Evaluation of the VITEK® 2 AST N-054 test card for the detection of extended-spectrum β-lactamase production in Escherichia coli with CTX-M phenotypes

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Objectives: A new VITEK® 2 antibiotic susceptibility testing (AST) card, AST N-054, was introduced for aerobic Gram-negative bacilli in 2007 and has been widely adopted for routine use in the UK. We evaluated its performance for detecting extended-spectrum β-lactamase (ESBL) production in Escherichia coli.

Methods: ESBL-producing faecal isolates of E. coli (n=137) from residents in nursing homes were tested using the AST N-054 card on VITEK® 2 and with MASTDISCS® ID ESBL detection disc diffusion tests (Mast Diagnostics, Bootle, UK). The susceptibility result recommended by the VITEK® 2 software was also recorded.

Results: The AST N-054 card detected ESBL production in 93 of the 137 isolates tested [test sensitivity 67.9% (95% CI, 59.7–75.1)]. E. coli strain A, a widespread lineage in the UK with a low-level CTX-M enzyme production, accounted for most of the detection failures, with 35/73 strain A isolates incorrectly reported versus 9/64 non-strain A isolates (P < 0.0001). The MASTDISCS® correctly detected ESBL in 135/137 isolates [test sensitivity 98.5% (95% CI, 94.5–99.9)]. Of the 44 isolates found to be negative for ESBL production by VITEK® 2, the Advanced Expert System misreported 29 as susceptible to cefotaxime and all as susceptible to ceftazidime and aztreonam.

Conclusions: These data suggest that the AST N-054 card for the VITEK® 2 system is less reliable than other previously reported cards for the detection of CTX-M β-lactamase-producing E. coli circulating in the UK, particularly strain A isolates.

Keywords: automated susceptibility testing, susceptibility testing, beta-lactamases

Introduction

The Health Protection Agency (HPA) in the UK has published a National Standard Method on the Laboratory detection and reporting of bacteria with extended-spectrum β-lactamase (ESBL) enzymes.1 A strategy of screening followed by confirmation, typically based on an antibiotic disc method, is recommended. The document states that automated systems, for example, VITEK (bioMérieux S.A., Marcy l’Etoile, France) and Phoenix (Becton Dickinson Diagnostic Systems, Sparks, MD, USA), which incorporate ESBL detection tests or strategies, are an alternative to the present recommendations. This view was supported by successful detection of 126 (92%) of 137 ESBL producers in a validation trial of the VITEK® 2 Advanced Expert System (AES) with antibiogram susceptibility testing (AST) card, AST N-010.2

A new AST card, AST N-054, was introduced for testing aerobic Gram-negative bacilli on VITEK® 2 systems in 2007 and has been widely adopted for routine use in the UK. We evaluated its performance for the detection of CTX-M ESBL production in Escherichia coli.
Methods

One hundred and thirty-seven ESBL-producing E. coli were recovered from faecal samples from nursing home residents during a separate epidemiological study (M. C. O’Leary, P. Donaghy, A. C. Loughrey, P. J. Rooney, J. A. Buchanan, M. M. Merron, B. Smyth, unpublished results). All gave ≥8-fold synergy between cefotaxime and clavulanate in agar dilution tests by the British Society for Antimicrobial Chemotherapy method, and all were inferred to produce CTX-M-type enzymes based on greater resistance to cefotaxime than to ceftazidime. PCR for a characteristic IS26-blaCTX-M-15 link3 showed that 73 belonged to strain A, which typically produces a low level of CTX-M-15 enzyme.4 64 were non-strain A and their CTX-M enzymes remain to be fully identified. The isolates were stored in pure culture in horse blood at −80°C.

After thawing, isolates were cultured on Columbia blood agar (Oxoid, Basingstoke, UK) at 35°C for 18–24 h. These cultures were then used to make suspensions in 0.45% saline, with a turbidity equivalent to a 0.5 McFarland standard, which were tested (i) using the AST N-054 card on the VITEK®2 automated system and (ii) with the MASTDISCS® ID ESBL detection disc diffusion test (Mast Diagnostics, Bootle, UK), with the latter performed on Mueller–Hinton agar (Oxoid) in accordance with the CLSI guidelines.5

The AST N-054 card tests 20 antimicrobials: ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefuroxime, cefalotin, cefotaxime, ceftazidime, ceftipime, cefoxitin, ertapenem, meropenem, aztreonam, gentamicin, amikacin, tobramycin, nalidixic acid, ciprofloxacin, trimethoprim and nitrofurantoin, and results were interpreted by the software version WSVT2-R04.03. This system presents both the direct result and that as interpreted by the AES. Testing was repeated if suggested by the AES. For the purposes of comparison with the MASTDISCS® result, all interpretations that included ‘ESBL’ were categorized as successful. The susceptibility results recommended for cefuroxime, cefotaxime, ceftazidime and aztreonam by the AES were also recorded.

The MASTDISCS® ID ESBL detection test employs discs with cefpodoxime 30 μg, cefuroxime 30 μg, cefotaxime 30 μg and combinations of these agents with clavulanic acid 10 μg. Zone sizes were recorded using the MASTSCAN Elite (Mast Diagnostics) zone reader and software, with a positive ESBL result taken as a cefotaxime zone diameter ≤27 mm or a ceftazidime zone diameter ≤22 mm and with the zone of inhibition of one or more cephalosporins increased by ≥5 mm in the presence of clavulanic acid.

