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Forty clinical isolates of Enterobacter spp. were identified as extended-spectrum β-lactamase (ESBL) producers by disk diffusion. The VITEK 2 Advanced Expert System (AES) identified the ESBL phenotype in only 25 isolates (62.5%), and erroneously reported cephalosporin susceptibility in 11 isolates (28%). Refinements in the AES are required in order to improve ESBL detection in Enterobacter.

Accurate identification of extended-spectrum β-lactamase (ESBL) production is essential for the appropriate reporting of antimicrobial susceptibility results, since ESBLs render penicillins, cephalosporins, and aztreonam inadequate for treatment of serious infections (2). While guidelines exist for ESBL detection in Enterobacter spp. were identified as extended-spectrum β-lactamase (ESBL) producers by disk diffusion. The VITEK 2 Advanced Expert System (AES) identified the ESBL phenotype in only 25 isolates (62.5%), and erroneously reported cephalosporin susceptibility in 11 isolates (28%). Refinements in the AES are required in order to improve ESBL detection in Enterobacter.

ESBL family Primer, sequence Gene product length (bp)
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TEM Forward, 5'-KACAAATACCGTAAATTCGCTG-3' Reverse, 5'-AGATATATATGACATAAGTCG-3' 936
SHV Forward, 5'-TATACGCTCAGCAGTGCTG-3' Reverse, 5'-CGTCGCCCAGATATAAGG-3' 930
OXA-1 Forward, 5'-ACACAATACATACATGACGTCG-3' Reverse, 5'-AGTGTGTTATAGATGTCACG-3' 813
OXA-2 Forward, 5'-TCCAAGGCGCGGGAGCACG-3' Reverse, 5'-TCCGCGTTATCAGGCGCTG-3' 702
OXA-10 Forward, 5'-CGTGTTATGAAAAGATCAGG-3' Reverse, 5'-CATATTTTTTGGGAGGAGG-3' 651
CTX-M (degenerate) Reverse, 5'-CGGCAGTCTTATAACT-3' 550
CTX-M-2 Forward, 5'-ATGATGACATCGAGAATCAG-3' Reverse, 5'-TTATGTGCTCAGCGCGC-3' 884
CTX-M-8 Forward, 5'-ATGATGACATCGAGAATCAG-3' Reverse, 5'-CGGTGAGATTGCTTCGAG-3' 864
CTX-M-10 Forward, 5'-GCTGACGACTTCGACGTG-3' Reverse, 5'-TTTACAACTGTTGTGACG-3' 683
CTX-M-25 Forward, 5'-ACACGAAATGAGTGATCAGG-3' Reverse, 5'-TACCTACATGTTGGAG-3' 924

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were subjected to isoelectric focusing to locate the isoelectric point (pI) of the \(\beta\)-lactamases produced, according to methods we have previously described (12).

The VITEK 2 AES identified 25 of 40 isolates (62.5%) as ESBL producers (Table 2). For 17, ESBL production was the only resistance mechanism suggested, and for 8, production of a high-level cephalosporinase was also proposed. Of the 15 isolates not identified by AES as ESBL producers (Tables 3 and 4), 11 (73%) were reported to be susceptible to at least one cephalosporin, contrary to published guidelines for susceptibility reporting in ESBL producers (2). The following alternative resistance mechanisms were reported for these isolates by AES: high-level cephalosporinase (13 isolates), impermeability (2 isolates), and other \(\beta\)-lactamases (3 isolates).

In 6 of the 15 discrepant isolates, PCR amplified an ESBL gene (Table 3). Three PCR products belonging to the SHV family were confirmed as the ESBL gene \(\text{bla}_{\text{SHV-12}}\) by sequencing. The remaining three PCR products belonged to the CTX-M family of ESBL genes. ESBL activity was phenotypically confirmed in cell lysates of the nine discrepant isolates for which an ESBL gene was not amplified by demonstrating ceftiraxone hydrolysis that was inhibited in the presence of clavulanate (Table 4). Results of isoelectric focusing were consistent with the production of at least one \(\beta\)-lactamase by each of these isolates. Their pI values are reported in Table 4.

