

Evaluation of the BD Phoenix™ Automated Microbiology System for Fluoroquinolone Susceptibility Testing of Gram Negative Bacilli

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REVISED ABSTRACT

■ The capability of the BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) as a means for performing same-day susceptibility testing with fluoroquinolones versus gram-negative bacilli was evaluated in six geographically diverse medical center laboratories. Clinical isolates and a collection of challenge strains were tested in both Phoenix and the NCCLS broth microdilution reference method. When results obtained with ciprofloxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, and ofloxacin were combined, the Phoenix System yielded 98.5% essential accord (EA) (range = 97.8-99.1% with individual drugs) and 95.5% categorical agreement (range = 94.5-97.8% with individual drugs) in comparison to results obtained with reference panels for approximately 3,000 isolates. Very major error rates with individual fluoroquinolones ranged from 0.4-1.5%. Major errors were uncommon (i.e., 0.2%) and minor error rates ranged from 2.1-5.4%. When individual organism groups were analyzed separately versus specific fluoroquinolones, only *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Enterobacter* spp. other than *E. aerogenes* and *E. cloacae*, miscellaneous non-enteric gram-negative bacilli, and *Burkholderia cepacia* isolates yielded overall categorical agreement rates of <90% with at least three fluoroquinolones. These were generally due to minor errors which were largely within EA. Very major error rates of greater than 5% were noted only with *Serratia marcescens* and gatifloxacin (4/20), levofloxacin (1/8), lomefloxacin (2/27), moxifloxacin (1/19), and norfloxacin (1/12) and with *Stenotrophomonas maltophilia* and ofloxacin (1/19). In conclusion, the BD Phoenix™ Automated Microbiology System was shown to be a reliable means for performing same-day fluoroquinolone susceptibility testing with *Enterobacteriaceae* and non-enteric gram-negative bacilli.

INTRODUCTION

Automated antimicrobial susceptibility test (AST) systems are commonly used in clinical microbiology laboratories to generate information that is used in the management of patients with infection. Advantages of such systems versus manual methods include possible labor savings, potential linkage to hospital information systems permitting easy reporting of pertinent results, opportunities for review of results by artificial intelligence systems, and the potential for improved intra- and inter-laboratory analytical reproducibility. In addition, improved turn-around time may be achieved. Previous studies suggest that rapid generation of results from automated AST systems can have a significant impact on both timely changes in antimicrobial therapeutic regimens and patient outcome.

Current examples of automated short-incubation susceptibility test systems include the VITEK system (bioMérieux Inc., Hazelwood, MO) and the MicroScan WalkAway System (Dade Behring, Inc., West Sacramento, CA). This study evaluated the capacity of the short-incubation Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) as a means of providing accurate fluoroquinolone susceptibility test results with gram-negative bacilli. Minimum inhibitory concentration (MIC) values and interpretative categories obtained with the Phoenix System were compared to those determined with the NCCLS reference broth microdilution method.

MATERIALS AND METHODS

Organisms

Two medical center laboratories in California, and one each in Iowa, Louisiana, New Jersey, and North Carolina examined between 450 and 550 distinct isolates of gram-negative bacilli. Approximately 43% of these organisms were fresh clinical isolates (less than 7 days old), with remaining organisms consisting of a combination of approximately 25% recent clinical isolates (7-60 days old), approximately 28% frozen stock clinical isolates (greater than 60 days old), and a collection of challenge organisms provided by the instrument manufacturer. Organisms were propagated on trypticase soy agar containing 5% defibrinated sheep blood (TSA II; BD Diagnostic Systems) with 18-24 h incubation in 35°C ambient air.

Antimicrobial susceptibility testing

Isolated colonies were suspended in BD Phoenix™ ID Broth to a turbidity equivalent to a 0.5 or 0.6 McFarland standard. One drop of BD Phoenix™ AST Indicator was added to BD Phoenix™ AST Broth tubes prior to addition of 25 µL of the test organism suspension. Phoenix panels were inoculated with this suspension within 30 minutes of initial preparation and placed into the BD Phoenix™ Automated Microbiology System for incubation at 35°C and continuous reading.

The NCCLS broth microdilution procedure served as the reference method for this study. PASCO (PASCO Laboratories, BD Diagnostic Systems) manufactured the frozen broth microdilution panels. Manufacture and testing followed NCCLS

recommendations. A 500 µL aliquot of the initial suspension in Phoenix™ ID Broth was transferred into a 12.5 mL tube of PASCO diluent broth. PASCO panels were inoculated with this suspension and incubated for 16-20 h at 35°C in ambient air. Results obtained with ciprofloxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, and ofloxacin were compared to those obtained with the Phoenix System.

Quality control strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and ATCC 35032, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213 were included in each run.

MIC interpretation and data analysis

FDA MIC breakpoints were used in assigning categories of susceptible (S), intermediate (I), or resistant (R). Results from Phoenix™ panels (PHX) were compared to corresponding NCCLS broth microdilution (REF) results. Essential accord was defined as MIC values with PHX being equal to or within ± 1 dilution of the corresponding REF value. Categorical agreement occurred when the same susceptibility category was obtained with both PHX and REF. Very major errors were defined as a REF result of R and a PHX result as S. The percentage of very major errors was calculated only for REF resistant isolates. Major errors were defined as a REF result of S and a PHX result of R. The percentage of major errors was calculated only for REF susceptible isolates. All other category discrepancies were considered minor.

