

Comparative Study of Identification and Antimicrobial Susceptibility Testing of *Enterobacteriaceae* and Non-Fermentative Gram-Negative Organisms with the Phoenix™ Automated Microbiology System and Microscan Walkaway.

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REVISED ABSTRACT

The Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) is designed for the rapid identification (ID) and antimicrobial susceptibility testing (AST) of clinically significant human bacterial pathogens. In this side-by-side study, we evaluated the performance accuracy of the Phoenix system with the Microscan Walkaway (Dade Behring, Sacramento, CA) for ID and AST of a broad range of Gram-negative clinical and challenge isolates of *Enterobacteriaceae* (N=25 *E. coli*, 124 non-*E. coli*, 14 challenge isolates) and non-fermentative Gram-negative bacilli (N=26 *P. aeruginosa*, 19 non-*Pseudomonas*, 8 challenge isolates). Panels used included: Phoenix Gram-negative NMIC/ID-108, and Microscan dried overnight Neg BP Combo Panel Type 30. All panels were inoculated per the respective manufacturer's directions. Identification results were compared for genus and species agreement. The API 20E and API 20 NE (bioMérieux, Durham, NC) were used to resolve discordant ID results. The Bauer Kirby Disc Diffusion method served as the reference for resolving discordant AST results. The overall Phoenix ID agreement with the Microscan to the genus and species level was as follows: *Enterobacteriaceae*: 98% and 97%, respectively; *P. aeruginosa* including non-aeruginosa species 100% and 97.7%, respectively; non-pseudomonal challenge isolates (37.5%, 0%). Both systems incorrectly identified the majority of the non-fermentor challenge isolates. For AST results, the essential agreement for *Enterobacteriaceae* was 99.5%; the very major, major, and minor error rates were 0.3%, 0.2%, and 3.2%, respectively. For non-fermentors, the essential agreement was 95%, the very major, major, and minor error rates were 0.7%, 1.3%, and 4.5%. Of the 14 ESBL producing organisms, 6 were flagged as ESBL producers by the Phoenix system expert rules; the Microscan flagged all 14. Overall, there was good correlation between the Phoenix and Microscan Walkaway for ID and AST of *Enterobacteriaceae* and non-fermentative Gram-negative microorganisms. The Phoenix System is reliable for ID and AST of the majority of clinical strains encountered in the clinical microbiology laboratory.

INTRODUCTION

The Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, MD) is designed for the rapid identification (ID) and antimicrobial susceptibility testing (AST) of clinically significant human bacterial pathogens. The system includes an instrument, software, disposable panels, broths for ID and AST, and an AST indicator. The ID method employs modified conventional, fluorogenic, and chromogenic substrates. The AST method is a broth-based microdilution test that utilizes a redox indicator to enhance the detection of organism growth. The instrument capacity is 100 test panels. The disposable test panels contain 136 microdilution wells and are available in ID, ID/AST, and AST only formats. The panels are read at 20-minute intervals by the instrument. IDs, minimal inhibitory concentrations (MICs), and category interpretations are generated. Final results are available in 2–12 hours for ID and 4–16 hours for AST.

In this head-to-head study, we compared the Phoenix System to our current system, the Microscan Walkaway (Dade Behring, Sacramento, CA), for the ID and/or AST of *Enterobacteriaceae* and non-fermenters.

MATERIALS AND METHODS

Clinical strains. Fresh clinical isolates were tested and reflected our laboratory's routine as to the species mix. To ensure the inclusion of a broad range of isolates, the number of *E. coli* and *P. aeruginosa* tested was limited to 25, respectively. The remaining isolates consisted of 124 non-*E. coli*, 14 challenge isolates for the detection of Extended Spectrum Beta Lactamase (ESBL), 25 non-*Pseudomonas*, and 8 non-fermentative challenge isolates. The respective "challenge isolates" were received as "unknowns" and kindly provided by Drs. Ken Thomson, Creighton University, and Peter Gilligan, U. North Carolina.

Each isolate for ID/AST testing was setup concurrently in the Phoenix System and in the Microscan Walkaway for genus/species identification and category susceptibility determinations, respectively.