A number of studies have sought to determine the reliability of the VITEK system for ESBL detection in Enterobacteriaceae isolates, most with satisfactory results (4–7, 9, 14, 16). These studies, however, have involved primarily isolates of \(E.\) coli and \(Klebsiella\) spp. ESBL detection in Enterobacter by automated systems is more complicated because of the production of chromosomally encoded AmpC-type enzymes, which, unlike ESBLs, are not inhibited by clavulanate and may even be induced by it and therefore may nullify the ability of the VITEK system to identify ESBL production based on the clavulanic acid effect. Indeed, in one study looking specifically at ESBL detection in \(E.\) coli and \(Klebsiella\) spp. (17), of 31 ESBL-producing isolates, the VITEK detection test, using cefotaxime and ceftazidime alone and in combination with clavulanic acid, was positive for only 2 (6.5%).

The AES enhances the ability of the VITEK system to identify ESBLs by basing its phenotype determination on the distribution of MICs for various \(\beta\)-lactam antibiotics rather than simply on neutralization by clavulanic acid (1, 6, 11). Sanders et al. found a high degree of accuracy of the AES in resistance mechanism detection in Enterobacter (92%), but insufficient data preclude a determination of the accuracy of the AES in ESBL detection specifically (11). Two groups have reported \(\geq 90\%\) agreement between the VITEK 2 AES and reference genotype data in ESBL detection overall in Enterobacteriaceae, including in AmpC-inducible species, though in each study, only a few \(Enterobacter\) isolates were tested (1, 6).

It seems apparent that the VITEK 2 AES, while an appropriate diagnostic tool for ESBL detection in \(E.\) coli and \(Klebsiella\) spp., is less reliable for their detection in \(Enterobacter\) spp. In our study,
AES had a sensitivity of 62.5% in identifying ESBL-producing Enterobacter spp., compared with disk diffusion. In 15 discrepant cases in which molecular testing was performed, PCR confirmed the presence of an ESBL gene in 40%, and ceftriaxone hydrolysis inhibited by clavulanate in cell lysate confirmed ESBL production in the remainder. Since the definition of ESBLs is phenotypically based (2), these isolates should be considered ESBL producers despite the absence of amplification of the main ESBL gene families by PCR. Whether this absence is indicative of ESBL genes not belonging to the common gene families remains to be determined. An unknown percentage of ESBL-producing Enterobacter species will not be detected by either VITEK or disk diffusion. Thus, the true sensitivity of the AES in ESBL detection is likely even lower than we report.

Misidentification of ESBL producers by the AES led to erroneous reporting of cephalosporin susceptibility in nearly three-quarters of discrepant cases. Refinements in the AES are therefore required to improve the accuracy of ESBL detection in Enterobacter spp. Inclusion of cefepime or cefpirome alone and with a β-lactamase inhibitor in susceptibility testing may improve performance, as these agents are less efficiently hydrolyzed by AmpC enzymes than are earlier-generation cephalosporins (17). Theoretically, tazobactam may be a more appropriate β-lactamase inhibitor than clavulanic acid, as it is a weaker inducer of AmpC enzymes. Additional studies are required before the VITEK 2 AES can be used as a sole method of detection of ESBL production in Enterobacter spp.

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REFERENCES


### TABLE 4. Results for isolates not identified by AES as ESBL producers, for which PCR did not amplify an ESBL gene

<table>
<thead>
<tr>
<th>Bacterium&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference antibiotic(s)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Interception of VITEK AES</th>
<th>Ceftriaxone hydrolysis inhibited by clavulanate&lt;sup&gt;d&lt;/sup&gt;</th>
<th>pl</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. aerogenes</em></td>
<td>CTX, CAZ, FEP</td>
<td>Wild (cephalosporinase)</td>
<td>+</td>
<td>8.0</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX, CAZ</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>7.6</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX, CAZ</td>
<td>High-level cephalosporinase + impermeability</td>
<td>+</td>
<td>7.8, 8.2</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX, CAZ</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>5.4</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>7.8, 8.2, 8.8</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX, CAZ</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>5.4</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>8.8</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>CTX, CAZ</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>7.8</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>CTX, CAZ</td>
<td>High-level cephalosporinase</td>
<td>+</td>
<td>8.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> A total of nine isolates (one from blood and eight from other sites) were tested. The results given are for individual isolates.

<sup>b</sup> * reported by AES as susceptible to at least one cephalosporin.

<sup>c</sup> CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime.

<sup>d</sup> +, hydrolysis and inhibition occur.