Evaluation of the New VITEK 2 Extended-Spectrum Beta-Lactamase (ESBL) Test for Rapid Detection of ESBL Production in Enterobacteriaceae Isolates

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Extended-spectrum beta-lactamases (ESBLs) are a large, rapidly evolving group of enzymes that confer resistance to oxyimino cephalosporins and monobactams and are inhibited by clavulanate. Rapid reliable detection of ESBL production is a prerequisite for successful infection management and for monitoring resistance trends and implementation of intervention strategies. We evaluated the performance of the new VITEK 2 ESBL test system (bioMérieux, Inc, Hazelwood, Mo.) in the identification of ESBL-producing Enterobacteriaceae isolates. We examined a total of 1,129 clinically relevant Enterobacteriaceae isolates (including 218 that had been previously characterized). The ESBL classification furnished by the VITEK 2 ESBL test system was concordant with that of the comparison method (molecular identification of beta-lactamase genes) for 1,121 (99.3%) of the 1,129 isolates evaluated. ESBL production was correctly detected in 306 of the 312 ESBL-producing organisms (sensitivity, 98.1%; positive predictive value, 99.3%). False-positive results emerged for 2 of the 817 ESBL-negative isolates (specificity, 99.7%; negative predictive value, 99.3%). VITEK 2 ESBL testing took 6 to 13 h (median, 7.5 h; mean ± SD, 8.2 ± 2.39 h). This automated short-incubation system appears to be a rapid and reliable tool for routine identification of ESBL-producing isolates of Enterobacteriaceae.

Extended-spectrum beta-lactamases (ESBLs) are a large, rapidly evolving group of enzymes that confer resistance to oxyimino cephalosporins and monobactams. They are inhibited by clavulanate (CA), sulbactam, or tazobactam. Originally observed in Escherichia coli and Klebsiella spp., ESBL production has now been documented in other gram-negative bacilli, including Enterobacter spp., Proteus mirabilis, and Providencia stuartii (4, 23, 25, 30, 45).

Laboratory detection of ESBL production can be problematic (4, 23, 27, 30, 33, 45, 49, 52). The presence of these enzymes does not always elevate MICs of oxyimino cephalosporins and monobactams to levels indicative of resistance defined by the Clinical Laboratory Standards Institute (CLSI) (2, 10). Furthermore, because expression of resistance is affected by multiple factors, the same ESBL can produce different resistance phenotypes, depending on the bacterial carrier and test conditions (17, 23). There are also increasing reports of more-complex ESBL phenotypes that include additional mechanisms of resistance, such as AmpC-type enzyme production (both chromosomal and plasmid-mediated), TEM and SHV beta-lactamases with reduced affinities for beta-lactamase inhibitors, hyperproduction of penicillinase, and porin changes (4, 6, 8, 17, 23, 26, 32, 34, 39, 49, 52–54).

Several molecular methods are available for research and epidemiological studies, but they are not appropriate for routine detection of ESBL production in clinical settings (9, 36). Two phenotypic strategies can be used to detect ESBL expression in clinical settings. One involves analysis of MIC patterns with specific software, such as that used by the Advanced Expert System of the VITEK 2 system (bioMérieux, Inc, Hazelwood, MO). The second is the two-step approach advocated by the CLSI (10), which involves screening for reduced susceptibility to more than one of the indicator antimicrobials (cefotaxime [CTX], ceftriaxone, ceftazidine [CAZ], cefpodoxime, and aztreonam). Positive results are then confirmed by the demonstration of synergy between the ceftazidine and cefotaxime and the beta-lactamase inhibitor CA. According to CLSI guidelines (10), ESBL production is confirmed in E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, or P. mirabilis if testing in the presence of CA decreases the ceftazidine and cefotaxime MICs by at least three twofold dilutions or increases the diameter of the inhibition zone for these drugs by at least 5 mm (compared with results obtained with the cephalosporin alone). This strategy is also the basis for the double-disk test, the three-dimensional test, the Etest ESBL test, and several other commercial systems (7, 12, 16, 18, 20–22, 28, 40, 41, 43, 46–48, 50, 51).