AST. All panels, Phoenix NMIC/ID-108 and Microscan dried overnight Neg BP combo Panel Type 30, were inoculated per the respective manufacturer's directions. In addition, specific detection of ESBL producing organisms was performed and interpretations were based on the respective ESBL detection rules for each system, BDXpert system (Phoenix) and LabPro Alert (Microscan). AST evaluation was only performed for isolates with concordant/resolved results. Category concordance was evaluated after application of Phoenix Expert System with only antibiotics that are tested in both systems.

Discrepancy Resolution. The identification results from the Phoenix System and the Microscan System were compared for genus agreement and species agreement. The ID was considered correct when the two systems agreed to the genus and species level. If the species were not in agreement for the two systems, the isolate was retested (in duplicate) in parallel in both systems. If discrepancy remained, the organism was tested with API-20E or API 20NE (bioMérieux, Durham, NC). Whichever of the discrepant IDs (Phoenix or Microscan) for the isolate agreed with the ID result of the third method the ID was considered to be correct. If the third result did not agree with either of the original discrepant identifications, the results for the isolate in question was rejected from the study.

If the AST results were discrepant, retests were performed in parallel in both systems and the respective Expert system applied for category concordance determination for the repeat results. If the AST results remained discrepant, the isolate was tested using the Bauer Kirby Disc Diffusion. Error rates (Very Major Error, Major Error and Minor Error) were calculated for each system compared to disk diffusion; only Very Major and Major errors were resolved.

Quality Control. Quality control stains were tested in all systems according to the respective manufacturer's recommendations.

RESULTS

Table 1. Identification Results for *Enterobacteriaceae* and Non-Fermenters

organism	% of Phoenix ID			API 20E ID
	n	concordant	discordant	
Enterobacteriaceae:				API 20E ID
<i>Klebsiella pneumoniae</i>	45	100	0	
<i>E. coli</i>	25	100	0	
<i>Enterobacter cloacae</i>	25	100	0	
<i>Proteus mirabilis</i>	18	100	0	
<i>Enterobacter aerogenes</i>	11	100	0	
<i>Serratia marcescens</i>	10	100	0	
<i>Klebsiella oxytoca</i>	9	100	0	
<i>Proteus vulgaris</i>	1	100	0	
<i>Morganella morganii</i>	1	100	0	
<i>Salmonella</i> sp.	1	100	0	
<i>Citrobacter freundii</i>	1	100	0	
<i>Pantoea (Enterobacter) agglomerans</i>	1	100	0	
<i>Yokenella (Koserella) regensburgei</i>	1	0	100	<i>Hafnia alvei</i> ¹
<i>Serratia fonticola/Enterobacter aerogenes</i>	1	0	100	<i>Enterobacter aerogenes</i> ²
TOTAL:	149			
Non-fermenters:				API 20NE ID
<i>Pseudomonas aeruginosa</i>	26	92.3	7.7	<i>mucoïd Pseudomonas aeruginosa</i> ³
<i>Acinetobacter baumannii</i>	8	100	0	
<i>Stenotrophomonas maltophilia</i>	8	100	0	
<i>Chromobacter violaceum</i>	1	100	0	
<i>Moraxella</i> spp.	1	0	100	<i>Kingella kingae</i> ⁴
<i>Pseudomonas fluorescens/putida</i>	1	100	0	
TOTAL:	45			

Overall Concordance: 98.7% (*Enterobacteriaceae*), 97.7% (Non-fermenters)

¹ Phoenix ID: *Hafnia alvei*

² Phoenix ID: *Enterobacter aerogenes/cloacae*

³ Phoenix ID: *Pseudomonas putida/aeruginosa (Pseudomonas fluorescens/putida upon repeat testing)*

⁴ Phoenix ID: *Kinaella kinae*

Table 2. Summary of Identification Results of Challenge Isolates

Microscan ID	Phoenix ID	Reference ID
<i>Ralstonia pickettii</i>	<i>Ralstonia pickettii</i>	<i>Ralstonia mannitolytica</i>
<i>Ralstonia pickettii</i> (51%) <i>Burkholderia cepacia</i> (42%)	<i>Burkholderia cepacia/Ralstonia pickettii</i>	<i>Ralstonia mannitolytica</i>
<i>Burkholderia cepacia</i>	No ID	<i>Burkholderia multivorans</i>
<i>Alcaligenes xylosoxidans</i>	<i>Moraxella</i> spp.	<i>Pandoraea pnomenus</i>
No ID	<i>Burkholderia cepacia</i>	<i>Burkholderia cenocepacia</i>
<i>Vibrio/Chryseobacterium</i>	<i>Burkholderia cepacia</i>	<i>Burkholderia cenocepacia</i>
No ID	No ID	<i>Burkholderia cenocepacia</i>
<i>Acinetobacter baumannii</i>	<i>Burkholderia</i> spp./ <i>Ralstonia</i> spp.	<i>Burkholderia gladioli</i>

