

Comparison of the Phoenix™ Automated Microbiology System to the Dade-MicroScan WalkAway-40 for Antimicrobial Susceptibility Testing with Gram-Negative and Gram-Positive Bacterial Isolates

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REVISED ABSTRACT

■ **OBJECTIVES:** The Phoenix™ System (BD Diagnostic Systems, Sparks, Maryland, USA), a rapid automated ID/AST system, was comparatively evaluated with the Dade-MicroScan WalkAway-40 System (Dade-MicroScan, W. Sacramento, California, USA) for accuracy of antimicrobial susceptibility test (AST) results with a varied group of gram-negative bacilli (GNB) and gram-positive cocci (GPC).

METHODS: A total of 306 clinical bacterial isolates, comprised of 186 GNB (family *Enterobacteriaceae*), and 123 GPC (79 staphylococci including 39 methicillin-resistant (MR) strains, and 40 enterococci) isolates were tested in standard commercially available Phoenix (PHX) and MicroScan (MSC) AST panels. Each inoculum suspension was prepared from the same subculture plate for both test systems. PHX and MSC panels were each inoculated, then incubated and automatically read in the system's instrument following each manufacturer's recommended procedure. Thirteen antimicrobics were evaluated for GNB, 13 for *Staphylococcus* species (ST), and 8 for *Enterococcus* species (ENT). The minimal inhibitory concentration (MIC) of each antimicrobial/organism combination was interpreted based on the 2001 NCCLS Standards. Discrepant AST results were repeated in both systems, and arbitrated using the NCCLS-recommended broth microdilution method. Essential agreement (EA), MIC results within +/- one doubling dilution, and categorical agreement (CA) were determined for the Phoenix System.

RESULTS: Overall, 3,620 AST combinations were evaluated in both systems. For GN isolates, the rate of EA was 99.2%, while the CA rate was 95.9%. False-susceptible (VM) and false-resistant (M) rates for PHX were 0.4% and 0.4%, respectively. Arbitration of 25 GNB disagreements resolved with 22 in agreement with the Phoenix MIC. For ST isolates, the EA and CA rates were 96.4% and 94.7%, respectively. All 39 MR strains were correctly identified as such by PHX. With ENT species, the EA was 99.3%, and the CA 95.8%. High-level aminoglycoside detection were between 97.7 and 100% equivalent for the two systems. PHX also correctly identified 4 enterococcal strains as vancomycin-resistant.

CONCLUSIONS: The rapid AST performance of the Phoenix System is nearly equivalent to that of the MicroScan Walk-Away overnight system, and provides an alternative in automated AST testing of GNB and GPC for the clinical laboratory.

INTRODUCTION

The Antimicrobial Susceptibility Testing (AST) of gram-negative and gram-positive clinical bacteria represents the largest proportion of AST tests performed in an average clinical microbiology laboratory. Automation of these tests can reduce the human workload of the laboratory. Rapid AST systems can have a beneficial impact on patient welfare through early recognition of bacterial resistance. This can lead to therapeutic modifications which benefit an infected patient, and is critical for the epidemiologic control of nosocomial infections. Clinical laboratories are also under more and more pressure to increase labor efficiency while providing timely and medically useful information to the medical staff. These laboratorians are, thus, considering the purchase of new systems offering enhanced levels of automation and more rapid test results. However, in making such a purchase decision, the accuracy of the susceptibility testing is crucial for laboratories to evaluate. For without accuracy the advantages of automation and rapid test results are lost in regards to patient care.

In this study, we evaluated a new automated identification (ID) and AST system, Phoenix, in comparison to our current routine method, MicroScan overnight system. The study compared the accuracy of AST of some commonly isolated gram-negative and gram-positive bacteria.

METHODS AND MATERIALS

Organisms

A combination of fresh clinical isolates and some frozen stock cultures were tested including gram-negative and gram-positive bacteria. The gram-negative bacteria included a representative sample of 186 Enterobacteriaceae strains in our hospital. Pseudomonas and other non fermentative bacteria were not included because it is our laboratory practice to test these organisms with other systems (Sensititre, E-test). The gram-positive bacteria included a routine mix of 79 Staphylococci and 44 Enterococci (*E. faecalis* & *E. faecium*).

Phoenix

(BD Diagnostic Systems, Sparks, MD): gram-negative panels NMIC/ID-1 [Cat no. 448506] and gram-positive panels PMIC/ID-1 [Cat no. 448502] were used. Table 1 indicates the antibiotics and test concentrations in each panel. The gram-negative panels included a test for the presence of ESBL using a principle similar to the NCCLS confirmatory test. The gram-positive panels contained a test for Staphylococcal penicillinase (β -Lactamase). The results of these tests were integrated into the antibiogram through the BDXpert System.

