umonstrated that this procedure may be valuable for detection of ESBL-E from blood cultures (9) (Online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/3/14-1277-Techapp1.pdf). The detailed MALDI-TOF MS protocol using the VITEK MS system (bioMérieux) is described in Detailed Methods in the Technical Appendix.

We performed antibiotic susceptibility testing (AST) by the disk diffusion method using bacterial colonies grown from blood cultures according to the Clinical Laboratory Standards Institute (CLSI) recommendations (10). The same MALDI-TOF technology and the API Gram negative Identification product (bioMérieux) were used for confirmatory identification of bacteria. AST results, obtained 48 h after blood cultures were identified as positive, were interpreted according to the CLSI breakpoints, as updated in 2014 (10). MIC of cefotaxime, ceftazidime, and cefepime were determined on Muller-Hinton (MH) agar and MH agar supplemented with 4 μg/mL of tazobactam (final concentration).

We used the double-disk synergy test (DDST) for the phenotypic detection of ESBL producers (11), according to the CLSI recommendations. For each sample, 1 disk contained cefotaxime, ceftazidime, or cefepime, and a second disk contained ticarcillin and clavulanate (10). The DDST was also performed on MH agar plates (bioMérieux) containing cloxacillin (150 mg/L) to inhibit cephalosporinase activity of natural producers of those inducible cephalosporinase. Because the DDST was performed in parallel to the AST, results were obtained 48 h later. The DDST was considered to be the reference standard for the detection of ESBL-E.

We also used molecular biology techniques to identify the ESBL genes. We used PCR to amplify DNA, which we then sequenced using the ESBL genes. We used PCR to amplify DNA, which we then sequenced using blaTEM, blaSHV, and blaCTX-M primers (9).

During the study, 245 blood specimens that were collected from patients hospitalized in any unit of the hospital
were cultured and grew GNB (Table 1). The 245 cases of bacteremia were attributed to Enterobacteriaceae (211, 86.1%), nonfermentative GNB (31, 12.7%), and anaerobic GNB (3, 1.2%) (Table 2). Three blood cultures (1.2%) were positive for 2 enterobacterial species (Table 2). Escherichia coli was the predominant enterobacterial species (118/211, 55.9%); the next most prevalent were Klebsiella pneumoniae (37/211, 17.5%) and Enterobacter cloacae (20/211, 9.5%). Pseudomonas aeruginosa (24/31, 77.4%) was the predominant non-fermentative GNB (Table 2). Anaerobic GNB belonged to the Bacteroides fragilis group (Bacillus fragilis and Bacillus vulgatus).

We identified bacteria directly from blood culture using the MALDI-TOF technique for 237 (96.7%) isolates; results corresponded to bacterial identification after culture ing the MALDI-TOF technique for 237 (96.7%) isolates; results corresponded to bacterial identification after culture.

Table 2. Results of bacterial identification after culture.

<table>
<thead>
<tr>
<th>Enterobacteriaceae</th>
<th>Total</th>
<th>Escherichia coli</th>
<th>Other</th>
<th>ESBL-negative</th>
<th>ESBL-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>4 (13.3)</td>
<td>0 (0)</td>
<td>4 (13.3)</td>
<td>0 (0)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>2 (6.7)</td>
<td>2 (6.7)</td>
<td>0 (0)</td>
<td>2 (6.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (26.7)</td>
<td>0 (0)</td>
<td>8 (26.7)</td>
<td>0 (0)</td>
<td>8 (26.7)</td>
</tr>
<tr>
<td>Total</td>
<td>77 (25.3)</td>
<td>0 (0)</td>
<td>77 (25.3)</td>
<td>0 (0)</td>
<td>77 (25.3)</td>
</tr>
</tbody>
</table>

The ESBL NDP test revealed a cefotaxime-hydrolyzing enzyme that was not inhibited by tazobactam for 1 K. pneumoniae isolate that produced an acquired cephalosporinase, 3 of the 5 E. cloacae that overproduced chromosome-encoded AmpC, and 2 Bac teroides spp. strains (data not shown).

The ESBL NDP test used with blood cultures had a sensitivity of 100% (95% CI: 92.4%–100%), a specificity of 100% (95% CI: 97.7%–100%), a positive predictive value of 100% (95% CI: 99.2%–100%) and a negative predictive value of 100% (95% CI: 97.8%–100%) for the detection of ESBL-E.

Conclusions
Detection of ESBLs that are the main source of cephalosporin resistance in Enterobacteriaceae still relies on antibiotic susceptibility testing, results of which usually take 24–48 h. We show that the ESBL NDP test directly performed on positive blood cultures is a reliable technique to identify ESBL-E within 30 min. Although these results are promising, they should be further confirmed in other countries where the prevalence and the epidemiology of ESBL-E might be different. A strong correlation between intermediate susceptibility or resistance to cefotaxime and positivity of the ESBL NDP test was observed (Table 3). Similar correlation between resistance to cefotaxime and ESBL production in Enterobacteriaceae was obtained in the United States (12). A concomitant use of the Carba NP test (13) directly from blood culture will also identify carbapenemase producers (such as K. pneumoniae carbapenemase producers) that also confer clavulanic-acid–inhibited resistance to cephalosporins.

