Evaluation of the VITEK 2 AST-N111 card for detection of extended-spectrum beta-lactamases (ESBLs) in and compared to ESBL Etests and combination disk methods

G. Valenza, S. Müller, C. Schmitt, D. Turnwald, T-T. Lam, M. Frosch, M. Abele-Horn, Y. Pfeifer

► To cite this version:

G. Valenza, S. Müller, C. Schmitt, D. Turnwald, T-T. Lam, et al.. Evaluation of the VITEK 2 AST-N111 card for detection of extended-spectrum beta-lactamases (ESBLs) in and compared to ESBL Etests and combination disk methods. *European Journal of Clinical Microbiology and Infectious Diseases*, 2011, 30 (7), pp.869-872. 10.1007/s10096-011-1169-2 . hal-00663401

HAL Id: hal-00663401

<https://hal.science/hal-00663401>

Submitted on 27 Jan 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Evaluation of the VITEK 2 AST-N111 card for detection of extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* compared to ESBL- Etests and combination disk methods

Running Title: Evaluation of the VITEK 2 AST-N111 card for ESBL detection

Keywords: Evaluation, VITEK 2, ESBL detection, *Escherichia coli*, *Klebsiella* spp.

Giuseppe Valenza¹, Sophia Müller¹, Corinna Schmitt¹, Doris Turnwald¹, Thien-Tri Lam¹, Matthias Frosch¹, Marianne Abele-Horn¹, Yvonne Pfeifer²

¹Institute of Hygiene and Microbiology, University of Wuerzburg, Germany

²Robert Koch Institute, Wernigerode, Germany

Corresponding author:

Giuseppe Valenza

Institute of Hygiene and Microbiology

University of Wuerzburg

Josef-Schneider-Str. 2 / E1

97080 Wuerzburg

Tel.: ++49-(0)931-201 46901

Fax: ++49-(0)931-201 46445

email: gvalenza@hygiene.uni-wuerzburg.de

Abstract

The VITEK 2 AST-N111 card was evaluated for detection of extended-spectrum beta-lactamases (ESBLs) was evaluated by testing 51 ESBL positive and 50 ESBL negative isolates of *E. coli*, *K. pneumoniae*, and *K. oxytoca*. The occurrence of beta-lactamase genes was confirmed by PCR and sequencing. The advanced expert system (AES) of the VITEK 2 system achieved sensitivity and specificity values of 100% and 96.0%, respectively. The ESBL test of the VITEK 2 AST-N111 card showed a sensitivity of 92.1% and a specificity of 90.0%. Contradictory results obtained with the two VITEK 2 tools could be clarified by combination disk tests in nine of 11 isolates. The combined use of AES and ESBL test of the AST-N111 card in association with combination disk tests in case of contradictory results seems to be a reliable method for ESBL detection.

Extended-spectrum beta-lactamase (ESBL)-production is the main cause of 3rd generation cephalosporin resistance in nosocomial, Gram-negative pathogens. Outbreaks caused by ESBL-producing *Enterobacteriaceae* have been reported worldwide [1]. Therefore, a rapid and reliable ESBL detection is indispensable for an appropriate antibiotic treatment and infection control.

Several automated systems for phenotypic ESBL detection have been developed and evaluated [2-5]. The VITEK 2 system (bioMérieux, Marcy l'Etoile, France) provides two different ESBL identification tools. The first one includes analysis and interpretation of minimal inhibitory concentration (MIC) of several beta-lactams using a specific software, the advanced expert system (AES). The second one (ESBL test) is based on simultaneous detection and assessment of antibacterial activity of cefepime, cefotaxime and ceftazidime, alone and in combination with clavulanic acid [6].

The new antimicrobial susceptibility testing card AST-N111 (bioMérieux) has been recently introduced for testing aerobic Gram-negative bacilli on VITEK 2. Using this card ESBL production can be detected by both AES and ESBL test.

Objective of this study was to evaluate the suitability of the VITEK 2 AST-N111 card for detection of ESBLs in *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*.

Therefore, a total of 101 non duplicate isolates of *E. coli* (n=56), *K. pneumoniae* (n=34), and *K. oxytoca* (n=11) were investigated.