Phoenix panels were tested strictly following manufacturer's instructions. Briefly, blood agar plates were inoculated with the test cultures and were incubated for 18-24 hours at 35°C. Isolated colonies were used to prepare a suspension of the bacteria in the Phoenix ID broth to match a 0.5 MacFarland.

AST broth was supplemented with one drop of indicator dye and 25 μ L of the ID suspension. Panels were auto-inoculated by pouring the AST suspension into the AST port of the panel. After closing, the panels were logged and loaded into the instrument. The instrument automatically incubated and optically scanned the panels at 20 min intervals. Dedicated algorithms kinetically calculated the MICs after sufficient incubation time. These results were automatically interpreted following NCCLS recommendations, and additionally evaluated for consistency and resistance mechanisms with the on-board BDXpert system.

MicroScan

Overnight panels were used for all testing. Prompt inoculation procedures were strictly followed. Panels were incubated and interpreted in the MicroScan WalkAway 40 system. The panels were additionally read visually to verify the results. MSC software version was 23.11-ISN.102 W/A 23.10-ISN.104.

Arbitration

AST test results that were retested using microbroth dilution system following the NCCLS recommendations as arbitrator method if the Phoenix vs. MicroScan result exhibited a major or very major discrepancy. The system that agreed with the arbitrator was considered the correct result.

Table 1
Antibiotics and concentrations evaluated using Phoenix and MicroScan AST panels

Gram-Negative	Phoenix	MicroScan
Amikacin	4-64	4 - 32
Amoxicillin/Clavulanate	2/1-32/16	8/4 - 16/8
Ampicillin	2-32	1 - 16
Cefazolin	4-32	2 - 16
Cefotaxime	2-32	2 - 8, 32
Ceftazidime	4-32	2 - 16
Cefuroxime	4-32	2 - 16
Ciprofloxacin	0.25-4	1 - 2
Gentamicin	1-16	2 - 8
Nitrofurantoin	32-128	32 - 64
Piperacillin	4-128	8 - 16, 64
Tobramycin	1-16	2 - 8
SXT	0.5/9.5-16/304	2/38

Gram-Positive	Phoenix	MicroScan
Cefazolin	2-16	4 - 16
Ciprofloxacin	1-4	1 - 2
Clindamycin	0.5-8	0.5 - 2
Gentamicin	1-16	4 - 8
Gentamicin-Synergy	500	500
Meropenem*	2-8	4 - 8
Ofloxacin*	1-4	2 - 4
Oxacillin	0.5-4	0.25, 1 - 2
Penicillin	0.06-16	0.12 - 8
Rifampin	1-4	1 - 2
Streptomycin-Synergy	1000	1000
Tetracycline	0.5-16	4 - 8
Teicoplanin	1-32	4 - 16
SXT	2/38-16/304	2/38
Vancomycin	0.5-32	2 - 16

*Not all organisms were tested against Meropenem and Ofloxacin

Table 2
Organisms evaluated using Phoenix and MicroScan AST panels

Gram-Negative Bacteria		Resistance Mechanism		
Organism	# Tested	ESBL	F-Quin ^R	Aminoside ^R
<i>Citrobacter braakii</i>	3			1
<i>Citrobacter freundii</i>	3	1		
<i>Citrobacter koseri</i>	9			
<i>Citrobacter spp.</i>	1			
<i>Enterobacter aerogenes</i>	10			
<i>Enterobacter cloacae</i>	19		1	
<i>Escherichia coli</i>	19	1	9	4
<i>Klebsiella oxytoca</i>	15	1	2	
<i>Klebsiella pneumoniae</i>	21	4	1	2
<i>Klebsiella spp.</i>	1			
<i>Morganella morganii</i>	17		5	3
<i>Proteus mirabilis</i>	18		1	2
<i>Proteus vulgaris</i>	4			
<i>Providencia stuartii</i>	5		2	
<i>Pseudomonas stutzeri</i>	1			
<i>Salmonella species</i>	18			
<i>Serratia liquefaciens</i>	1			
<i>Serratia marcescens</i>	15			1
<i>Shigella flexneri</i>	3			
<i>Shigella sonnei</i>	2			
<i>Yersinia enterocolitica</i>	1			
Total	186	7	21	13

