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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Received 12 May 2008; returned 3 June 2008; revised 8 July 2008; accepted 8 July 2008

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 antibiotic 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.

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The detection result for ESBL in the VITEK®2 AST N-054 card was 93% (95% CI, 88.9–96.1%) sensitivity. Of the 137 ESBL-positive isolates, 35/35 (100%) were correctly reported as positive, whereas 62/102 (60.8%) of the ESBL-negative isolates were incorrectly reported as negative. The sensitivity of the VITEK®2 AST N-054 card for ESBL detection was calculated with 95% confidence intervals around the sensitivity estimate, which were 91% (95% CI, 86.3–94.4%) sensitivity for strain A isolates and 99% (95% CI, 97.2–100%) sensitivity for non-strain A isolates.

The AST N-054 card detected ESBL production in 35 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 ceftazidime and all as susceptible to cefotaxime 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’.

Discussion

Prompt and accurate detection of ESBL production in Entrobacteriaceae is important, as treatment failure and death have been associated with cefalosporin 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 cefalosporins.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.

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

<table>
<thead>
<tr>
<th>System/method</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 success</td>
<td>93 (68%)a</td>
<td>38 (52%)</td>
<td>55 (86%)</td>
</tr>
<tr>
<td>failure</td>
<td>44 (32%)b</td>
<td>35 (48%)b</td>
<td>9 (14%)b</td>
</tr>
<tr>
<td>MASTDISCS® success</td>
<td>135 (98.5%)c</td>
<td>73 (100%)</td>
<td>62 (97%)</td>
</tr>
<tr>
<td>failure</td>
<td>2 (1.5%)</td>
<td>0</td>
<td>2 (3%)</td>
</tr>
</tbody>
</table>

a95% confidence interval 59.7–75.1.

bP < 0.0001.

c95% confidence interval 94.5–99.9.
VITEK®2 AST N-054 for ESBLs


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®2 AES to detect ESBL-producing organisms with low MICs when the AST cards contained neither cefpodoxime nor a specific ESBL test.7,9 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 blaCTX-M-15 gene and its normal promoter in ISEcp13 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.3 The manual disc diffusion method appeared to be more sensitive in this study than previously reported.10 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 β-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.

Acknowledgements

We are grateful to Maureen O’Leary, Eastern Health and Social Services Board, for providing access to bacterial isolates. Part of this work was presented at the 18th ECCMID (Barcelona, April 2008; poster P 851).

Funding

The study was funded in full by the Department of Microbiology, The Royal Hospitals, Belfast.

Transparency declarations

None to declare.

References


