

Comparison of Phoenix and Vitek 2 ESBL Confirmatory Tests Against *E. coli* and *Klebsiella* Isolates with Well-characterized β -lactamases

Kenneth S. Thomson, Ph.D. and Ellen Smith Moland, B.S.M.T. (ASCP)

¹Center for Research in Antiinfectives and Biotechnology ("CRAB"), Department of Medical Microbiology and Immunology
Creighton University School of Medicine • Omaha, Nebraska

INTRODUCTION

The study was designed to compare the abilities of the Phoenix and Vitek 2 ESBL confirmatory tests to discriminate between ESBL-positive and negative strains of *E. coli*, *K. pneumoniae*, and *K. oxytoca* with both instruments equipped with software that is currently available in the United States.

The strains were chosen to identify strengths and weaknesses of the instruments. They were not routine clinical isolates and included strains for which CLSI tests are unreliable. All were previously characterized by appropriate biochemical, molecular and phenotypic procedures to determine their types of β -lactamase production. They produced a wide variety of ESBLs, clavulanate-susceptible non-ESBLs, plasmid-mediated AmpC β -lactamases, chromosomal AmpC β -lactamases, and class A carbapenemases. Many produced multiple β -lactamases.

Testing was done at two Omaha sites with the test inoculum for each isolate prepared on the same day from the same plate culture. The Phoenix testing was done at Creighton University and the Vitek 2 testing at Methodist Hospital. Copies of all reports issued by the instruments have been previously provided to BD.

METHODOLOGY

The strains comprised 76 ESBL producers and 26 non-ESBL producers, including clinical isolates of all three species and laboratory strains of *E. coli*. At least 38 distinct ESBLs, possibly more, were produced either alone or in combination with other β -lactamases, especially other β -lactamases known to obscure phenotypic detection of ESBLs (e.g. AmpC and KPC β -lactamases). Some isolates produced multiple ESBLs (up to 3) and some isolates produced up to five different β -lactamases. To our knowledge, the study included the widest range of ESBLs ever used in an evaluation of an automated susceptibility testing instrument.

The distinct (i.e. definitively identified) ESBLs were:
TEM-3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 16, 24, 26, 28, 43, 47, 50, 52, 61
SHV-2, 3, 4, 5, 6, 7, 9, 10, 12

CTX-M-9, 14, 16, 17, 18, 19, 44 (Toho-1), 45 (Toho-2), Toho-3, PER-1
There may have been additional ESBLs. Some were not sequenced, but only identified by biochemical and PCR tests and then designated with the affix "like" to indicate the type of ESBL that they most closely resembled. For example an enzyme designated SHV-2 like was confirmed as an ESBL with an isoelectric point of 7.6 and was SHV PCR +ve - attributes that are also shared by SHV-6, 7, 8, 13, 16, 19, 20, 21, 26, and 38.

The non-ESBLs that produced in the absence of an ESBL were: TEM-1, 2, SHV-1, LEN-1, K1, OXA-3, 4, 5, 6, KPC-2, and five AmpC β -lactamases (CMY, DHA-1, MIR-1, MOX-1, and the *E. coli* chromosomal AmpC). Strains producing these enzymes included hyperproducers of these enzymes and strains that produced multiple non-ESBLs. Porin mutants were also included. Some additional non-ESBLs not listed above were also produced by the ESBL-positive isolates.

ESBL confirmatory tests and expert systems currently available in the U.S. were used. The Phoenix card NMIC/ID-108 was run with software version 5.02H/V4.11B. The Vitek 2 card AST-GN13 was run with software version WSVT2-R04.01. In addition, two normally inactive Phoenix expert rules intended to enhance ESBL detection, based on antibiogram (rules 325 and 1437), were activated.

Testing occurred at two sites with the test inoculum for each strain prepared from the same plate culture on the same day. The Phoenix testing was done at Creighton University and the Vitek 2 testing at Methodist Hospital, Omaha, NE. Repeated tests were performed for the following reasons:

- to determine if errors were reproducible,
- if there was insufficient growth or the test was not finalized,
- if repeat testing was suggested by the expert system,
- if there was a discrepancy between the ESBL confirmatory test and the expert system, and
- because ESBLs may have been lost from two strains prior to testing with the Vitek 2.

