

# Detection of Extended Spectrum Beta-Lactamase Among *Enterobacteriaceae* Using Phoenix™ Automated Microbiology System with BDXpert™ System

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## ABSTRACT

**BACKGROUND:** The reliability of automated antibiotic susceptibility testing systems for the detection of Extended-Spectrum  $\beta$ -Lactamase (ESBL) among *Enterobacteriaceae* has been a concern recently. The BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) was evaluated for the detection of ESBL production in clinically significant *Enterobacteriaceae* using the Phoenix ESBL test\* and the BDXpert™ System.

**METHODS:** A total of 133 strains, including *Escherichia coli* (61), *Klebsiella* (53), *Citrobacter* (4), *Enterobacter* (5), *Morganella* (1), *Proteus* (3), *Serratia* (3), and *Salmonella* species (3) were tested. The Phoenix ESBL test, a proprietary 5-well confirmatory test, was evaluated. Specially designed BDXpert rules for the detection of ESBL were also evaluated. Reference methods included: the NCCLS phenotypic confirmatory tests for ESBLs using standard broth microdilution and disk diffusion methods; THE SOCIETE FRANCAISE DE MICROBIOLOGIE disk approximation method for the detection of ESBL; PCR procedures for the detection of TEM and SHV genes.

**RESULTS:** Of the total, 83 strains tested positive as determined by the reference methods. Phoenix ESBL test with the BDXpert rules correctly detected 78 strains. The Phoenix System for the detection of ESBL showed a sensitivity of 94% and specificity of 96%. The average time to result was  $8.5 \pm 2.7$  h in the Phoenix System.

**CONCLUSIONS:** These results indicate that the Phoenix System with the BDXpert rules provides reliable and rapid detection of ESBL production in clinically important *Enterobacteriaceae*.

## INTRODUCTION AND PURPOSES

Accurate detection of Extended-Spectrum Beta-Lactamases (ESBLs) among *Enterobacteriaceae* has been a focal point for many clinicians and laboratorians lately. The National Committee for Clinical Laboratory Standards (NCCLS) has provided guidelines and interpretive criteria for overnight standard broth microdilution (SBM) and disk diffusion methods for screening and confirmation of ESBL production in *E. coli*, *K. pneumoniae* and *K. oxytoca* (M100-S12). The NCCLS confirmatory method is based on the inhibitory effect of clavulanic acid (CA) on ESBL when tested with Ceftazidime (CAZ) and Cefotaxime (CTX) with or without clavulanic acid. The SFM has also defined a double disk methodology for the detection of ESBL producing strains among clinically significant *Enterobacteriaceae* applying the synergistic effect between amoxicillin/clavulanic acid and several extended-spectrum beta-lactams.

Commercially available automated AST systems, such as MicroScan® (Dade Behring Inc., West Sacramento, CA) or Vitek® (bioMérieux Vitek, Inc. Hazelwood, Missouri), have offered ESBL screens on a routine panel/card, but they usually require some additional tests for the confirmation of ESBL production. The reliability of automated antimicrobial susceptibility test systems for the detection of ESBL among *Enterobacteriaceae* other than *E. coli*, *Klebsiella* species remains a concern. The BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) was evaluated for the detection of ESBL production in clinically significant *Enterobacteriaceae* using the Phoenix ESBL test and the BDXpert™ System. The Phoenix ESBL test uses the differences in growth response to select second or third generation cephalosporins, with or without clavulanic acid in order to detect the production of ESBL. Specially designed BDXpert rules with pre-defined antibiogram pattern for the detection of ESBL producing strains were also evaluated in conjunction to the Phoenix ESBL test results. The NCCLS confirmatory, SFM double disk diffusion tests and PCR amplification of TEM or SHV genes were used as the reference methods.

## METHODS



Figure 1.  
Phoenix™ Automated  
Microbiology System

**BACTERIAL STRAINS.** A total of 133 strains of clinical or stock isolates, including *Escherichia coli* (61 strains), *Klebsiella* (53), *Citrobacter* (4), *Enterobacter* (5), *Morganella* (1), *Proteus* (3), *Serratia* (3), and *Salmonella* species (3) were tested in this study. The two NCCLS quality control organisms, *E. coli* ATCC 25922 and *K. pneumoniae*, ATCC 700603 were included in all experiments.