The VITEK 2 ESBL test (bioMérieux) is a new tool for rapid detection of ESBL production which is based on simultaneous assessment of the inhibitory effects of cefepime, ceftaxime, and ceftazidine, alone and in the presence of CA. The present study was designed to evaluate its performance in the identification of ESBL-producing isolates of Enterobacteriaceae and the advantages it can offer for routine clinical testing.
MATERIALS AND METHODS

Study design. The study was conducted on a total of 1,129 bacterial isolates: *E. coli* (n = 534), *K. pneumoniae* (n = 193), *Enterobacter cloacae* (n = 88), *P. mirabilis* (n = 85); Enterobacter aerogenes (n = 56); *K. oxytoca* (n = 38); Citrobacter freundii (n = 36); *P. stuartii* (n = 33); *Serratia marcescens* (n = 28); Morganella morganii (n = 14); *Citrobacter koseri* (n = 8); and *Salmonella enterica* spp. (n = 6). Two hundred eighteen of the isolates had been collected and characterized as part of a previously published nationwide survey (41). The remaining 911 were consecutive nonduplicate clinically relevant isolates of *Enterobacteriaceae* recovered from blood cultures of hospitalized patients between January 1999 and December 2003. They had not been characterized at the time of the study.

Identical methods were used to characterize all 1,129 study isolates. ID32E galleries (bioMérieux, Marcy l’Etoile, France) and/or the VITEK 2 system (bioMérieux) were used for species-level identification. Susceptibilities to beta-lactam antibiotics were evaluated by using the Etest (Upjohn; Solna, Sweden), as previously described (41). *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 25783 were included as quality control strains in all sessions. MICs were classified according to CLSI criteria (10).

Characterization of bacterial isolates. All isolates (except ampicillin-susceptible *E. coli*, *P. mirabilis*, and *S. enterica* isolates) were subjected to isoelectric focusing (IEF) analysis of beta-lactamase production profiles, as described previously (41), and results were interpreted using published criteria (44, 49, http://www.lahey.org/studies/web.htm). The same isolates then underwent PCR amplification of *bla*TEM, *bla*SHV, *bla*OXY, *bla*CTX, *bla*GES, *bla*PER, and *ampC*-type genes as well as sequencing of both strands of the PCR products, as previously described (1, 3, 31, 34). The molecular findings were compared with those of IEF analysis and resistance phenotypes (e.g., an ESBL phenotype or a cephalosporinase phenotype) based on susceptibility testing (described above). Any discrepancies that emerged were resolved by repeat testing with all three methods. The final result for each isolate was a molecular profile of beta-lactamase production supported by compatible findings in IEF and in vitro susceptibility analyses, and these profiles were used as the comparison method for our assessment of the performance of the VITEK 2 ESBL test.

All strains found to harbor ESBL genes were subjected to the Etest to determine the MICs for cefotaxime, ceftazidime, and cefepime, alone and with CA. The sensitivity, specificity, and positive and negative predictive values for the VITEK 2 ESBL test were calculated according to the results of the molecular comparison method described above. When discrepancies emerged, VITEK 2 ESBL testing, biochemical and molecular characterization, and Etestng were repeated. The performance of the new system was assessed for all 1,129 isolates tested and separately for each bacterial species.

RESULTS

Molecular and phenotypic testing results. Molecular testing revealed ESBL genes in 312/1,129 test isolates. Most ESBL producers (251/312; 80%) were *E. coli*, *K. oxytoca*, *K. pneumoniae*, and *P. mirabilis*, but 61 belonged to species for which there are currently no CLSI guidelines and rules for interpretation of ESBL testing results (e.g., *Citrobacter spp.*, *Enterobacter spp.*, *M. morganii*, *P. stuartii*, and *S. marcescens*) (10). A total of 361 different ESBL genes were identified in the 312 isolates (Table 1): 179 (49.6%) *bla*_TEM genes, 131 (36.3%) *bla*_SHV genes, and 51 (14.1%) *bla*_CTX-M genes. Half of the ESBL-producing isolates also carried genes for at least one broad-spectrum beta-lactamase (e.g., *TEM-1/2*, *SHV-1*, *SHV-11*).