Table 1. Performance of VITEK® 2 AST N-054 and MASTDISCS® for ESBL detection

<table>
<thead>
<tr>
<th>System/detection result</th>
<th>All isolates (n = 137)</th>
<th>CTX-M 15 strain A isolates (n = 73)</th>
<th>Non-strain A isolates (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST N-054</td>
<td>Success</td>
<td>93 (68%)</td>
<td>38 (52%)</td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>44 (32%)</td>
<td>35 (48%)</td>
</tr>
<tr>
<td>MASTDISCS®</td>
<td>Success</td>
<td>135 (98.5%)</td>
<td>73 (100%)</td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>2 (1.5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

a 95% confidence interval 59.7–75.1.
b P < 0.0001.

Discussion

Prompt and accurate detection of ESBL production in Enterobacteriaceae is important, as treatment failure and death have been associated with cephalosporin therapy for infections with ESBL producers that appeared susceptible in vitro.1,6 Failure to detect ESBL production in this study led to isolates being reported as susceptible to cefotaxime, ceftazidime and aztreonam, contrary to the current UK national guidance.1

The ability of the VITEK®2 AES to detect ESBL production in E. coli with the AST N-054 card (sensitivity 67.9%) was poorer than previously reported by other investigators,2,7–9 who mostly used a heterogeneous mix of ESBL producers and other AST cards with different combinations of cephalosporins.2,7–9

The VITEK®2 AST N-010 card, which (unlike the AST N-054 card) includes cefpodoxime, successfully detected ESBL production in 5/5 E. coli strain A, 4/4 non-A E. coli with CTX-M-15

Statistical analysis

The sensitivity of each test method was calculated with 95% confidence intervals placed around the sensitivity estimate. Differences in proportions were evaluated using Fisher’s exact test.

Results

VITEK®2 AST N-054 results

The AST N-054 card detected ESBL production in 93 of the 137 isolates tested [test sensitivity 67.9% (95% CI, 59.7–75.1)]. Strain A accounted for a significant majority of the 44 ESBL detection failures (Table 1), with 35/73 strain A isolates incorrectly reported compared with 9/64 non-strain A isolates (P < 0.0001). Of the 44 isolates misreported as negative for ESBL production by the AST N-054 card, the AES reported 29 as susceptible to cefotaxime and all as susceptible to ceftazidime and aztreonam.

When the AST N-054 card did detect ESBL production, all susceptible results for cefuroxime, cefotaxime, ceftazidime and aztreonam on reports were changed by the AES to ‘intermediate’.

Disc testing results

All the isolates (137/137) gave positive disc diffusion screening results with cefotaxime and ceftazidime, based on cefotaxime zone diameters ≤27 mm. The median zone diameter for cefotaxime was 18 mm (range 6–25), whereas that for ceftazidime was 24 mm (range 12–30). The MASTDISCS® method confirmed ESBL production in 135 of the 137 isolates tested [test sensitivity 98.5% (95% CI, 94.5–99.9)]. The median zone diameter enhancement for cefpodoxime by clavulanic acid was 11 mm (range 0–20), for cefotaxime 10 mm (range 1–23) and for ceftazidime 5 mm (range 0–16). For two isolates, the zone diameter enhancement with clavulanic acid was <5 mm for all three cephalosporins. One of the two isolates was found to be positive by the AST N-054 card, the other was negative by both methods. Using the MASTDISCS® method alone, both would have been reported as susceptible to ceftazidime, with one being reported as intermediate to cefotaxime and the other resistant.

We conclude that the sensitivity of ESBL detection by VITEK®2 may depend on both the AST card used and the ESBLs present. Concerns have been raised previously about the ability of the VITEK® AES to detect ESBL-producing organisms with low MICs when the AST cards contained neither cefpodoxime nor a specific ESBL test. These conditions are very relevant to E. coli strain A, where the CTX-M-15 enzyme expression is reduced by an IS26 insertion between the \( \text{bla}_{\text{CTX-M-15}} \) gene and its normal promoter in \text{ISeCp1} and for the AST N-054 card, which lacks cefpodoxime or a specific ESBL test.

Strain A isolates were significantly associated with ESBL detection failure, compared with non-strain A isolates \((P < 0.0001)\). This failure is important because strain A, one of the five related E. coli ST131 clones with CTX-M-15 enzyme, is nationally distributed in the UK and is dominant in some areas. The manual disc diffusion method appeared to be more sensitive in this study than previously reported. This may be a consequence of selection bias from the method used to screen the initial faecal samples for ESBL-producing E. coli.Suspicious isolates were selected using growth on cystine lactose electrolyte deficient agar (Oxoid), containing 1 mg/L ciprofloxacin and resistance to cefpodoxime as markers of possible ESBL production, with confirmation by the MASTDISCS combination disc method. Nonetheless, disc diffusion testing concomitant with VITEK®2 testing provided phenotypic confirmation and the assurance that the isolates used did not represent an unusual sample.

In summary, in those areas of the UK where E. coli strain A is the dominant producer of CTX-M-15 \( \beta \)-lactamase, it seems prudent to use an alternative method to screen E. coli for ESBL production; this might include disc diffusion testing or an alternative VITEK®2 AST card.

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Transparency declarations

None to declare.

References