Gram-Positive Bacteria		Resistance Mechanism			
Organism	# Tested	Va ^R	HLAR	MRS	
<i>Enterococcus faecalis</i>	21	1	11		
<i>Enterococcus faecium</i>	18	3	4		
<i>Enterococcus irae /avium</i>	1				
<i>Staphylococcus species</i>	1				
<i>Staphylococcus aureus</i>	26			10	
<i>Staphylococcus epidermidis</i>	18			15	
<i>Staphylococcus haemolyticus</i>	15			14	
<i>Staphylococcus hominis</i>	1				
<i>Staphylococcus lugdunensis</i>	9				
<i>Staphylococcus saprophyticus</i>	8				
<i>Staphylococcus sciuri</i>	1				
Total	119	4	15	39	

RESULTS

The results of the performance of the PHX system in comparison to MSC for clinical routine isolates and some stock cultures of gram-negative and gram-positive bacteria are shown in Tables 3 to 5.

Gram-negatives: the overall concordance of PHX compared with MSC for EA was 99.7 and for CA was 96.2. Arbitration of the 4 major discrepancies favoured each system twice. The discrepancies in favor of PHX were significant as these involved an ESBL positive strain.

Staphylococci: the overall concordance of PHX compared with MSC for EA was 96.4 and for CA was 94.7. There were 3 strains

of *S. lugdunensis* that were oxacillin resistant in MSC but susceptible in PHX. All 3 were tested for *mecA* and were found to be negative. This is consistent with a recent report by Zafar Hussain *et al.* (JCM 2000, vol. 38, pp. 752-754). Thus we have to assume both systems are correct.

Enterococci: The overall concordance of PHX compared with MSC for EA was 99.3 and for CA was 95.8. For Penicillin, PHX in 2 cases interpreted susceptible results whereas MSC interpreted resistant results. Arbitration testing was not performed with these two strains, so it is not possible to evaluate which system was likely to be correct.

Table 3
Comparison of Phoenix AST to MicroScan AST with 186 GNB Strains

Antimicrobial	Phoenix				MicroScan			Essential Agreement		Categorical Agreement		PHX=S MS=R		PHX=R MS=S		minor	
	N	S	I	R				N	% EA	N	% CA	N	%	N	%	N	%
Ampicillin	184	33		151	32	1	150	182	98.9	184	100.0						
Amoxicillin /Clav	183	93	7	83	97	5	81	183	100.0	173	94.5			10	5.5		
Amikacin	182	160		22	159		23	182	100.0	182	100.0						
Ceftazidime*	183	155		28	153	1	29	182	99.5	181	98.9			1	3.4	1	0.5
Cefotaxime*	183	155		28	154	2	27	180	98.4	181	98.9			1	3.7	1	0.5
Ciprofloxacin	182	150	7	25	149	11	22	181	99.5	174	95.6					8	4.4
Cefuroxime	183	102	5	76	108	12	63	182	99.5	168	91.8					15	8.2
Cefazolin	183	88	2	93	86	5	92	182	99.5	176	96.2					7	3.8
Gentamicin	182	143	3	36	144		38	182	100.0	179	98.4					3	1.6
Nitrofurantoin	181	88	29	64	77	36	68	180	99.4	154	85.1					27	14.9
Tobramycin	182	140	10	32	146	3	33	182	100.0	175	96.2					7	3.8
Piperacillin §	184	120	11	53	112	10	62	177	96.2	170	92.4	6	5.4			8	4.3
SXT °	183	143		40	142		41	180	98.4	181	98.9	1	0.7	1	2.4		
Total*	2375	1570	74	731	1559	86	729	2355	99.2	2278	95.9	7	0.4	3	0.4	87	3.7

Arbitration outcome:

*The Phoenix System detected an ESBL in 7 strains (1 *C. freundii*, 1 *E. coli*, 4 *K. pneumoniae* and 1 *K. oxytoca*) which were confirmed with manual tests. MicroScan did not have this information available. Consequently, two perceived major errors with *K. oxytoca* in PHX were arbitrated in favor of PHX (=VME in MSC).

§ All 6 piperacillin discrepancies arbitrated in favor of PHX (= ME in MSC).

°Both SXT categorical errors arbitrated in favor of MSC (one VME with *S. marcescens* and one ME with *P. mirabilis*).