Eighty of all isolates were previously collected from clinical samples at the Institute of Hygiene and Microbiology of the University of Wuerzburg. Furthermore, we included 21 control isolates producing different beta-lactamases [7-9] (table 1).

All isolates included in this study were investigated for ESBL production by using the VITEK 2 AST-N111 card. Both AES interpretation and ESBL test results were recorded. Furthermore, ESBL production was tested by cefotaxime and ceftazidime ESBL Etests

(AB Biodisk, Solna, Sweden) and combination disk diffusion tests involving cefotaxime (30 µg), ceftazidime (30 µg), and cefpodoxime (30 µg), with and without the β-lactamase inhibitor clavulanate (10 µg) (MAST Diagnostica, Reinfeld, Germany). ESBL and further beta-lactamase genes harbouring the 101 test isolates were identified by molecular methods (PCR and sequencing) as previously described [7].

Thirty-three of the 80 clinical isolates from Wuerzburg harboured ESBL genes of type CTX-M-1, CTX-M-9, CTX-M-14, CTX-M-15, and SHV-12. The remaining 47 clinical isolates were ESBL negative. However, in 22 of them penicillinase genes of OXY-, SHV-, and TEM-type were identified.

Among the 21 control isolates eight harboured only ESBL genes, four were characterized by ESBLs and penicillinases, six contained a combination of ESBLs and other beta-lactamases such as plasmidic AmpC (CMY-4, CMY-6) and **carbapenemases (OXA-48, KPC-2, metallo-beta-lactamase type VIM-1)**. The remaining three isolates produced only metallo-beta-lactamases (VIM-1) or AmpC (DHA-1).

The ESBL detection results of the different tools adopted in this study are shown in table 1 and 2. The best performance could be achieved by the AES of the VITEK 2 system and the combination disk method (sensitivity values: 100% and 94.1%, respectively; specificity values: 96.0% and 100%, respectively). Furthermore, the ESBL test of the VITEK 2 AST-N111 card and the ESBL tests showed sensitivities of 92.1% and 90.1% and specificities of 90.0% and 92.2%, respectively. The performance of the ESBL test of the VITEK 2 AST-N111 card is in line with previous reports evaluating ESBL tests of other VITEK 2 cards [2, 4, 5, 10].

The five false-positive results obtained by ESBL test were due to three OXY-1 producing *K. oxytoca*, one SHV-1 producing *K. pneumoniae*, and one TEM-1 producing *E. coli*. All five isolates were correctly identified as ESBL negative by VITEK 2 AES and the

combination disk tests. However, several ESBL Etests failed to correctly identify the three OXY-1 producing *K. oxytoca* and the SHV-1 producing *K. pneumoniae* as ESBL-negative (Table 1). Problems with discrimination of these resistance mechanisms have already been described and are probably associated with hyperexpression of the corresponding genes [11, 12].

Regarding the 21 control isolates, four ESBL positive isolates which co-expressed CMY-4, KPC-2 or VIM-1 (n=2) were falsely detected as ESBL negative by the ESBL test of the VITEK 2 AST-N111 card and by ESBL Etests. In addition, the combination disk tests failed to correctly identify ESBL-production in the two isolates which co-expressed MBLs.

Moreover, a further isolate which co-expressed CTX-M-15 and OXA-48 was falsely identified as ESBL negative by the ESBL test of the VITEK 2 AST-N111 card and by ESBL Etests. This finding could be associated with a porin loss as OXA-48 should not be able to mask an ESBL. All above mentioned isolates were correctly identified by the VITEK 2 AES. However, the VITEK 2 AES identified two isolates producing VIM-1 and DHA-1 as ESBL positive. The missing carbapenemase warning for several VIM-1 or OXA-48 containing isolates is probably due to variable carbapenemase levels resulting only in increased (not resistant) MIC values to carbapenems. This is alarming as the co-expression of ESBL and AmpC or carbapenemases such as MBL, KPC, and OXA-48 is increasing in *Enterobacteriaceae* isolates worldwide [8, 13, 14].

