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 patients in our intensive care unit doubled the number of ESBL+ bacteria detected in samples obtained from patients in our intensive care unit.
16 March to 17 May 2007. Specimens were obtained with an
Amies swab (Copan, Brescia, Italy). On the day that the sur-
veillance cultures were obtained, the patient’s swabs were first
streaked on BLSE agar and then inserted into 5 ml of antibi-
otic-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 produc-
tion with three methods: by the double-disc synergy test with
an amoxicillin (amoxicillin) clavulanate tablet in the center,
surrounded by cefpodoxime, ceftazidime, and cefotaxime tab-
lets; by the combined-disc diffusion test with cefepime and
cefepime clavulanate tablets (all tablets from Rosco Diagnos-
tica, 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 en-
richment, spiked fecal suspensions showed numbers of colo-
nies 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 colo-
nies on BLSE agars than they did without enrichment (P <
0.05; Wilcoxon signed-rank test). TSB enrichment of K. pneu-
moniae 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 demon-
strate that the growth of ESBL⁺ strains in enrichment broth is
not inhibited by fecal flora; enrichment in TSB can even im-
prove 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 pa-
tients in our hospital receive selective decontamination of the
digestive tract (SDD) (an antibiotic cocktail containing poly-
myxin E, tobramycin, and amphotericin B, with cefotaxime
intravenously administered on the first 3 days) to reduce ven-
tilator-associated infections (1). Surveillance cultures are rou-
tine 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 pa-
tients 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 characteris-
tics (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⁺ and conventional strains equally well. The difference in detection be-
tween 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 de-
tected 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 enrich-
ment (Table 1). In two of the patients that were positive with
both culture methods, ESBL⁺ strains were detected approxi-
mately 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, cul-
ture 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
improves the detection of ESBL+ strains and permits earlier recognition and isolation of patients that carry these strains.

REFERENCES