Fig. 1. Enterobacteriaceae AST Results

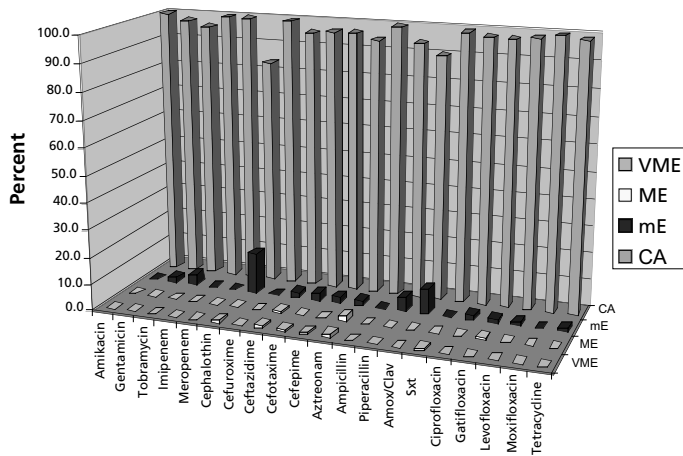


Fig. 2. Non-fermenter AST Results

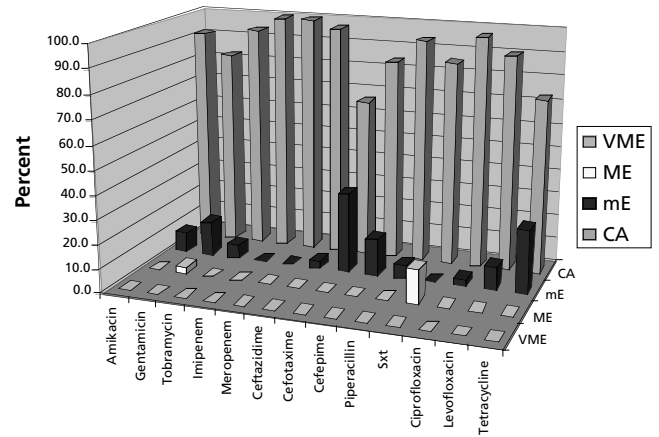


Table 3. Detection of ESBL Producing Challenge and Clinical Isolates

Organism	MicroSCAN	Phoenix	Disk Confirmation	ESBL	Other Beta Lactamase
CHALLENGE STRAINS					
<i>Klebsiella pneumoniae</i>	?ESBL	1505		SHV-3-like	SHV-1-like
<i>Klebsiella pneumoniae</i>	?ESBL	1505		CTX-M-19	SHV-1-like and TEM-1-like
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert		SHV-4-like	PSE-like, FOX-like AmpC, and SHV-1-like
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert		SHV-5 and SHV-3	ACT-1 AmpC, SHV-1, and TEM1
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert		SHV-3-like	SHV-1-like
<i>Klebsiella oxytoca</i>	?ESBL	No Alert		SHV-3-like	DHA-like AmpC, K1, and TEM-1-like
<i>Klebsiella oxytoca</i>	?ESBL	1505		SHV-1-like	K1 and TEM-1-like
<i>Klebsiella oxytoca</i>	?ESBL	1505		TEM-3-like	K1
<i>Escherichia coli</i>	?ESBL	1505		Tem-12	TEM-1
<i>Escherichia coli</i>	?ESBL	1505		TEM-10	
<i>Serratia marcescens</i>	?ESBL ¹	No Alert		SHV-4-like	AmpC (chromosomal)
<i>Enterobacter cloacae</i>	?ESBL ¹	No Alert		SHV-5-like	AmpC (chromosomal) and TEM-1
<i>Citrobacter koseri</i>	?ESBL ¹	No Alert		SHV-7	TEM-1-like and OXA-like
<i>Proteus mirabilis</i>	?ESBL ¹	No Alert		CTX-M-2	TEM-1-like
CLINICAL STRAINS					
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert	ND		
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert	ND		
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert	Negative		
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert	Negative		
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert	Negative		
<i>Klebsiella pneumoniae</i>	?ESBL	No Alert	Negative		
<i>Klebsiella pneumoniae</i>	?ESBL	1505	Positive		
<i>Escherichia coli</i>	?ESBL	1505	Negative		
<i>Escherichia coli</i>	?ESBL	No Alert	Negative		

