

# The Importance of Extended-Spectrum $\beta$ -Lactamase (ESBL) Screening Tests in Rapid Automated Antimicrobial Susceptibility Testing (AST)

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## ABSTRACT (revised)

**BACKGROUND:** A known weakness of all AST systems, in particular rapid AST systems is their inability to detect some resistance mechanisms without special adjustment of nutrient medium formulations or inclusion of screening tests (i.e., Methicillin resistant *Staphylococcus aureus* [MRSA], or ESBL).

**METHODS:** In this study we report on an evaluation of 3 rapid AST systems, VITEK, VITEK2 (bioMérieux, Lyon, France), and Phoenix™ (BD Diagnostic Systems) to detect ESBL. The NCCLS reference broth micro-dilution method including the ESBL “confirmatory” test was used as reference. Organisms tested included 55 challenge strains of *E. coli*, *K. pneumoniae* and *K. oxytoca* which contained an accessory beta-lactamase (BL) for which most had been previously characterized at molecular level (50/55 strains). The ESBL positive strains included 16 different molecular classes of ESBL, including 15 strains with TEM (>3), and 9 SHV (>1). Also included were 3 strains of *K. oxytoca* that hyper-produced chromosomal K1 BL. The ESBL negative strains contained accessory BL including 3 TEM (<3), 2 SHV(1), 3 IRT, 2 PSE, 2 OXA, 2 AmpC isolates and 2 porin (OMP) deficient strains.

**RESULTS:** The sensitivity of the ESBL screening tests were 93.1%, 79.3%, and 98.3% for VITEK, VITEK2 and Phoenix, respectively. The specificity was 95.8% for all three systems. The very major errors (VME) to antibiotics that would have been corrected if the ESBL result was correct for the strains was 4 for VITEK, 8 for VITEK2, and 0 for Phoenix. Had there been no ESBL screen in the system panels, there would have been the following additional VME: 39 for VITEK, 20 for VITEK2, and 21 for Phoenix.

**CONCLUSIONS:** The results highlight the importance of an accurate ESBL test for correct interpretation of rapid automated AST test systems.

## INTRODUCTION

The increasing emergence of clinical isolates of the family *Enterobacteriaceae* with ESBL phenotypes remains a major reason for antimicrobial resistance problems, especially in intensive care units (1). Therefore reliable detection of ESBL production is a difficult task facing microbiologists (3). A known weakness of all AST systems, in particular rapid systems, is their inability to detect some resistance mechanisms without special adjustment of nutrient medium formulations or inclusion of screening tests (4). In this study the ability of three automated rapid AST systems to detect the ESBL phenotypes were evaluated [Phoenix System (BD Diagnostic Systems, Maryland, USA) VITEK and VITEK2 (bioMérieux, Lyon, France)].

## MATERIALS AND METHODS

**BACTERIAL ISOLATES.** This study was conducted at two sites: at the University of Muenster the Phoenix system (PHXMü) was compared to the VITEK 2 instrument, and in the Laboratory Group Heidelberg where the Phoenix (PHXHD) was compared to the Vitek system. Fifty five challenge strains from the Centers for Disease Control (CDC), French National Reference Center (SFM) and BD internal collection of *Escherichia coli*, *Klebsiella pneumoniae* and *K. oxytoca* which contained an accessory beta-lactamase were tested. Fifty strains had been characterized at a molecular level: the ESBL positive strains included 16 different molecular classes; also ESBL negative strains were included, containing 7 different beta-lactamases characterized at a molecular level. (Table 1).

**ANTIMICROBIALS.** The following  $\beta$ -lactam antibiotics were shared in both the Phoenix panels and the VITEK cards: ampicillin, ampicillin/sulbactam, mezlocillin, piperacillin, piperacillin/tazobactam, cefazolin, cefuroxime, cefotaxime, ceftazidime.

**ESBL DETECTION.** The Phoenix and the VITEK used a dedicated ESBL test which utilized various cephalosporins with and without clavulanic acid. The VITEK 2 used its Advanced Expert System (AES) to predict the ESBL phenotype by interpretative reading of the MIC patterns.

**REFERENCE SYSTEM.** The NCCLS reference broth microdilution ESBL confirmatory test was used as a reference method (3).

Table 1. Results of ESBL Screening Tests.