Although it was proposed that identifications would be manually entered at the time of testing, the Phoenix provided identifications for each isolate (two of which needed to be corrected).

RESULTS

The results were analyzed as 1) initial results and 2) results after repeat testing. The initial results represent the routine laboratory situation. Repeat tests were performed on 28 of the 102 strains. The majority of repeated tests were performed for reasons relevant only to the study and not applicable to the clinical laboratory situation.

Initial Results: 76 ESBL-producing Strains

As shown in Table 1, the Phoenix ESBL confirmatory test and currently available expert system detected ESBLs in 96% of the ESBL-producing strains. Activation of the normally inactive expert rules 325 and 1437 increased ESBL detection to 99%. The Vitek 2 ESBL confirmatory test detected 91% of the ESBL-producing strains and its expert system detected 89%. The slightly lower expert system detection rate for Vitek 2 occurred because one *E. coli* strain that produced TEM-26 was not recognized as an ESBL producer by the expert system even though the ESBL confirmatory test was positive. This error was corrected on repeat testing.

Table 1. Results with 76 ESBL-producing Strains

No. Correct on Initial Test (%)		
	ESBL Test	Expert System
Phoenix	73 (96)	73 (96)
Phoenix*	73 (96)	75 (99)
Vitek 2	69 (91)	68 (89)
No. Correct on Repeat Test (%)		
Phoenix	74 (97)	73 (96)
Phoenix*	74 (97)	75 (99)
Vitek 2	70 (92)	70 (92)

Phoenix*: Results after activation of two normally inactive Phoenix expert rules (rules 325 and 1437) intended to enhance ESBL detection, based on antibiogram.

Initial Results: 26 ESBL-Negative Strains

As shown in Table 2, the Phoenix ESBL confirmatory test and expert system using currently available rules yielded correct results (i.e. ESBL negative) for 81% of the ESBL negative strains (i.e. false positive rate of 19%). Activation of expert rules 325 and 1437 reduced accuracy to 46% (false positive rate of 54%). The Vitek 2 ESBL confirmatory test and expert system were correct for 85% of ESBL negative strains (false positive rate of 15%).

Table 2. Results with 26 ESBL-negative Strains

No. Correct on Initial Test (%)		
	ESBL Test	Expert System
Phoenix	21 (81)	21 (81)
Phoenix*	21 (81)	12 (46)
Vitek 2	22 (85)	22 (85)
No. Correct on Repeat Test (%)		
Phoenix	20 (77)	19 (73)
Phoenix*	20 (77)	11 (38)
Vitek 2	23 (88)	22 (85)

Phoenix*: Results after activation of two normally inactive Phoenix expert rules (rules 325 and 1437) intended to enhance ESBL detection, based on antibiogram.

Confirmatory Tests: Comments and Details

Considering the challenging nature of the strains, both ESBL confirmatory tests were very sensitive. The relatively high percentages of false positive tests with the 26 ESBL-negative strains reflected the challenging nature of these strains and also the high mathematical impact of an incorrect result when testing a relatively small number of strains. Nonetheless, it should be possible for the Phoenix expert system to override four of the five false positive ESBL confirmatory tests that occurred in this study. If this was done, the false positive rate would have been reduced from 19% to only 3.8%. This issue is addressed below – see Expert Systems Comments and Details 4d).

1. ESBLs not detected by the Phoenix confirmatory test

- TEM-28 in laboratory strain of *E. coli* (Misc 358)
- SHV-4 like enzyme in *K. pneumoniae* that also produced SHV-1, a pI 5.6 enzyme, and the plasmid-mediated AmpC, FOX-5 (CoudM621)
- SHV-3 like enzyme in *K. oxytoca* (01VCH72) that also produced K1, TEM-1, and a plasmid-mediated DHA-1 like AmpC