**MEDIA.** All test strains were sub-cultured onto trypticase soy agar plates with 5% defibrinated sheep blood (TSA II, BDDS) and incubated at 35±1°C for 18-20 h. Mueller-Hinton II Broth or Agar (BDDS) was used in all antibiotic susceptibility testing.

**PHOENIX AST METHOD AND BDXpert.** Bacterial suspensions were prepared from an overnight culture on TSA II and adjusted to a 0.5 – 0.6 McFarland standard in Phoenix ID broth using the CrystalSpec™ Nephelometer (BDDS). A final inoculum density of 5x10<sup>5</sup> cfu/mL, equivalent to the NCCLS recommendations, was used. A drop of Phoenix AST Indicator was added to each Phoenix AST Broth tube before testing. The inoculated Phoenix panels were then placed into the Phoenix instrument for incubation and continuous reading until complete. Every Phoenix gram-negative panel includes the Phoenix ESBL confirmatory test. The BDXpert rules were activated in the instrument to interpret the MIC results using NCCLS categorical interpretation criteria. Several specially designed BDXpert rules were used to recognize the phenotypic antibiogram of ESBL among *Enterobacteriaceae*. The following pattern was used for phenotypic detection of ESBL strains: susceptible to a carbapenem and resistant to ureidopenicillins and any of the following drugs: ceftriaxone, cefotaxime, ceftazidime, cefpodoxime or aztreonam. The criteria is applied to all enterics except species producing intrinsic inducible cephalosporinase such as *Enterobacter cloacae*, *Citrobacter freundii* etc.

## REFERENCE ESBL METHODS

- 1. NCCLS Standard Broth Microdilution (SBM) and Disk Diffusion Methods.** All strains tested were also evaluated with SBM panels and ESBL confirmatory disk diffusion methods. Frozen microdilution panels were prepared and inoculated according to NCCLS recommended guidelines (M7-A5) using the same inoculum prepared for Phoenix inoculation above. After 18–20 h of incubation at 35±1°C in ambient air, the reference SBM panels were interpreted by trained technologists. The MIC and ESBL results were interpreted in accordance with NCCLS criteria (M100-S12). The drugs tested with SBM (and concentration ranges in micrograms/mL) were ceftazidime (CAZ, 0.25–128), ceftazidime/clavulanate (CCZ, 0.25/4–128/4), cefotaxime (CTX, 0.25–64), and cefotaxime/clavulanate (CCX, 0.25/4–64/4). The NCCLS based disk diffusion method was performed with CAZ (30 µg), CCZ (30/10 µg), CTX (30 µg) and CCX (30/10 µg) using BBL™ Sensi-Disc (BDDS). The NCCLS M100-S12 interpretation criteria were used for all results.
- 2. SFM Double Disk Diffusion Method.** THE COMITE DE L'ANTIBIOGRAMME DE LA SOCIETE FRANCAISE DE MICROBIOLOGIE Report 2000–2001 outlines a double disk diffusion test using a disk of amoxicillin/clavulanic acid (AMC) centered to several discs with extended-spectrum beta-lactam. A synergistic effect observed between AMC and any one or more of the tested beta-lactams indicates the production of ESBL in the test isolate. The following BBL™ Sensi-Disc (BDDS) were used in the study: AMC (20/10 µg), CAZ (30 µg), CTX (30 µg), aztreonam (ATM 30 µg), ceftriaxone (CRO 30 µg) and cefepime (FEP 30 µg).
- 3. PCR for the Detection of TEM and SHV genes.** PCR procedures were used to detect the presence of TEM or SHV genes in the test isolates (Rasheed et. al. Antimicrobial Agents and Chemotherapy, 1997, 41:647-653). DNA template was prepared using Bio-Rad InstaGene™ Matrix (Hercules, CA). Amplification primer pair 1 and 2 bla<sub>TEM</sub>, or primer pair 4 and 5 bla<sub>SHV</sub> were used (AAC, 1997, 41:647-653). All amplifications were performed in 100 µL reaction mixtures containing 20 mM Tris-HCL pH 8.4, 50mM KCL, 1.5mM MgCl<sub>2</sub>, 200 µmol; dNTP, 2.5 U Taq DNA polymerase, 1µmol oligonucleotide primers and 1 µL of the DNA template. PCR assays were performed as follows: initial denaturation at 96°C for 5 min; 35 cycles of denaturation at 96°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min; and a final extension at 72°C for 10 minutes. A volume of 10 µL PCR product was electrophoresed in 1% gel and 1X TBE buffer, stained with ethidium bromide and photographed under UV lighting. The PCR amplification procedure yielded an 867 bp product for either bla<sub>TEM</sub> or bla<sub>SHV</sub> genes. The PCR results were used to determine the final ESBL results in additional to SBM, disk diffusion and double disk diffusion results.