Interestingly, both AES and AST-N111 card could correctly identify 44 isolates as ESBL positive and 46 isolates as ESBL negative. Contradictory results obtained with the two VITEK 2 tools could be clarified by ESBL combination disk tests in nine of the remaining 11 isolates. In contrast, only in one case contradictory results of the AES and ESBL test could be resolved by ESBL Etests.

1 Therefore, the combined use of AES and ESBL test of the VITEK 2 AST-N111 card in
2 association with the combination disk tests in case of contradictory results seems to be a
3 reliable method for detection of ESBLs.

4 **Acknowledgements**

6 Neil Woodford, and Jesus Mingorance are acknowledged for donation of the control
7 strains. We are grateful to Gabi Heinze and Simone Pusch for expert technical assistance.

References

1. Canton R, Novais A, Valverde A, et al (2008) Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. *Clin Microbiol Infect* 14 Suppl 1:144-53
2. Spanu T, Sanguinetti M, Tumbarello M, et al. (2006) Evaluation of the new VITEK 2 extended-spectrum beta-lactamase (ESBL) test for rapid detection of ESBL production in Enterobacteriaceae isolates. *J Clin Microbiol* 44:3257-62
3. Wiegand I, Geiss HK, Mack D, Sturenburg E and Seifert H (2007) Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. *J Clin Microbiol* 45:1167-74
4. Thomson KS, Cornish NE, Hong SG, Hemrick K, Herdt C and Moland ES (2007) Comparison of Phoenix and VITEK 2 extended-spectrum-beta-lactamase detection tests for analysis of *Escherichia coli* and *Klebsiella* isolates with well-characterized beta-lactamases. *J Clin Microbiol* 45:2380-4
5. Farber J, Moder KA, Layer F, Tammer I, Konig W and Konig B (2008) Extended-spectrum Beta-lactamase detection with different panels for automated susceptibility testing and with a chromogenic medium. *J Clin Microbiol* 46:3721-7
6. Drieux L, Brossier F, Sougakoff W and Jarlier V (2008) Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect* 14 Suppl 1:90-103
7. Grobner S, Linke D, Schutz W, et al. (2009) Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. *J Med Microbiol* 58:912-22
8. Cendejas E, Gomez-Gil R, Gomez-Sanchez P and Mingorance J (2010) Detection and characterization of Enterobacteriaceae producing metallo-beta-lactamases in a tertiary-care hospital in Spain. *Clin Microbiol Infect*;16:181-3
9. Woodford N, Fagan EJ and Ellington MJ (2006) Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. *J Antimicrob Chemother* 57:154-5
10. Robin F, Delmas J, Schweitzer C and Bonnet R (2008) Evaluation of the Vitek-2 extended-spectrum beta-lactamase test against non-duplicate strains of Enterobacteriaceae producing a broad diversity of well-characterised beta-lactamases. *Clin Microbiol Infect* 14:148-54
11. Rice LB, Carias LL, Hujer AM, et al (2000) High-level expression of chromosomally encoded SHV-1 beta-lactamase and an outer membrane protein change confer resistance to ceftazidime and piperacillin-tazobactam in a clinical isolate of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* ;44:362-7

- 1 12. Potz NA, Colman M, Warner M, Reynolds R and Livermore DM (2004) False-positive
2 extended-spectrum beta-lactamase tests for *Klebsiella oxytoca* strains hyperproducing K1
3 beta-lactamase. *J Antimicrob Chemother* 53:545-7
4
- 5 13. Gulmez D, Woodford N, Palepou MF, et al (2008) Carbapenem-resistant *Escherichia*
6 *coli* and *Klebsiella pneumoniae* isolates from Turkey with OXA-48-like carbapenemases
7 and outer membrane protein loss. *Int J Antimicrob Agents* 31:523-6
8
- 9 14. Pournaras S, Poulou A, Voulgari E, Vrioni G, Kristo I and Tsakris A (2010) Detection
10 of the new metallo-beta-lactamase VIM-19 along with KPC-2, CMY-2 and CTX-M-15 in
11 *Klebsiella pneumoniae*. *J Antimicrob Chemother* 65:1604-7
12
13

Table 1. Identification of different resistance phenotypes by the advanced expert system (AES) and the ESBL test of the VITEK 2 AST-N111 card compared to the ESBL Etests and ESBL combination disk tests for 101 *Enterobacteriaceae* isolates