?ESBL – Positive ESBL Screen as defined by software using CLSI guidelines (no confirmation available on panel)
 ?ESBL¹ – Positive ESBL Screen as defined by user (no confirmation available on panel)
 1505 – Phoenix alert for a confirmed ESBL producer
 ND – Not Done

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SUMMARY OF RESULTS

I. Bacterial Identification

- Results of identification for the *Enterobacteriaceae* and non-fermentative gram-negative bacilli are summarized in Table 1. When compared to the Microscan, the Phoenix system correctly identified (genus and species) 98.7% of the *Enterobacteriaceae*. The two discrepant identifications associated with the Phoenix system and initially identified by the Microscan as *Yoknella (Koserella) regensburgei* and *Serratia fonticola/Enterobacter aerogenes* required additional testing by the reference method. Following confirmatory testing, the Phoenix system correctly identified both isolates, which resulted in a final identification concordance rate of 100%.
- Of the 45 non-fermentative gram-negative bacilli, two discrepant identifications were noted with the Phoenix system. One isolate, identified correctly by the Microscan as *P. aeruginosa* (mucoid strain) was incorrectly identified by the Phoenix as *P. putida/aeruginosa*, *P. fluorescens/putida* (repeat testing). The second discrepant isolate, following additional testing with the reference method, was correctly identified by the Phoenix as *Kingella kingae*; the Microscan incorrectly identified the isolate as *Moraxella*. The overall accuracy of the Phoenix system for identifying common non-fermentative gram-negative bacilli was 97.7%.
- All challenge isolates were incorrectly identified, primarily at the species level, by both systems (Table 2). This observation is not unexpected since these species and the genus, *Pandoraea*, are not currently included in the taxonomical databases of the respective instruments.

II. AST

- AST results for the *Enterobacteriaceae* were as follows: essential agreement: 99.5%; the rates of Very Major, Major, and Minor discrepancies were 0.3%, 0.2%, and 3.2%, respectively (Fig. 1).
- AST results for the non-fermentative gram-negative bacilli were as follows: essential agreement: 95%; Very Major, Major, and Minor discrepancies were, 0.7%, 1.3%, and 4.5%, respectively (Fig. 2).
- Of the 14 ESBL producing challenge isolates, previously characterized with regard to ESBL type and other β -lactamases, the Microscan flagged all 14 as potential ESBL isolates that would require confirmatory testing whereas the Phoenix AST expert system accurately detected six (43%, Table 3). One potential problem that may have accounted for some ESBLs to be undetectable is that AmpC β -lactamases can mask detection of ESBLs. It should also be noted that in the United States, the Phoenix only detects ESBL production for *E. coli*, *K. pneumoniae*, and *K. oxytoca* based on CLSI screening guidelines. Since many of the default settings associated with the Phoenix BDXpert system were disabled during this study this would account for the failure of the Phoenix to detect the four respective ESBL producing isolates of *Serratia*, *Enterobacter*, *Citrobacter*, and *Proteus*.
- Based on the Microscan screening software, 9 clinical strains were detected as possible ESBL producing microorganisms. Following confirmatory testing, 6 were negative, one was positive and confirmatory testing was not done on two isolates. The Phoenix correctly detected the confirmed ESBL producing *K. pneumoniae*, and no alert was generated for the confirmed negative strains in addition to the two isolates for which confirmatory testing was not done (Table 3).

CONCLUSION

- The Phoenix system compared favorably with the Microscan Walkaway and is an acceptable alternative for the ID and AST testing of gram-negative organisms.