

Variability in *In Vitro* Susceptibility Test Results for *E. coli* Isolates and Piperacillin/Tazobactam Using Commercial and Reference AST Methods

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ABSTRACT

BACKGROUND: Following inconsistent results in a recent multi-center study, the antimicrobial combination, piperacillin/tazobactam (TZP), a β -lactam and β -lactamase inhibitor, was evaluated against a challenge set of *E. coli* (EC) isolates for *in vitro* resistance detection in three commercial AST systems: Phoenix™ (PHX; BD Diagnostics, Sparks, MD), Vitek® Legacy (VTK; BioMerieux, Hazelwood, MO) and the Microscan® Walkaway (MS; Dade-Behring, Sacramento, CA), as well the CLSI-recommended standard broth microdilution (SBM) method. Reproducibility of the TZP results was also assessed in each of the systems.

METHODS: In the primary evaluation, 78 TZP-resistant (TZP-R) EC (clinical and stock isolates) were tested in each of the four AST systems. Bacterial suspensions for each test and QC strain were adjusted to a 0.5 McFarland standard, and then inoculated into each system (per the manufacturer's instructions) and the SBM reference method. For the reproducibility study, 14 EC isolates (11 TZP-R) were tested in each system daily over a 20-day period using the same procedures as in the primary evaluation. TZP breakpoints were based on CLSI M100-S15.

RESULTS: For the 78 TZP-R EC, the rate of categorical agreement (CA) was 96% for PHX, 62% VTK and 61% MS. Very major error (VME) rates were 4.0%, 22.9% and 6.8% for PHX, VTK and MS, respectively. Variable results were observed in all systems in the 20-day reproducibility study. When compared to the SBM mode, the CA was 74%, 47% and 44% for PHX, VTK and MS, respectively. VMEs ranged from 6% in PHX to 69% in VTK. Nearly 10% of the SBM TZP reproducibility tests had to be excluded as a result of 'skipped' wells, where additional growth occurs past the determined MIC.

CONCLUSIONS: Resistant strains of *E. coli* may demonstrate variable *in vitro* susceptibility test results with TZP in the SBM reference method as well as in commercial AST systems. Compared to the SBM, PHX was the most accurate system for TZP-R detection.

INTRODUCTION

Piperacillin/Tazobactam (Zosyn®, Wyeth® Pharmaceuticals, Collegeville, PA) is a potent broad-spectrum antibacterial combination of a beta-lactam antibiotic and a beta-lactamase inhibitor compound. Piperacillin sodium exerts bactericidal activity by inhibiting septum formation during cell-wall synthesis in susceptible bacteria. Tazobactam sodium acts as a β -lactamase inhibitor of the Richmond-Sykes class III (Bush class 2b & 2b') penicillinases and cephalosporinases. Though tazobactam alone has little clinical application (due to its reduced affinity to penicillin-binding proteins), the combination of the two acts as a powerful and often-prescribed therapeutic agent in the treatment of moderate to severe gram-negative (GN) infections. As with all antimicrobial agents, detection of resistance to TZP in GN bacteria is vital to patient care, and requires accurate reporting by antimicrobial susceptibility testing (AST) methods. Our study was conducted to determine this level of AST system performance in two phases. In the initial Accuracy phase, 78 TZP-resistant *E. coli* (EC) strains were tested against TZP in three commercial AST systems: Phoenix™ (PHX; BD Diagnostics, Sparks, MD), Vitek, Legacy (VTK; BioMerieux, Hazelwood, MO) and Microscan, Walkaway (MS; Dade-Behring, Sacramento, CA), as well as in the CLSI-recommended standard broth microdilution (SMB) reference method. In the Reproducibility phase, 14 of the 78 strains used in the first phase were tested in the three AST systems and SBM daily over a 20-day period.

MATERIALS AND METHODS

ACCURACY PHASE:

Bacterial Strains. A total of 78 TZP-resistant (TZP-R) *EC* isolates consisting of clinical and stock strains were tested in each of the three AST systems (Table 1). The TZP interpretive result was determined by no less than 20 daily replicates in SMB for each strain. Eighteen of these strains were also confirmed as ESBL-positive strains by various reference methods.

Quality Control (QC). The gram-negative QC strains recommended by the Clinical Laboratory Standards Institute (CLSI) were tested daily in each system (Table 1).