2. ESBLs not detected by the Vitek 2 confirmatory test

- SHV-2 like enzyme in *E. coli* that also produced TEM-1 and the class A carbapenemase, KPC-3 (*E. coli* 233)
- SHV-4 like enzyme in *K. pneumoniae* that also produced SHV-1, a pI 5.6 enzyme, and the plasmid-mediated AmpC, FOX-5 (CoudM621)
- SHV-5 in a laboratory strain of *E. coli* (Misc 289)
- SHV-5 like enzyme in *K. pneumoniae* that also produced TEM-1, a pI 5.6 enzyme, and a FOX like plasmid-mediated AmpC (01VCH55)
- SHV-5 like enzyme in *K. pneumoniae* that also produced SHV-1, TEM-1, a pI 5.25 enzyme, and the class A carbapenemase, KPC-2 (01BH79)
- TEM-28 in a laboratory strain of *E. coli* (Misc 358)
- CTX-M-44 (Toho-1) in *E. coli* (Misc 382)

3. False positive results with the Phoenix confirmatory test

- High level chromosomal AmpC - one *E. coli* (strain 01WM15)
- High level K1 β -lactamase - two *K. oxytoca* (strains 01BH59, 01EUHI62)
- High level SHV-1 - one *K. pneumoniae* (strain GB91)
- Production of the KPC-2 carbapenemase - one *K. pneumoniae* (strain UMM3)

4. False positive results with the Vitek 2 confirmatory test

- Production of the KPC-2 carbapenemase - one *K. pneumoniae* (strain UMM3)
- High level K1 β -lactamase - one *K. oxytoca* (strain 01BH59)
- High SHV-1 - one *K. pneumoniae* (strain GB91), one *E. coli* (strain 01LSAI20)

5. Excellent results with the Phoenix confirmatory test

- Detection of the complex mutant TEM ESBL, TEM-50, in strain Misc 372. This enzyme is not strongly inhibited by clavulanate and therefore is less readily detected by clavulanate-based ESBL tests. (The Vitek 2 also detected this enzyme).
- Detection of an SHV-5 like ESBL in *K. pneumoniae* 01VCH55 that produced four β -lactamases including a FOX-like AmpC. The Vitek 2 could not cope with this isolate, initially suggesting retesting due to irresolvable correction possibilities and on retesting incorrectly suggested carbapenem resistance.

- c. Detection of an SHV-5 like ESBL in *K. pneumoniae* 01BH79 that produced five β -lactamases including KPC-2, and commenting on the unusual carbapenem resistance. The Vitek 2 did not detect the ESBL (but did comment on the unusual carbapenem resistance).
- d. Detection of CTX-M ESBLs.

Expert Systems: Comments and Details

Ideally, an expert system should provide important interpretations and recommendations that do not require additional input by laboratory personnel. Inconclusive or incorrect comments can cause confusion and may delay or prevent the issue of an accurate and useful report to the clinician. The following summarizes the expert system findings in this study.

1. The **currently available Phoenix expert system** recognized unusually elevated carbapenem MIC in tests with two of three *K. pneumoniae* strains producing the KPC-2 class A carbapenemase and one *E. coli* strain producing the KPC-3 carbapenemase. In recognizing three of four such strains, the Phoenix expert system may have an advantage over the Vitek 2 expert system, which recognized reduced carbapenem susceptibility in two of the four strains.
2. Suboptimal attributes of currently available Phoenix expert system:
 - a. For ESBL-producing strains, it changed all susceptible or intermediate amoxicillin/clavulanate test results to resistant. This conflicts with CLSI ESBL recommendations. Although it may be clinically prudent to do this, the reference cited as justifying this rule does not substantiate it.
 - b. Yielded two fewer correct results on repeat testing.
 - c. Did not specifically suggest the possibility of a carbapenemase when an elevated carbapenem MIC was detected. This is an important need. Many United States laboratories are currently floundering because their diagnostic tests are inadequate and isolates producing KPC carbapenemases are spreading through their patient populations. Thousands of patients have been infected or colonized with these isolates in New York City.
 - d. For one *K. oxytoca* that hyperproduced the K1 β -lactamase, it changed a susceptible result for cefotaxime to resistant (strain Kleb 67). This conflicts with CLSI recommendations.
 - e. Did not correct a false positive ESBL test due to production of KPC-2 carbapenemase (in absence of an ESBL) in *K. pneumoniae* strain UMM3.
 - f. Did not convert false positive ESBL tests to negative for high level production of (i) the K1 β -lactamase in *K. oxytoca* (strains 01BH59, 01EUHI62), (ii) SHV-1 in *K. pneumoniae* (strain GB91), (iii) the chromosomally-encoded AmpC β -lactamase of *E. coli* (strain 01WM15) and (iv) for production of KPC-2 in *K. pneumoniae* (strain UMM3). Comments and some possible strategies for correcting these errors:
 - i. High K1 production in *K. oxytoca* can be distinguished from ESBL production because the ceftriaxone MIC is at least three dilutions higher than the cefotaxime MIC whereas ESBL production is associated with similar MICs of these drugs (usually differing by no more than one dilution). In addition, the ceftazidime MIC is usually elevated by ESBL production ($\geq 8 \mu\text{g/mL}$) but not by the K1 enzyme (typically $< 2 \mu\text{g/mL}$).
 - ii. False positive ESBL calls due to SHV-1 hyperproduction is a problem with most ESBL confirmation tests. This is because SHV-1 weakly hydrolyzes ceftazidime. However most SHV-1 hyperproducers have cefpodoxime MICs of $\leq 1 \mu\text{g/mL}$, whereas ESBL producers have cefpodoxime