Strain No.	Strain	Phenotype	Expected	ESBL detection			
				Phoenix Mu	Phoenix HD	VITEK	VITEK2
AMGKY1	<i>E. coli</i>		-	-	-	-	-
AMGLY4	<i>E. coli</i>		-	-	-	-	-
C2008	<i>E. coli</i>		-	-	-	-	-
C2174	<i>K. pneumoniae</i>		-	-	-	-	-
C2176	<i>K. pneumoniae</i>		-	-	-	-	-
FR112	<i>E. coli</i>	AmpC	-	-	-	-	-
11154A	<i>E. coli</i>	AmpC_high	-	-	-	-	-
FR114	<i>E. coli</i>	ANT(2'), OXA_3	-	-	-	-	-
FR93	<i>K. oxytoca</i>	Bla	-	-	-	-	-
FR148	<i>E. coli</i>	d_OmpC, d_OmpF, R_FOX, R	-	-	+	-	-
FR141	<i>E. coli</i>	IRT 2, TEM_30	-	-	-	-	-
FR142	<i>E. coli</i>	IRT 5, TEM_33	-	-	-	-	-
FR143	<i>E. coli</i>	IRT 7, TEM_36	-	-	-	-	-
10909	<i>K. oxytoca</i>	K1_high	-	-	-	-	-
BLAC8	<i>E. coli</i>	OXA_1	-	-	+	-	+
BLAC9	<i>E. coli</i>	OXA_2	-	-	-	-	-
BLAC12	<i>E. coli</i>	PSE_1	-	-	-	-	-
BLAC10	<i>E. coli</i>	PSE_2	-	-	-	+	-
11046	<i>E. coli</i>	R_FOX	-	-	-	-	-
BLAC6	<i>E. coli</i>	SHV_1	-	-	-	-	-
FR103	<i>K. pneumoniae</i>	SHV_1, TEM_1	-	-	-	-	-
BLAC2	<i>E. coli</i>	TEM_1	-	-	-	-	-
C2103	<i>E. coli</i>	TEM_1	-	-	-	-	-
BLAC3	<i>E. coli</i>	TEM_2	-	-	-	-	-
C2263	<i>K. oxytoca</i>		+	+	+	+	-
11060	<i>K. pneumoniae</i>		+	+	+	+	+
11063	<i>K. pneumoniae</i>		+	+	+	+	+
FR90	<i>E. coli</i>	TEM_3	+	+	+	+	+
C2249	<i>K. pneumoniae</i>	OSBL, SHV_2, TEM_1_	+	+	+	+	+
11156	<i>K. oxytoca</i>	K1_high	equivocal*	+	+	-	-
11161	<i>K. oxytoca</i>	K1_high	equivocal*	+	+	+	-
11005	<i>K. pneumoniae</i>	SHV_2	+	+	+	+	+
11019	<i>K. pneumoniae</i>	SHV_3	+	+	+	+	+
11002	<i>E. coli</i>	SHV_4	+	+	+	+	+
11001	<i>K. pneumoniae</i>	SHV_4	+	+	+	+	+
11010	<i>K. pneumoniae</i>	SHV_4	+	+	+	+	+
11009	<i>E. coli</i>	SHV_5	+	+	+	+	-
11013	<i>E. coli</i>	SHV_5	+	+	+	+	-
11003	<i>K. pneumoniae</i>	SHV_5	+	+	+	+	+
FR146	<i>E. coli</i>	SHV_6	+	+	+	-	-
11007	<i>E. coli</i>	TEM_10	+	+	+	+	+
11012	<i>K. pneumoniae</i>	TEM_11	+	+	+	+	+
11015	<i>E. coli</i>	TEM_12	+	+	+	+	+
11008	<i>K. pneumoniae</i>	TEM_24	+	+	+	+	+
11020	<i>E. coli</i>	TEM_25	+	+	+	+	-
11006	<i>K. pneumoniae</i>	TEM_26	+	+	+	-	+
BLAC4	<i>E. coli</i>	TEM_3	+	-	+	+	+
11000	<i>K. pneumoniae</i>	TEM_3	+	+	+	+	+
FR137	<i>E. coli</i>	TEM_5	+	+	+	+	+
10999	<i>K. pneumoniae</i>	TEM_5	+	+	+	+	+
FR138	<i>E. coli</i>	TEM_6	+	+	+	+	+
11016	<i>E. coli</i>	TEM_8	+	+	+	+	+
FR139	<i>E. coli</i>	TEM_8	+	+	+	+	+
11011	<i>K. pneumoniae</i>	TEM_9	+	+	+	+	+
FR91	<i>K. oxytoca</i>	TLS	+	+	+	+	-

\*equivocal; strains were positive using NCCLS confirmatory test, however, the hyper K1 phenotype is not considered a classical ESBL.