Beta-lactamase genes	n	VITEK 2 AES interpretation	No. of ESBL positive results		
			VITEK 2 ESBL-test	ESBL Etest ^a	ESBL combination disk test ^b
Clinical isolates (Wuerzburg)					
<i>E. coli</i>					
CTX-M-1 (ESBL)	1	ESBL	1	1	1
CTX-M-1 (ESBL) +TEM-1	1	ESBL	1	1	1
CTX-M-9 (ESBL)	1	ESBL	1	1	1
CTX-M-14 (ESBL) + TEM-1	5	ESBL	5	5	5
CTX-M-15 (ESBL)	12	ESBL	12	12	12
CTX-M-15 (ESBL) + TEM-1	5	ESBL	5	5	5
SHV-12 (ESBL)	1	ESBL	1	1	1
TEM-1	8	penicillinase	1	0	0
TEM-40	1	penicillinase	0	0	0
no gene	15	wild type	0	0	0
<i>K. pneumoniae</i>					
CTX-M-15 (ESBL) + TEM-1	4	ESBL	4	4	4
CTX-M-15 (ESBL) + TEM-1 + SHV-1	2	ESBL	2	2	2
SHV-1	4	wild type/penicillinase	0	0	0
	1	SHV-1 hyperproduction	1	1	0
TEM-1	1	penicillinase	0	0	0
no gene	8	wild type	0	0	0
<i>K. oxytoca</i>					
OXY-1	1	wild type/penicillinase	0	0	0
	3	high level penicillinase	3	3	0
OXY-2	3	wild type/penicillinase	0	0	0
no gene	2	wild type	0	0	0
Control isolates					
<i>E. coli</i>					
CTX-M-2 (ESBL)	1	ESBL	1	1	1
CTX-M-9 (ESBL)	1	ESBL	1	1	1
CTX-M-15 (ESBL)	1	ESBL	1	1	1
CTX-M-15 (ESBL) + CMY-4	1	ESBL + carbapenemase	0	0	1
TEM-3 (ESBL)	1	ESBL	1	1	1
VIM-1 + TEM-1	1	penicillinase + cephalosporinase	0	0	0

K. pneumoniae

CTX-M-2 (ESBL) + TEM-1 + VIM-1	2	ESBL + carbapenemase	0	0	0
CTX-M-15 (ESBL) + OXA-48	1	ESBL	1	0	0
SHV-4 (ESBL)	1	ESBL	1	1	1
SHV-5 (ESBL)	2	ESBL	2	2	2
SHV-5 (ESBL) + TEM-1	1	ESBL	1	1	1
SHV-12 (ESBL) + TEM-1	1	ESBL	1	1	1
SHV-12 (ESBL) + CMY-6	1	ESBL	1	0	1
SHV-12 (ESBL) + SHV-11 + KPC-2	1	ESBL + carbapenemase	0	0	1
SHV-18 (ESBL)	1	ESBL	1	1	1
TEM-52 (ESBL) + SHV-76	1	ESBL	1	1	1
DHA-1	1	ESBL + carbapenemase	0	0	0

K. oxytoca

SHV-5 (ESBL) + TEM-1	1	ESBL	1	1	1
VIM-1	1	ESBL	0	0	0

^aESBL Etest positive if a ≥ 3 twofold concentration decrease in a MIC for either antimicrobial agent tested in combination with clavulanic acid vs its MIC when tested alone occurs

^bESBL production confirmed if a ≥ 5 mm increase in a zone diameter for one antimicrobial agent tested in combination with clavulanic acid vs its zone when tested alone occurs

Table 2. Sensitivities (%) and specificities (%) of different phenotypic methods for detection of ESBL production using molecular identification as reference method for all isolates

	<i>E. coli</i>		<i>K. pneumoniae</i>		<i>K. oxytoca</i>		all	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
VITEK 2 AES	100	100	100	93.3	100	90.0	100	96.0
VITEK 2 ESBL-test	96.7	96.0	84.2	93.3	100	70.0	92.1	90.0
ESBL Etest	96.7	100	73.7	93.3	100	70.0	90.1	92.0
ESBL combination disk test	100	100	84.2	100	100	100	94.1	100