JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2006, p. 241–243 Vol. 44, No. 1
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.1.241–243.2006

Utility of the VITEK 2 Advanced Expert System for Identification of Extended-Spectrum β-Lactamase Production in *Enterobacter* spp.

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Received 8 July 2005/Returned for modification 13 August 2005/Accepted 6 November 2005

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 *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* isolates only (2), these enzymes are produced by a wide variety of other gram-negative organisms as well, including *Enterobacter* spp. (3, 8, 12, 13).

The VITEK 2 Advanced Expert System (AES; bioMérieux, Durham, NC) is an automated system that uses the antimicrobial susceptibility data generated to suggest the phenotype of the tested isolate and thereby determine susceptibility or resistance to antibiotics not tested (10). It has been used successfully to determine ESBL presence in *E. coli* and *Klebsiella* spp. (14, 16). Although ESBL detection in *Enterobacter* spp. by earlier automated systems, including VITEK, was hampered by the production of AmpC β-lactamase (17), the more recently developed AES has not been evaluated for *Enterobacter* specifically. Given the high proportion of ESBL production among *Enterobacter* isolates at our institution and elsewhere (8, 12) and the fact that the AES includes ESBL production among the resistance mechanisms it suggests for *Enterobacter* isolates, we sought to determine the accuracy of the AES in ESBL detection among clinical isolates of *Enterobacter*.

Forty unique-patient isolates of *Enterobacter* were included (30 *E. cloacae* isolates and 10 *E. aerogenes* isolates). These epidemiologically unrelated isolates were identified phenotypically as ESBL producers via the disk diffusion method established for *E. coli*, *Klebsiella* spp., and *P. mirabilis* (2, 3), using 30-μg cefotaxime-, ceftazidime-, and cefepime-impregnated disks with and without 10 μg clavulanic acid (cefotaxime- and ceftazidime-containing disks made by Oxoid [Basingstoke, Hampshire, England]; cefepime-containing disks were prepared in-house). Although CLSI does not recommend the disk diffusion method for *Enterobacter*, we and others have previously noted a strong correlation between demonstration of a clavulanic acid effect and the presence of an ESBL gene (3, 12, 15). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls for ESBL production, respectively.

Susceptibility profiles and suggested resistance mechanisms were recorded by the AES for each isolate. All isolates not identified as ESBL producers by VITEK were subjected to further study in order to demonstrate ESBL presence. Screening for the common ESBL gene families (TEM, SHV, OXA, and CTX-M) was performed by PCR (primers recorded in Table 1). Isolates for which PCR failed to amplify a known ESBL gene were subjected to further phenotypic testing to confirm the presence of a β-lactamase able to hydrolyze ceftriaxone and inhibited by clavulanate, i.e., an ESBL. β-Lactamase activity of sonicated cells was measured spectrophotometrically using 25 μM ceftriaxone in 50 mM phosphate buffer (pH 7.0) as the substrate. Inhibition by 2.5 μM clavulanate was measured after 5-min preincubation. In addition, these isolates

TABLE 1. Primers used in PCR runs for tested *Enterobacter* isolates

ESBL family	Primer, sequence	Gene product length (bp)
TEM	Forward, 5′-KACAATAACCCTGRTAAATGC-3′ Reverse, 5′-AGTATATATGAGTAAACTTGG-3′	936
SHV	Forward, 5′-TTTATCGGCCYTCACTCAAGG-3′ Reverse, 5′-GCTGCGGGCCGGATAACG-3′	930
OXA-1	Forward, 5′-ACACAATACATATCAACTTCGC-3′ Reverse, 5′-AGTGTGTTTAGAATGGTGATC-3′	813
OXA-2	Forward, 5′-TTCAAGCCAAAGGCACGATAG-3′ Reverse, 5′-TCCGAGTTGACTGCCGGGTTG-3′	702
OXA-10	Forward, 5′-CGTGCTTTGTAAAAGTAGCAG-3′ Reverse, 5′-CATGATTTTGGTGGGAATGG-3′	651
CTX-M (degenerate)	Forward, 5′-CGYTTTSCIATGTGCAG-3′ Reverse, 5′-ACCGCRATATCRTTGGT-3′	550
CTX-M-2	Forward, 5′-ATGATGACTCAGAGCATTCG-3′ Reverse, 5′-TTATTGCATCAGAAACCGTG-3′	884
CTX-M-8	Forward, 5′-ATGATGAGACATCGCGTTAAG-3′ Reverse, 5′-CGGTGACGATTTTCGCGGCAG-3′	864
CTX-M-10	Forward, 5′-GCTGATGAGCGCTTTGCG-3′ Reverse, 5′-TTACAAACCGTTGGTGACG-3′	683
CTX-M-25	Forward, 5′-CACACGAATTGAATGTTCAG-3′ Reverse, 5′-TCACTCCACATGGTGAGT-3′	924