Approximately 40% of the 312 ESBL carriers were classified by the Etest as susceptible (defined by a MIC of ≤8 μg/ml) to one of the oximino cephalosporins (usually cefepime); 35% were susceptible to two, and 9% were susceptible to all three. Four isolates (two *E. coli* and two *K. pneumoniae* isolates) had oximino cephalosporin MICs of ≤2 μg/ml (ceftazidime MIC, 2 μg/ml; cefotaxime MIC, 1 to 2 μg/ml; cefepime MIC, 0.5 μg/ml). For all 312 ESBL producers, the MICs of ceftazidime, cefotaxime, and/or cepime decreased by ≥3 twofold dilutions in the presence of CA. All *E. coli*, *Klebsiella* spp., and *P. mirabilis* isolates also fulfilled the CLSI criteria for confirmation of ESBL production (i.e., disk diffusion zone diameters increased by ≥5 mm around ceftazidime and cefotaxime disks in the presence of CA) (10).

The 817 isolates with no evidence of ESBL production in molecular testing included 212 (Enterobacter spp. and *Citrobacter spp.* in most cases) that were positive only for the *ampC*-type gene. Two of the 36 non-ESBL-producing *K. oxytoca* isolates displayed profiles suggestive of hyperproduction of K1 beta-lactamate, i.e., resistance/low susceptibility to ceftazidime, aztreonam, and ceftriaxone (MICs of 128, 8, and 16 μg/ml, respectively) and full susceptibility to cefazidime and cefotaxime (MICs of 1 μg/ml). PCR analysis of these isolates yielded a 155-bp amplicon that is consistent with the OXY-2 enzyme subtype (14, 41). The other 563 isolates lacking ESBLs (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *S. enterica* spp.) included 156 ampicillin-susceptible and 407 ampicillin-resistant isolates producing broad-spectrum beta-lactamases belonging to Bush group 2b, which harbored TEM-1 (105 isolates), TEM-2 (22 isolates), and SHV-1 (280 isolates). These enzymes hydrolyze penicillin and ampicillin and to a lesser degree carbencillin or cephalothin, but they have no significant effect on oximino cephalosporins or aztreonam. In fact, all but two of these isolates had Test MICs indicative of full susceptibility to both ceftazidime and cefotaxime (MICs ≤ 1 μg/ml) (23). The remaining six ESBL-negative isolates carried SHV-10, an inhibitor-resistant beta-lactamase belonging to Bush group 2b. OXA-1 enzyme (Bush group 2d) was also detected in 11 isolates.

None of the 1,129 test isolates harbored plasmid-mediated AmpC beta-lactamases.

VITEK 2 ESBL test results, VITEK 2 ESBL testing required from 6 to 13 h (median, 7.5 h; mean ± SD, 8.2 ± 2.39 h), and the results were concordant with those of the molecular comparison method for 1,121 (99.3%) of the 1,129 isolates evaluated (Table 2). The VITEK 2 ESBL test system correctly identified 306 of the 312 ESBL-producing organisms (sensitivity, 98.1%; positive predictive value, 99.3%). False-negative results emerged for six isolates, including two SHV-2-producing isolates of *E. coli* and two of *K. pneumoniae*, one containing SHV-2 and the
other harboring SHV-5. These isolates displayed low levels of
resistance to oxyimino cephalosporins (MICs from 0.5 to 2
μg/ml), but in all four cases, CA synergy (MIC reduced by
3 twofold dilutions) was clearly observed in Etest results. The
remaining discrepancies involved two *E. aerogenes* isolates, one
producing SHV-12 and the other producing CTX-M-1. These
isolates had Etest MICs for the oxyimino cephalosporins plus
CA that were higher (i.e., >1 μg/ml) than the drug concentra-
tions included in the VITEK 2 ESBL test.

Two of the 817 ESBL-negative isolates were falsely flagged
as ESBL producers (specificity, 99.7%; negative predictive
value, 99.3%). For these isolates (both *E. coli*), Etest MICs of