MICs $\geq 2 \mu\text{g/mL}$, often $\geq 8 \mu\text{g/mL}$. With an appropriate cefpodoxime MIC testing range and an appropriate software modification, the Phoenix could correctly distinguish between most high level SHV-1 producers and ESBL producers.

- iii. In most instances where rules 325 and 1437 were not activated, high level production of AmpC did not cause false positive ESBL confirmatory tests. This issue does not seem to be a major problem.
- iv. An elevated carbapenem MIC is an important indicator of carbapenem-hydrolyzing enzymes and should not be ignored by an expert system. An imipenem MIC $\geq 1 \mu\text{g/mL}$ for *E. coli* or *K. pneumoniae* is significantly elevated and is an important diagnostic clue. This result should invoke a comment to warn of possible carbapenemase production. It should also prevent the microbiologist from blindly accepting expert system interpretations that may otherwise be valid e.g. ESBL production. This is clinically important because a false positive ESBL call could lead to a patient being incorrectly treated with a carbapenem when the pathogen is carbapenemase producer.
- g. Incorrectly identified two strains – *K. oxytoca* as *K. pneumoniae* (kleb250) and *K. pneumoniae* as *E. cloacae* (01UCHI36).
- h. Indicated that ampicillin and moxifloxacin results were invalid for two strains of *K. oxytoca* and that the strains should be retested or that another test method should be used (strains 01EUH47 and 01VCH72).
3. The **Phoenix expert system utilizing rules 325 and 1437** increased the ESBL detection rate but was associated with very high false positive rate. In essence it converted the system into a highly sensitive ESBL screen, not a confirmatory test. In addition it:
 - a. Unnecessarily suggested retesting of ampicillin or that another susceptibility test method be used for two ESBL-producing *K. oxytoca* strains.
 - b. Incorrectly over-ruled the negative ESBL confirmatory test for one *K. oxytoca* strain that hyper-produced its K1 β -lactamase and called it ESBL positive, but then did not make the corresponding susceptibility modification of changing ceftazidime and cefepime results to resistant.
 - c. Provided false positive ESBL interpretations for three strains but did not change susceptible results for cefepime to resistant and did not change the susceptible cefotaxime result to resistant for one of these strains.
4. The **Vitek 2 expert system**:
 - a. Correctly recognized production of a CTX-M ESBL by *E. coli* 01TH63.
 - b. Correctly over-ruled a positive ESBL confirmatory test for *K. oxytoca* strain 01BH59 that produced a high level of K1 β -lactamase and was ESBL-negative
 - c. Correctly identified high SHV-1 production in two of three strains, one of which yielded a false positive ESBL confirmatory test. On repeat testing the third strain was correctly identified as a high level ESBL producer.
 - d. Correctly recognized production of plasmid-mediated AmpC β -lactamases by two of nine strains possessing this resistance mechanism. The enzymes were MIR-1 and MOX-1.
5. Suboptimal attributes of the Vitek 2 expert system:
 - a. Slightly reduced the sensitivity of ESBL detection due to over-ruling a positive confirmatory test.
 - b. Incorrectly indicated production of a CTX-M ESBL in a laboratory strain of *E. coli* (MISC 289) producing SHV-5.

This error was not clinically error, but indicated that the Vitek 2 does not reliably distinguish CTX-M ESBLs from other ESBLs.