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TABLE 2. Results for isolates identified by VITEK 2 AES as ESBL producers[a]

Bacterium	Reference antibiotic(s)[b]	Interpretation of VITEK AES
E. aerogenes	CTX, CAZ, FEP	ESBL
E. cloacae	CTX, CAZ	ESBL
E. cloacae	CTX	ESBL + high-level cephalosporinase
E. cloacae	CTX, CAZ	ESBL
E. cloacae	CTX, CAZ, FEP	ESBL
E. cloacae	CAZ	ESBL + high-level cephalosporinase
E. aerogenes	CTX, CAZ, FEP	ESBL
E. cloacae	CTX	ESBL + high-level cephalosporinase
E. cloacae	CTX, CAZ, FEP	ESBL
E. aerogenes	CTX, CAZ, FEP	ESBL
E. aerogenes	CTX, CAZ, FEP	ESBL
E. cloacae	CTX, CAZ	ESBL
E. cloacae	CTX, CAZ	ESBL
E. cloacae	CTX	ESBL + high-level cephalosporinase
E. cloacae	CAZ	ESBL + high-level cephalosporinase
E. cloacae	CTX, CAZ	ESBL
E. cloacae	CTX, CAZ	ESBL
E. aerogenes	CTX, CAZ	ESBL
E. aerogenes	CAZ	ESBL
E. cloacae	CTX	ESBL
E. cloacae	CTX, CAZ	ESBL
E. cloacae	CTX, CAZ	ESBL + high-level cephalosporinase
E. cloacae	CAZ	ESBL + high-level cephalosporinase
E. cloacae	CTX, CAZ	ESBL + high-level cephalosporinase
E. cloacae	CTX, CAZ	ESBL

[a] A total of 25 isolates (4 from blood and 21 from other sites) were tested. The results given are for individual isolates.
[b] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime.

were subjected to isoelectric focusing to locate the isoelectric point (pI) of the β-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 β-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 $bla_{\text{SHV-12}}$ 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 ceftriaxone hydrolysis that was inhibited in the presence of clavulanate (Table 4). Results of isoelectric focusing were consistent with the production of at least one β-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 *Enterobacter* 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 β-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 ≥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,

TABLE 3. Results for isolates not identified by AES as ESBL producers, for which PCR amplified an ESBL gene[a]

Bacterium[b]	Reference antibiotics[c]	Interpretation of VITEK AES	ESBL or ESBL type whose gene was amplified
*E. cloacae**	CTX, CAZ, FEP	High-level cephalosporinase	SHV-12
E. aerogenes	CTX, CAZ, FEP	High-level cephalosporinase + carbapenem resistance	CTX-M-10 group
*E. aerogenes**	CTX, FEP	Acquired penicillinase	CTX-M-25 group
*E. cloacae**	CAZ	High-level cephalosporinase	SHV-12
*E. cloacae**	CTX, CAZ	High-level cephalosporinase	SHV-12
E. cloacae	CTX	High-level cephalosporinase + impermeability	CTX-M-2 group

[a] A total of six isolates (two from blood and four from other sites) were tested. The results given are for individual isolates.
[b] *, reported by AES as susceptible to at least one cephalosporin.
[c] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime.

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

Bacterium[b]	Reference antibiotic(s)[c]	Interpretation of VITEK AES	Ceftriaxone hydrolysis inhibited by clavulanate[d]	pI
*E. aerogenes**	CTX, CAZ, FEP	Wild (cephalosporinase)	+	8.0
*E. cloacae**	CTX, CAZ	High-level cephalosporinase	+	7.6
E. cloacae	CTX, CAZ	High-level cephalosporinase + impermeability	+	7.8, 8.2
*E. cloacae**	CTX, CAZ	High-level cephalosporinase	+	5.4
*E. cloacae**	CTX	High-level cephalosporinase	+	7.8, 8.2, 8.8
E. cloacae	CTX, CAZ	High-level cephalosporinase	+	5.4
*E. cloacae**	CTX, CAZ	High-level cephalosporinase	+	8.8
*E. cloacae**	CTX	High-level cephalosporinase	+	7.8
*E. aerogenes**	CTX, CAZ	High-level cephalosporinase	+	8.2

[a] A total of nine isolates (one from blood and eight from other sites) were tested. The results given are for individual isolates.
[b] *, reported by AES as susceptible to at least one cephalosporin.
[c] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime.
[d] +, hydrolysis and inhibition occur.

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 hydrohlyzed 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.

This work was supported in part by a grant from the Sapir Fund, Mifal Hapayis, Israel.

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