## RESULTS

Table 1. Numbers of Strains Tested in This Study

Species	Number of Strains Tested
<i>Citrobacter braakii</i>	1
<i>Citrobacter freundii</i>	3
<i>Enterobacter aerogenes</i>	2
<i>Enterobacter cloacae</i>	3
<i>Escherichia coli</i>	61
<i>Klebsiella oxytoca</i>	12
<i>Klebsiella pneumoniae</i> ssp <i>pneumoniae</i>	41
<i>Morganella morganii</i> ssp <i>morganii</i>	1
<i>Proteus mirabilis</i>	2
<i>Proteus vulgaris</i>	1
<i>Salmonella newport</i>	1
<i>Salmonella</i> species	2
<i>Serratia marcescens</i>	3

Table 2. Comparison of ESBL Results from Different Reference Methods

Double Disk Diffusion	PCR <i>bla</i> <sub>TEM/SHV</sub>	Standard Broth Microdilution		
		Negative	Positive	Total
Negative	Negative	50	8*	58
Positive	Positive		75	75
Total		50	83	133

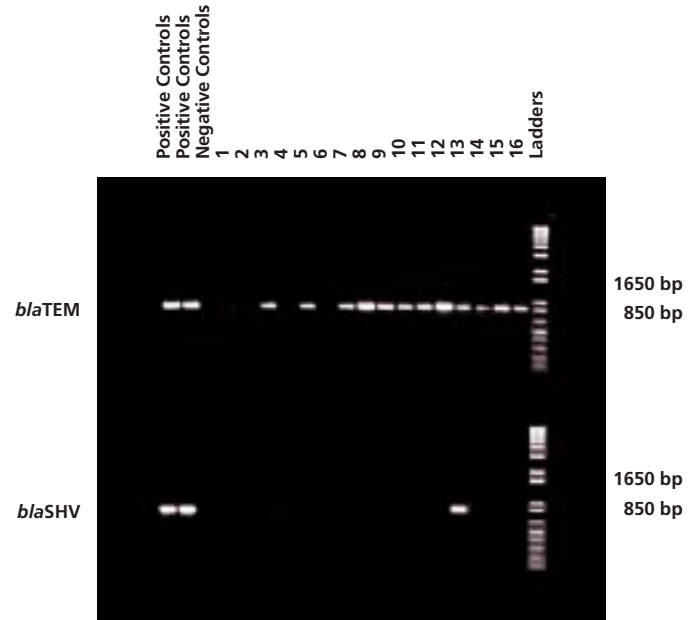
\* Isolates demonstrated resistance to at least one or more extended-spectrum beta-lactams.

Table 3. Results of Phoenix ESBL Compared to Resolved Reference

Phoenix System*	Final ESBL Results with Reference Methods		Sensitivity	94.3
	Positive	Negative		
Positive	78	2	Specificity	96.2
Negative	5	48	Accuracy of Test Method	94.7

Phoenix System – Final results as produced by the Phoenix ESBL test and BDXpert rule interpretations

Figure 2. PCR Results for the Detection of *bla*<sub>TEM</sub> or *bla*<sub>SHV</sub> genes



Lanes in top portion of gel contain product from *bla*<sub>TEM</sub> PCR. Lanes in middle portion of gel contain *bla*<sub>SHV</sub> product. All controls performed as expected. At top portion of gel, lanes 3, 5, and 7 thru 16 showed amplification of *bla*<sub>TEM</sub> gene at 867bp. In middle of gel, lane 13 showed amplification of *bla*<sub>SHV</sub> gene.

## CONCLUSIONS

- Eight strains showed discrepant results between SBM and double disk diffusion methods. These 8 strains were detected negative using the PCR primers for either *bla*<sub>TEM</sub> or *bla*<sub>SHV</sub> genes. However, these strains showed resistance to at least 1 extended-spectrum beta-lactam tested in this study.
- In this study, the Phoenix System demonstrated a 94% sensitivity and 96% specificity when compared to the reference methods with a time to result of 8.5±2.7 hours.
- The Phoenix System with BDXpert, provides reliable detection of ESBL production in commonly encountered *Enterobacteriaceae*.