## RESULTS AND DISCUSSION

The single test results of all instruments evaluated are shown in Table 1. The sensitivities of the ESBL screening tests were 93.1%, 79.3% and 96.9% for VITEK, VITEK 2, and Phoenix (study sites combined), respectively, the results of the specificity were 95.8% for each instrument (Table 2). The Phoenix system and the VITEK instrument showed good results. Whereas the AES of the VITEK 2 instrument failed to detect some ESBL mechanisms, although other authors were able to detect very seldom encountered beta-lactamase types (3). BioMérieux anticipates a special detection screen for ESBLs in the VITEK 2 in the near future. Non-detection of the ESBL phenotypes resulted in the following very major errors (VME): 4 for VITEK, 8 for VITEK 2 and 0 for Phoenix (Table 3). Without the ESBL screen detection tests or detection algorithms there would have been many additional VME's with regard to beta-lactam antibiotics, including 39 for VITEK, 20 for VITEK 2, 21 for PHXMü and 15 for PHXHD.

Table 2. Performance of the VITEK, VITEK2 and Phoenix System to Detect ESBLs in Comparison to NCCLS

Parameter	Result				
	Phoenix MÜ	Phoenix HD	Phoenix MÜ+HD	VITEK	VITEK2
ESBL strains correct	28	29	57	27	23
Non-ESBL strains correct	24	22	46	23	23
False negative results	1	0	1	2	6
False positive results	0	2	2	1	1
Sensitivity	96.6%	100.0%	98.3%	93.1%	79.3%
Specificity	100.0%	91.7%	95.8%	95.8%	95.8%

Reference Method  
(ESBL pos n=29, ESBL neg n=24)

Table 3. Drug VME Resulting from Non-Detection of ESBL in One or More Systems

Lab No.	Strain	Phenotype	Expected	PHOENIX Mu+HD		VITEK		VITEK2	
				Measured <sup>1</sup>	BDXpert <sup>2</sup>	Measured <sup>1</sup>	Expert <sup>2</sup>	Measured <sup>1</sup>	AES <sup>2</sup>
FR146	<i>E. coli</i>	SHV_6 Cefazolin	R	R	–	S	–	NR <sup>3</sup>	–
FR146	<i>E. coli</i>	SHV_6 Cefuroxime	R	R	–	S	–	S	–
FR146	<i>E. coli</i>	SHV_6 Piperacillin	R	R	–	S	–	S	–
11006	<i>K. pneumoniae</i>	TEM_26 Cefotaxime	R	S	R	S	–	S	1
11020	<i>E. coli</i>	TEM_25 Ceftazidime	R	S	R	S	R	S	–
11156	<i>K. oxytoca</i>	K1_high Cefotaxime	R	S	R	S	R	S	–
11161	<i>K. oxytoca</i>	K1_high Cefotaxime	R	1	R	1	R	S	–
FR146	<i>E. coli</i>	SHV_6 Cefotaxime	R	S	R	S	R	S	–
FR146	<i>E. coli</i>	SHV_6 Ceftazidime	R	S	R	S	R	S	–
FR91	<i>K. oxytoca</i>	TLS Cefotaxime	R	S	R	S	R	S	–
<b>Total VME</b>					0		4		8

<sup>1</sup> measured; interpretation based on the MIC determined by the instrument

<sup>2</sup> BDXpert, Expert, AES; interpretative result following modification based on each system's ESBL algorithm

<sup>3</sup> NR; no result

### CONCLUSIONS

- The importance of performing accurate ESBL screening tests in rapid automated systems is highlighted in this evaluation.
- Failure to accurately detect ESBL phenotypes can result in multiple VMEs in each antibiogram. Thus, errors in the ESBL affects the VME rate in an amplified way.
- All systems were equivalent for specificity, the Phoenix system demonstrated higher sensitivity.

### REFERENCES

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