Table 1. Origin of gram-negative bacilli identified in blood samples drawn from hospitalized patients and cultured by using rapid detection methods for bacterial blood cultures.

<table>
<thead>
<tr>
<th>Hospital department</th>
<th>No. gram-negative bacilli</th>
<th>Total</th>
<th>Escherichia coli</th>
<th>Other</th>
<th>ESBL-negative</th>
<th>ESBL-positive</th>
<th>No. (%) nonfermenting bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiology</td>
<td>5</td>
<td>4 (80)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>4 (100)</td>
<td>0</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Digestive surgery</td>
<td>9</td>
<td>8 (89)</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Orthopedic surgery</td>
<td>3</td>
<td>3 (100)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>3 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endocrinology</td>
<td>2</td>
<td>2 (100)</td>
<td>0</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gerontology</td>
<td>9</td>
<td>7 (78)</td>
<td>3 (43)</td>
<td>4 (57)</td>
<td>7 (100)</td>
<td>0</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Gynecology-obstetric</td>
<td>3</td>
<td>2 (67)</td>
<td>0</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Hepato-gastroenterology</td>
<td>20</td>
<td>18 (90)</td>
<td>8 (44)</td>
<td>10 (56)</td>
<td>15 (83)</td>
<td>3 (17)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Emergency</td>
<td>40</td>
<td>37 (93)</td>
<td>30 (81)</td>
<td>7 (19)</td>
<td>32 (86)</td>
<td>5 (14)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>28</td>
<td>25 (89)</td>
<td>16 (64)</td>
<td>9 (36)</td>
<td>17 (68)</td>
<td>8 (32)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Nephrology</td>
<td>28</td>
<td>21 (75)</td>
<td>8 (38)</td>
<td>13 (62)</td>
<td>11 (52)</td>
<td>10 (48)</td>
<td>7 (25)</td>
</tr>
<tr>
<td>Neurology</td>
<td>8</td>
<td>8 (100)</td>
<td>5 (63)</td>
<td>3 (38)</td>
<td>8 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pediatric unit</td>
<td>2</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>2 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pneumology</td>
<td>4</td>
<td>3 (75)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>67</td>
<td>57 (85)</td>
<td>24 (42)</td>
<td>33 (58)</td>
<td>42 (74)</td>
<td>15 (26)</td>
<td>10 (15)</td>
</tr>
<tr>
<td>Rhumatology</td>
<td>3</td>
<td>2 (67)</td>
<td>2 (100)</td>
<td>0</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Urology</td>
<td>14</td>
<td>12 (86)</td>
<td>8 (67)</td>
<td>4 (33)</td>
<td>10 (83)</td>
<td>2 (17)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>245</td>
<td>211 (86)</td>
<td>118 (56)</td>
<td>93 (44)</td>
<td>164 (78)</td>
<td>47 (22)</td>
<td>34 (14)</td>
</tr>
</tbody>
</table>

*ESBL, extended spectrum β-lactamase.
This inexpensive ESBL NDP test might be implemented worldwide. It may optimize rapid choices of antibiotics for treating bloodstream infections. It may also contribute to avoidance of overuse of carbapenems. Finally, a rapid detection of ESBL-E coupled with bacterial species identification will enhance identification of ESBL in species likely to be the source of nosocomial outbreaks (K. pneumoniae, Enterobacter spp.) and facilitate implementation of a rapid strategy for containment (14).

An international patent form for the ESBL NDP test has been filed on behalf of INSERM Transfert (Paris, France).

Dr. Dortet is an associate professor of medical microbiology, South-Paris University, Paris. His main interests include the genetics and molecular epidemiology of resistance in gram-negative rod-shaped bacteria.

References


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Rapid Detection of ESBL-Producing Enterobacteriaceae in Blood Cultures

Methods Overview

MALDI-TOF MS bacterial identification directly on positive blood culture samples and definitive identification of bacterial species

