on BLSE were counted after one night of 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 and rectum) of samples from mechanically ventilated patients in the ICU of our hospital were performed one to two times per week and collected from

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TABLE 1. ESBL-positive clinical samples with and without enrichment[a]

Patient with ESBL$^+$ strain	No. of cultured swabs from patient	Culture without enrichment in TSB[b]			Culture with enrichment in TSB[c]		
		No. of ESBL$^+$ swabs (cultured on BLSE agar)	No. of days in ICU until first positive swab	Identified ESBL$^+$ microorganism	No. of ESBL$^+$ swabs (cultured on BLSE agar)	No. of days in ICU until first positive swab	Identified ESBL$^+$ microorganism(s)
1	4	1	0	*E. coli*	2	0	*E. coli*
2	14	5	8	*E. coli*	7	1	*E. coli* and *Raoultella ornithinolytica*
3	19	1	7	*E. coli*	3	1	*E. coli* and *Proteus mirabilis*
4	4	2	0	*Enterobacter cloacae*	1	0	*E. cloacae*
5	2	1	1	*Citrobacter freundii*	1[d]	1	*C. freundii*
6	23	0			2	16	*E. coli*
7	6	0			2	0	*P. mirabilis*
8	9	0			1[d]	18	*Achromobacter xylosoxidans*
9	8	0			1	14	*E. coli*

[a] The times to the first positive swab were, on average, 3.2 days for culture without enrichment and 0.6 days for culture with enrichment.
[b] There were, in total, 10 swabs with ESBL$^+$ strains from 5 patients and 490 swabs without ESBL$^+$ strains from 83 patients.
[c] There were, in total, 20 swabs with ESBL$^+$ strains from 9 patients and 480 swabs without ESBL$^+$ strain from 79 patients.
[d] These were the only two swabs with ESBL producers from the throat; all other 18 positive cultures were from fecal swabs.

16 March to 17 May 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 enrichment in TSB, were incubated for 2 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: by the double-disc synergy test with an amoxicillin (amoxicilline) 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 an Etest 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 (*K. pneumonia* and *E. coli*, isolate 2) log dilutions. When cultured without TSB enrichment, spiked fecal suspensions showed numbers of colonies on BLSE agars that were similar to those in corresponding PBS dilutions of ESBL$^+$ strains on MacConkey agars. After TSB enrichment, the cultures produced significantly more colonies on BLSE agars than they did without enrichment ($P < 0.05$; Wilcoxon signed-rank test). TSB enrichment of *K. pneumoniae* and *E. coli* (isolate 1) also yielded growth 1 log dilution further than the level observed 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 2-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, with cefotaxime intravenously administrated on the first 3 days) 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 the number of cultures without enrichment; this corresponded to nine patients detected as carriers of ESBL$^+$ strains when culture with preenrichment was used, compared to five patients detected by conventional culture (Table 1). On the premise that differences in culture outcome were not affected by patient characteristics (with the null hypothesis not rejected by the 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 the 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 (Table 1). In two of the patients that were positive with both culture methods, ESBL$^+$ strains were detected approximately 1 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 1 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 preenrichment step in TSB

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improves the detection of ESBL$^+$ strains and permits earlier recognition and isolation of patients that carry these strains.

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