Phoenix.TM Phoenix Gram-negative panels containing TZP with a full range of doubling dilutions from 0.5/4-128/4 µg/mL were tested using the manufacturer's procedural recommendations. Inoculated Phoenix panels were placed into the PhoenixTM instrument for incubation and continuously read every 20 minutes to completion, up to a maximum of 16 hours.

Vitek[®] Legacy. Vitek GNS-122 cards containing a dilution range of 8/4-128/4 µg/mL were tested using the manufacturer's recommendations. Once inoculated, the cards were placed in the Vitek instrument for incubation and continuous overnight readings.

Table 1

Test Strains	n		% of Total
<i>Escherichia coli</i>	18	ESBL-producer	23%
	60	non-ESBL producer	77%
	78		100%
QC Strains (3)			
<i>Escherichia coli</i>		ATCC 25922	
<i>Escherichia coli</i>		ATCC 35218	
<i>Pseudomonas aeruginosa</i>		ATCC 27853	

MicroScan[®] Walkaway. MicroScan NEG-MIC Type 30 panels containing a dilution range of 8/4-64/4 µg/mL were tested. Panel preparation and suspensions were made according to manufacturer guidelines. Once inoculated, panels were incubated for 16-20 hours and automatically read by the instrument for MIC determinations.

Standard Broth Microdilution (SBM) Method. Reference SBM panels containing TZP with a range of 0.5/4-128/4 µg/mL were prepared and inoculated according to CLSI-recommended guidelines (M7-A6). Following inoculation, all SBM panels were incubated at 35°C in ambient air for 18-20 hours and read manually for MIC endpoints using the interpretive breakpoints listed in Table 2.

REPRODUCIBILITY PHASE:

Bacterial Strains. Fourteen *EC* isolates consisting of clinical and stock strains were tested in each of the three AST commercial systems and the reference SBM panels. Two of the 14 were morphological variants isolated from primary cultures of these strains.

Procedure. The 14 *EC* strains (5 TZP-S, 1 TZP-I and 8 TZP-R) were tested daily in the PHX, VTK and MS systems, as well as the reference SBM, for twenty days. Standardized inocula were prepared from the same primary TSA II plates (BD Diagnostics) for use in all four systems. Two *EC* ATCCTM strains, 25922 and 35218, were used as controls.

Table 2

Antimicrobial Agent	MIC Interpretive Breakpoints for <i>Enterobacteriaceae</i>			AST System	Dilution Range (mcg/mL)
	S	I	R		
Piperacillin/Tazobactam	<= 16/4	32/4 – 64/4	=> 128/4	SBM Reference	0.5/4 – 128/4
				Phoenix	0.5/4 – 128/4
				Vitek	8/4 – 128/4
				MicroScan	8/4 – 64/4

Accuracy Phase: Results for the 78 TZP-R *E. coli* isolates were first compared to the calculated reference SBM mode (Table 3), established by testing each strain daily in the SBM panel at least twenty days. When the daily SBM results were compared to the SBM modal result for the same strain, 74/78 (95%) were in categorical agreement (CA). Of the four strains not in CA, two (3%) yielded Very Major Errors (VME), and the other two produced minor errors (MIE). For the commercial systems, CAs compared to the modal SBM result were 92%, 56% and 59% for PHX, MS and VTK, respectively. The VME rate was lowest for PHX (4%), and highest for VTK (19%). There were no Major Errors (ME) as all strains were resistant in the reference.

The results of daily testing of each system to the SBM are shown in Table 4. The CA rate increased slightly for all three commercial systems while the VME rates decreased for PHX and VTK. Note that four (4) strains that were not resistant in the SBM during parallel testing were omitted (n = 74).

Reproducibility Phase: Interpretations compiled for the 20 replicates of the 14 test strains with TZP (8 TZP-R, 1 TZP-I and 5 TZP-S), along with CA and error rates for each system appear in Table 5. In this study phase, the SBM result from daily testing was compared to the modal SBM result calculated for the 20-day period. This resulted in only a 77%

RESULTS

Table 3. Piperacillin/Tazobactam Challenge Set Testing Results with 78 TZP-Resistant *E. coli* Compared to Reference Mode*

AST System	Agreement to Reference Mode*		
	CA (%)	VME (%)	MIE (%)
Reference (Daily)	74/78 (95%)	2/78 (3%)	2/78 (3%)
Vitek Legacy	46/78 (59%)	15/78 (19%)	17/78 (22%)
MicroScan	44/78 (56%)	5/78 (6%)	29/78 (37%)
Phoenix	72/78 (92%)	3/78 (4%)	3/78 (4%)