One milliliter of blood culture was transferred into an 1.5-mL Eppendorf tube. Fifty microliters of Triton 10% was added in the tube that was then briefly vortexed. After a centrifugation step at 13,000 x g for 2 min, the supernatant was discarded and the bacterial pellet was resuspended in 500 µL of distilled water. After a second centrifugation step at 13,000 x g for 2 min, the supernatant was discarded and the bacterial pellet was resuspended into 50 µL of 100% ethanol (Thermo Scientific, Villebon-sur-Yvette, France). One drop of this suspension was spotted in duplicate on the disposable plate. The dried spots were overlaid with 1 µL of the matrix solution (VITEK-MS CHCA; bioMérieux, La Balme-les-Grottes, France) and air-dried for 1–2 min at room temperature, as recommended by the manufacturer. The loaded slide was then inserted into the VITEK MS system. Spectra were analyzed and identifications were calculated automatically by the advanced spectrum classifier algorithm provided by the manufacturer. Definitive identification retained as the gold standard was obtained with cultured bacteria using the MALDI-TOF MS bacterial identification (VITEK-MS; bioMérieux) and the Api20E biochemical gallery (bioMérieux).
Detailed Methods

Rapid Detection of ESBL Activity in Enterobacteriaceae

ESBL NDP Test Directly from Blood Cultures

Protocol

1. Transfer 0.5 mL of Enterobacteriaceae–positive blood culture (using a syringe with needle) to each of 3 Eppendorf tubes, size 1.5 mL (tubes A, B, and C).

2. Add 50 µL of a solution of Triton 10% (vol/vol).

3. Vortex.

4. Incubate 5 min at room temperature.

5. Centrifuge at 13,000 × g for 2 min.

6. Discard the supernatant.

7. Resuspend the bacterial pellet in 500 µL of distilled water.

8. Check that bacterial colonies have been properly resuspended. If needed, mix up and down with a pipette.

9. Centrifuge at 13,000 × g for 2 min.

10. Discard the supernatant.

11. Resuspend the bacterial pellet in 100 µL of 20 mmol/L Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific, Pierce).

12. Check that bacterial colonies have been correctly resuspended. If necessary, mix up and down with a pipette.

13. Add 10 µL of a tazobactam concentrated solution (40 mg/mL) in the tube C.

14. Add (i) 100 µL of the revelation solution (Solution R) in the tube A and (ii) 100 µL Solution R + cefotaxime 6 mg/mL in the tubes B and C.

15. Incubate at 37°C for a maximum of 15 min.
16. Optical reading of the color of each tube.

**Principle of the Test:**

![Diagram showing the principle of the test]

**Interpretation:**

<table>
<thead>
<tr>
<th>No ESBL</th>
<th>ESBL</th>
<th>Overexpressed cephalosporinase +/- ESBL</th>
<th>Non interpretable</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Tube A" /></td>
<td><img src="image2" alt="Tube B" /></td>
<td><img src="image3" alt="Tube C" /></td>
<td><img src="image4" alt="Tube A" /></td>
</tr>
<tr>
<td>No antibiotic (tube A)</td>
<td>Cefotaxime (tube B)</td>
<td>Cefotaxime + tazobactam (tube C)</td>
<td>Non interpretable</td>
</tr>
<tr>
<td>No ESBL</td>
<td>Red</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>ESBL</td>
<td>Red</td>
<td>Orange/yellow</td>
<td>Red</td>
</tr>
<tr>
<td>Cephalosporinase or Cephalosporinase + ESBL</td>
<td>Red</td>
<td>Orange/yellow</td>
<td>Orange/yellow</td>
</tr>
<tr>
<td>Non interpretable</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

ESBL-producing *E. coli*. The *Enterobacteriaceae*–positive blood culture specimens contain A) no antibiotic; B) cefotaxime; C) cefotaxime and tazobactam.
Material

- 1.5-mL Eppendorf tubes
- Cefotaxime sodium salt (Sigma-Aldrich) or cefotaxime (drug used for patient treatment).
- Tazobactam sodium salt (Sigma-Aldrich, Cat: T-2820)
- B-PERII, Bacterial Protein Extraction Reagent (Thermo Scientific, Pierce), Cat: 78260.
- Concentrated solution of Triton X-100 (Sigma-Aldrich, Cat: T-8787)
- Negative (wild-type *E. coli*) and positive (*Klebsiella pneumoniae* CTX-M-15) controls.

Preparation and Storage of Solution R

1. Prepare a concentrated solution of red phenol 0.5% w/v
2. Mix 2 mL of the concentrated red phenol solution (strongly vortex before pipetting to resuspend the solution) in 16.6 mL of distilled water
3. Adjust the pH of the solution at a value 7.8 by adding drops of a NaOH solution (1 N)

Solution R is stable at room temperature for 1 week and may be kept at –20°C for several months.

Solution R + cefotaxime (6 mg/mL) has to be prepared extemporaneously.

However, batches of cefotaxime powders may be weighted in advance and kept at 4°C for 2 weeks if solution A is not added.

Preparation and Storage of Concentrated Tazobactam Solution (40 mg/mL)

1. Add 250 µL of distilled water in 10 mg of tazobactam sodium salt (Sigma-Aldrich, Cat: T-2820)
2. Prepare aliquots of 10 µL of this concentrated solution of tazobactam (40 mg/mL)

3. Those aliquots can be stored at −20°C for 1 month