Table 4
Comparison of Phoenix AST to MicroScan AST with 79 strains of Staphylococci

Antimicrobial	Phoenix				MicroScan			Essential Agreement		Categorical Agreement		PHX=S MSC=RM		PHX=R SC=S		minor	
	N	S	I	R	S	I	R	N	% EA	N	% CA	N	%	N	%	N	%
Clindamycin	79	47	1	31	46	1	32	78	98.7	78	98.7	1	3.1				
Ciprofloxacin	79	42	1	36	43		36	79	100.0	78	98.7					1	1.3
Cefazolin §	79	28		51	25		54	73	92.4	72	91.1	4	7.4	2	8.0		
Gentamicin	78	48		30	48		30	78	100.0	78	100.0						
Meropenem §	48	12		36	9		39	41	85.4	41	85.4	4	10.3	2	22.2		
Ofloxacin	49	21	1	27	22		27	48	98.0	48	98.0					1	2.0
Oxacillin *	79	27		52	25		54	78	98.7	73	92.4	4	7.4	2	8.0		
Penicillin	79	9		70	8		71	68	86.1	74	93.7	1	1.4	2	25.0		
Rifampin	79	68		11	69		10	77	97.5	76	96.2	1	10.0	2	2.9		
SXT	79	56		23	59		20	76	96.2	76	96.2	1	5.0	2	3.4		
Tetracycline	78	70		8	70	1	7	78	100.0	77	98.7					1	1.3
Teicoplanin	79	74	2	3	74	5		79	100.0	74	93.7					5	6.3
Vancomycin	79	78		1	79			79	100.0	78	98.7			1	1.3		
Total	964	580	5	379	577	7	380	929	96.4	913	94.7	18	4.7	13	3.4	8	0.8

MSC skips the 0.5 µg/mL dilution for oxacillin

Arbitration outcome:

* 3 of 4 VME with Oxacillin were with *S. lugdunensis* which were *MecA* negative and penicillin « S ». We believe NCCLS breakpoint inappropriate for this organism, and we have to assume both systems are correct. [Zafar Hussain *et al.* 2000. J. Clinical Microbiol. 38 (2):752-754].

§ 3 of 4 VME associated with Oxacillin discrepancies and associated expert rules. If Meropenem and Cefazolin were interpreted independent of the oxacillin result, both systems would be in agreement and would have been interpreted as susceptible.

Table 5
Comparison of Phoenix AST to MicroScan AST with 44 strains of Enterococci

Antimicrobial	Phoenix				MicroScan			Essential Agreement		Categorical Agreement		PHX=S MSC=R		PHX=R MSC=S		minor	
	N	S	I	R	S	I	R	N	% EA	N	% CA	N	%	N	%	N	%
Ciprofloxacin	40	14	2	24	14	3	23	40	100.0	37	92.5					3	7.5
Ofloxacin	12	3	1	8	3		9	12	100.0	11	91.7					1	8.3
Penicillin §	40	23		17	21		19	40	100.0	38	95.0	2	10.5				
HL Gentamicin*	40	20		20	21		19	40	100.0	39	97.5			1	4.8		
HL Streptomycin *	40	20		20	20		20	40	100.0	40	100.0						
Tetracycline °	40	18		22	17		23	40	100.0	39	97.5	1	4.3				
Teicoplanin	40	37	1	2	37		3	40	100.0	39	97.5					4	2.5
Vancomycin	40	36		4	36		4	40	100.0	40	100.0						
Total	292	171	4	117	169	3	120	287	99.3	277	95.8	3	1.0	1	0.3	8	2.8

Arbitration outcome:

§ Two strains PHX=S, MSC=R. In both cases, PHX MIC = 8 mcg/mL and MSC MIC >8 mcg/mL/ One strain arbitrated in favor of PHX and one strain in favor of MSC.

* Essential agreement cannot be performed as these are 1-well tests.

° The 1 PHX=R, MSC=S, resolved in Phoenix's favor.

CONCLUSIONS

For gram-negatives: PHX results were very comparable to MSC. Following arbitration, PHX was found to be slightly superior to MSC based on ESBL test in PHX.

For Staphylococcus: The performance of PHX was very comparable to MSC. Differences in the performance were mostly associated with interpretation of Oxacillin resistance in *S. lugdunensis* where interpretive guidelines such as NCCCLS are currently uncertain and likely to change.

For Enterococcus: The performance of PHX was nearly identical to MSC. There were 2 significant discordances between the systems with Penicillin which were unfortunately not arbitrated.

In either case, Penicillin is not the best indicator drug for penicillins and amino-penicillin class of antibiotics with Enterococci. The performance of the system with ampicillin would have been preferable, but this agent was not in either PHX or MSC panels used in this study.

The overall AST performance of the Phoenix System is nearly equivalent to that of the MicroScan WalkAway overnight system, and provides an alternative in automated AST testing of GNB and GPC for the clinical laboratory. Phoenix offers the advantages of a more rapid AST result, better automation (no reagent management, fewer moving parts) and better workflow.