* Reference modal MIC result established after => 20 days of testing

Table 4. Piperacillin/Tazobactam Challenge Set Testing Results with 74 TZP-Resistant *E. coli* Compared to Daily Reference*

AST System	Agreement (%)		
	CA	VME	MIE
Vitek Legacy	46/74 (62%)	11/74 (15%)	17/74 (23%)
MicroScan	45/74 (61%)	5/74 (7%)	26/74 (35%)
Phoenix	71/74 (96%)	0 (0%)	3/74 (4%)

* Four (4) strains yielded TZP-S results in the reference system during this test.

CA for the SBM, and included a 7% rate for both VME (11/147) and ME (6/88). Furthermore, 26 (9.3%) skipped wells for TZP occurred in the reference SBM. (Skipped wells were defined as any well with no growth occurring between one or more wells exhibiting growth.) The total number of results for each system was different based on the number of results omitted due to skipping, no growth, etc.

Of the three AST systems, PHX yielded CA and error rates most comparable to those of the reference. The 20-day results for two TZP-R strains are shown in Table 6. VTK was tested for only

16 days. One of these EC strains was determined to be an ESBL-producer, the other was not. Both strains yielded variable and/or discrepant results with all systems tested, except ENF12941 in PHX. The spread of the MICs occasionally covered all three interpretation (S-I-R) categories. Though the reference modal result was considered as “R” for both strains, over 30% of the SBM tests for the same strain were in error to the mode. All systems were in agreement based on modal results for the five susceptible strains tested.

Table 5. Results of 20-Day Reproducibility Study (14 Strains)

AST System	S	I	R	Total n**	Agreement (%)*			
					CA	VME	ME	Minor
Reference SBM*	88	18	147	253	195 (77)	11 (7)	6 (7)	41 (16)
Phoenix	89	20	153	262	193 (74)	9 (6)	13 (15)	47 (18)
Vitek Legacy	81	18	128	227	106 (47)	88 (69)	0	33 (15)
MicroScan	97	19	160	276	122 (44)	73 (46)	0	81 (29)

* Agreement rates for Reference SBM calculated against the reference modal result for each strain.

** Total n = 280 - (# of skipped wells and/or # not tested).

Categorical agreement (CA): Agreement of interpretive results between the test and reference modal result.

Very Major error (VME): Test = S, SBM = R; Major error (ME): Test = R, SBM = S; Minor error: Test = I, SBM = S or R, or Test = S or R, SBM = I.

Table 6. Examples of Reproducibility Issues with TZP-Resistant *E. coli* in 4 AST Systems

Test Strain	AST System	Minimal Inhibitory Conc. (MIC)																	
		S	I	R	Not tested	Skip	n	CA	Minor	VME	ME	S							
												< 8	8	16	32	64	128	>64	>128
BD ENF12941 (ESBL -)	Reference, SBM*	3	3	13		1	19	13	3	3	0		2	1		3	8		5
	Phoenix			20			20	20	0	0	0					8		12	
	Vitek Legacy	16			4		16	0	0	16	0	16							
	MicroScan	5	14	1			20	1	14	5	0	2		3	8	6		1	
BD ENF12912 (ESBL +)	Reference, SBM		6	14			20	14	6	0	0				6	8		6	
	Phoenix	5	12	3			20	3	12	5	0		2	3	5	7	2	1	
	Vitek Legacy	1	5	10	4		16	10	5	1	0			1	2	3		10	
	MicroScan	13	3	4			20	4	3	13	0	5		8	2	1		4	

* SBM = NCCLS-recommended standard broth microdilution method

■ = Modal reference SBM result over 20 day test period. Also AST System modal agreement.

■ = System modal result = minor error.

■ = System modal result = Very major error.

CONCLUSIONS

- Piperacillin/Tazobactam-resistant *E. coli* may produce variable *in vitro* categorical results when tested in the CLSI-recommended broth microdilution reference method, as well as in commercial AST systems.
- In this study, the Phoenix AST System was the most comparable to the reference method for detecting TZP-resistance in *E. coli*.
- Gram-negative species other than *E. coli* should be similarly challenged for accurate and reproducible detection of TZP-resistance.