### TABLE 1. Distribution of 361 extended-spectrum beta-lactamases in 312 isolates of *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Microorganism (no. of isolates)</th>
<th>SHV</th>
<th>TEM</th>
<th>SHV/TEM</th>
<th>CTX-M</th>
<th>CTX-M/SHV/TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. freundii</em> (5)</td>
<td>SHV-12 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. aerogenes</em> (11)</td>
<td>SHV-12 (8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. cloacae</em> (2)</td>
<td>SHV-12 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> (107)</td>
<td>SHV-2 (2)</td>
<td>TEM-5 (1)</td>
<td>SHV-12/TEM-52 (1)</td>
<td>CTX-M-1 (6)</td>
<td></td>
</tr>
<tr>
<td><em>K. oxytoca</em> (2)</td>
<td>SHV-12 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (86)</td>
<td>SHV-2a (9)</td>
<td>TEM-20 (5)</td>
<td>SHV-8/TEM-24 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. morganii</em> (7)</td>
<td>SHV-12 (3)</td>
<td>TEM-43 (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em> (56)</td>
<td>TEM-52 (33)</td>
<td>TEM-72 (22)</td>
<td>TEM-93 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. stuartii</em> (33)</td>
<td>TEM-52 (19)</td>
<td>TEM-72 (14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. marcescens</em> (3)</td>
<td>TEM-12 (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2. Performance of the VITEK 2 ESBL test for detection of ESBLs in 312 ESBL-producing and 817 non-ESBL-producing isolates

<table>
<thead>
<tr>
<th>Microorganism (no. of isolates)</th>
<th>No. of ESBL-positive isolates identified by VITEK 2 as:</th>
<th>No. of ESBL-negative isolates identified by VITEK 2 as:</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV(^a) (%)</th>
<th>NPV(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESBL positive</td>
<td>ESBL negative</td>
<td>ESBL positive</td>
<td>ESBL negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Citrobacter</em> spp.(^b) (44)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>39</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.(^c) (144)</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>131</td>
<td>84.6</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em> (534)</td>
<td>105</td>
<td>2</td>
<td>2</td>
<td>425</td>
<td>98.1</td>
<td>99.5</td>
</tr>
<tr>
<td><em>K. oxytoca</em> (38)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (193)</td>
<td>84</td>
<td>2</td>
<td>0</td>
<td>107</td>
<td>97.7</td>
<td>100</td>
</tr>
<tr>
<td><em>M. morganii</em> (14)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>P. mirabilis</em> (85)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>P. stuartii</em> (43)</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>S. marcescens</em> (28)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>S. enterica</em> spp. (6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>ND(^d)</td>
<td>ND</td>
</tr>
<tr>
<td>Total (1,129)</td>
<td>306</td>
<td>6</td>
<td>2</td>
<td>815</td>
<td>98.1</td>
<td>99.7</td>
</tr>
</tbody>
</table>

\(^a\) PPV, positive predictive value; NPV, negative predictive value.
\(^b\) *C. freundii* and *C. koseri*.
\(^c\) *E. aerogenes* and *E. cloacae*.
\(^d\) ND, not determined.
Discrimination between ESBL production and hyperproduction of K1 enzyme by *K. oxytoca* isolates can also be difficult (21, 30, 37, 41). Cefpodoxime-plus-CA disks have proved to be particularly sensitive and specific (100% each) for detecting ESBLs in this species because cefpodoxime inhibition is not enhanced by CA (7). M’Zali et al. (28) reported that 93% of ESBL-producing *K. oxytoca* isolates were correctly identified with the MAST double-disk test using both ceftazidime and cefpiramide, whereas use of either of these drugs alone was considerably less sensitive (86% and 65.5%, respectively). The two K1-hyperproducing isolates we tested were correctly identified by the VITEK 2 ESBL test as ESBL negative. Both had moderately high MICs of aztreonam and ceftriaxone (8 to 16 μg/ml) and low MICs for the other three oximino cephalosporins tested (≤1 μg/ml), which is consistent with previous reports (13). Further studies are needed, however, to evaluate the true specificity of VITEK 2 ESBL testing in *K. oxytoca*.

Infections caused by ESBL-producing organisms have a significant impact on mortality rates and hospital costs (4, 30). Kang et al. (19) maintain that delays in starting appropriate...
therapy have no significant effect on the outcome of ESBL bloodstream infections if therapy is promptly adjusted in accordance with in vitro susceptibility data. Therefore, these data must be reported to the physician as soon as possible, especially in high-risk cases. Although further studies are needed to evaluate its overall performance, our experience with this large series of Enterobacteriaceae isolates indicates that the VITEK 2 ESBL test system is a reliable time-saving tool for routine identification of ESBL-producing strains. It furnished results in 6 to 13 h (median, 7.5 h), compared with at least 18 h for CLSI-approved phenotypic screening and confirmatory assays (10). The one drawback is the absence of automated results for P. mirabilis isolates, and this problem should be addressed in future versions of the system.

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