- c. Suggested either retesting or that the laboratory should correct the results for 10 strains (*E. coli* strains Misc 239, 359, 199, 234, *E. coli* 204, JQ1, MG32, and BL6, and *K. oxytoca* strains Coudm488 and 01VCH72). This was a confusing message. It was frustrating if the same message occurred again after retesting.
- d. No finalized result for six *E. coli* strains. (Note – five of these were laboratory strains, not clinical isolates, and correct, finalized results were obtained on retesting for three of the strains).
 - i. For three of these strains, the ESBL test correctly detected an ESBL and the expert system correctly modified the susceptibility (Misc 208, 359, and 234). The failure to finalize reports for these three strains seemed inconsistent because reports were finalized for other strains yielding similar results.
 - ii. The tests were terminated due to insufficient growth for two strains (Misc 289 and Misc 382).
 - iii. No expert interpretation was provided for one strain.
- e. Inconclusive, confusing messages for three ESBL-positive *K. pneumoniae* strains in which the laboratory was asked to decide if the strains were ESBL-positive or ESBL-negative. The choices offered were:
 - i. ESBL or ESBL + impermeability or SHV-1 hyperproduction. The strain, Kleb 285, actually produced the CTX-M-19 ESBL + TEM-1 and SHV-1 like enzymes. Despite a suggested interpretation that

this isolate did not produce an ESBL, the ESBL-related corrections were applied to the susceptibility results.

- ii. ESBL + impermeability or SHV-1 hyperproduction. The strain, JW6, actually produced an SHV-2 like ESBL and a TEM-1 like enzyme.
- iii. ESBL + impermeability or SHV-1 hyperproduction. The strain, JW1, actually produced an SHV-2 like ESBL.
- f. Did not recognize production of class A carbapenemase (KPC-2) by *K. pneumoniae* UMM3 despite the significantly elevated imipenem MIC of ≥ 16 $\mu\text{g/mL}$. On retesting, the imipenem MIC was still ≥ 16 $\mu\text{g/mL}$ but the expert system over-rode this result reported ≤ 2 $\mu\text{g/mL}$.
- g. Did not reliably correct false positive ESBL tests due to high level production of SHV-1 or K1 β -lactamases, or due to the KPC-2 carbapenemase. It was inconsistent in handling high level production of SHV-1. The expert system gave the correct interpretation with one strain but was incorrect with two other strains (01LSAI20 and GB19).
- h. Did not suggest possibility of a carbapenemase when an elevated carbapenem MIC was detected (strain UMM30).
 - i. Had a problem with the MIR-1 plasmid-mediated AmpC β -lactamase. The ESBL test correctly classified this as a non-ESBL but the expert system over-rode the correct result and incorrectly classified it as an ESBL. The MIR-1 enzyme caused problematic tests with both *E. coli* and *K. pneumoniae*. On retesting the expert system gave the correct interpretation.
- j. Did not detect an ESBL that was co-produced with the KPC-2 β -lactamase in strain 01BH79.

CONCLUSIONS

- Considering the challenging nature of the strains, both systems were highly sensitive at ESBL detection with the Phoenix (96% sensitivity) being more sensitive than the Vitek 2 (89% sensitivity).
- The ESBL-producing strains for which the Phoenix exhibited greater sensitivity than the Vitek 2 produced multiple β -lactamases.
- The Phoenix had lower specificity (81%) than the Vitek 2 (85%), reflecting both the challenging nature of strains and also the high mathematical impact of an incorrect result when only 26 ESBL-negative strains were tested.
- Modifications should be made to the Phoenix expert system to over-ride false positive ESBL confirmatory tests and increase its specificity to possibly as high as 96.2% (against the strains of this study).
- While both expert systems provided helpful comments of high quality for some strains, they were unsatisfactory for others and need modifying and updating.
- Phoenix expert comments were simpler and generally more directly useful. The Vitek 2 more often suggested that strains should either be retested or that the lab should decide what the resistance mechanism was, or did not finalize results. This is likely to cause frustration, particularly in smaller laboratories where an expert may not be available to determine the correct report.
- Both expert systems should indicate the possibility of a carbapenemase when an elevated carbapenem MIC occurs.
- The Phoenix expert system with rules 325 and 1437 activated was unreliable for ESBL confirmation.