RESULTS

The mean length of time to provision of test results with the Phoenix System was 8.06 h (range = 4.51-16.01 h). Overall essential accord was noted with 98.5% of comparisons. Essential accord percentages for individual fluoroquinolones ranged from 97.8% (norfloxacin) to 99.1% (gatifloxacin; Table 1). Categorical agreement was seen with 95.5% of comparisons when all fluoroquinolones tested were combined (range = 94.5-97.8% with individual agents). The rate of very major errors when data from all fluoroquinolones were combined was 0.6%. The overall major error rate was 0.1%; minor error rates ranged from 2.1% with moxifloxacin to 5.4% with norfloxacin.

When results obtained with all fluoroquinolones tested in the Phoenix System were combined, five groups of organisms yielded results that differed significantly from those obtained with the reference method. *Burkholderia cepacia* yielded essential accord and categorical agreement values of 88.9% and 82.2%, respectively, and a minor error rate of approximately 18% (Table 2). *Enterobacter* species other than *E. aerogenes* or *E. cloacae*, miscellaneous non-enteric gram-negative bacilli, *Serratia marcescens* and *Stenotrophomonas maltophilia* yielded categorical agreement values of less than 90% (range 78.5%-89.0%) and minor error rates of greater than 10% (range 11.0%-20.0%).

Only 6 (3.0%) of the 197 different fluoroquinolone-organism com-

binations examined yielded essential accord values of less than 90%. These included *Aeromonas* species, *B. cepacia*, *Proteus vulgaris/penneri*, *Pseudomonas* species, and *S. maltophilia* versus a variety of fluoroquinolones. Essential accord values for these combinations ranged from 83.3% to 89.0% (data not presented).

Categorical agreement rates of less than 90% were obtained with *S. marcescens* versus six different fluoroquinolones (Table 3), a very major error rate of greater than 5% versus five fluoroquinolones (Table 4), and a minor error rate of greater than 10% when tested versus three fluoroquinolones (Table 3). Similar observations were made with *S. maltophilia*. *B. cepacia*, *Enterobacter* species other than *E. aerogenes* or *E. cloacae*, and miscellaneous non-enteric gram-negative bacilli yielded categorical agreement rates of less than 90% and minor error rates of greater than 10% (Table 3) with three different fluoroquinolones.

Only 3 (1.5%) of the 197 fluoroquinolone-organism combinations yielded major error rates of greater than 1% (data not presented). These combinations included levofloxacin versus *S. maltophilia* (2.5%) and miscellaneous non-enteric gram-negative bacilli (5.9%), and ofloxacin versus *S. maltophilia* (3.0%).

Table 1
Performance of the BD Phoenix™ Automated Microbiology System for fluoroquinolone susceptibility testing of approximately 3000 clinical isolates of gram-negative bacilli.

Fluoroquinolone	n	Essential Accord (%)	Categorical Agreement (%)	Very Major Error Rate (%)	Major Error Rate (%)	Minor Error Rate (%)
ciprofloxacin	2973	98.3	95.0	0.6	0.0	4.8
gatifloxacin	2169	99.1	96.2	1.5	0.0	3.6
levofloxacin	2978	98.8	96.1	0.7	0.2	3.6
lomefloxacin	2724	98.3	94.9	0.4	0.1	5.0
moxifloxacin	2229	98.6	97.8	0.5	0.0	2.1
norfloxacin	3000	97.8	94.5	0.6	0.0	5.4
ofloxacin	2970	98.8	94.8	0.4	0.2	5.0

Gram-Negative Bacillus Organism Group	n	Essential Accord (%)	Categorical Agreement (%)	Very Major Error Rate (%)	Major Error Rate (%)	Minor Error Rate (%)
<i>Acinetobacter baumannii</i>	430	97.7	96.3	0.4	0.0	3.5
<i>Acinetobacter</i> species	39	100.0	100.0	0.0	0.0	0.0
<i>Aeromonas</i> species	18	94.4	94.4	0.0	0.0	5.6
<i>Burkholderia cepacia</i>	45	88.9	82.2	0.0	0.0	17.8
<i>Citrobacter freundii</i>	579	98.6	92.6	0.0	0.0	7.4
<i>Citrobacter koseri</i>	329	100.0	99.1	0.0	0.0	0.9
<i>Citrobacter</i> species	222	100.0	98.2	0.0	0.0	1.8
<i>Enterobacter aerogenes</i>	819	98.4	97.6	0.0	0.0	2.4
<i>Enterobacter cloacae</i>	1939	99.3	96.0	0.0	0.0	4.0
<i>Enterobacter</i> species	154	99.4	89.0	0.0	0.0	11.0
<i>Escherichia coli</i>	4131	99.7	99.3	0.4	0.0	0.6
<i>Klebsiella oxytoca</i>	510	99.2	95.5	0.0	0.0	4.5
<i>Klebsiella pneumoniae</i>	2866	98.2	95.4	0.7	0.0	4.5
<i>Klebsiella</i> species	4	100.0	100.0	0.0	0.0	0.0
Miscellaneous enteric	110	100.0	100.0	0.0	0.0	0.0
Miscellaneous non-enteric	88	98.9	85.2	0.0	2.2	13.6
<i>Morganella morganii</i>	433	99.1	97.5	0.0	0.0	2.5
<i>Pantoea agglomerans</i>	110	96.4	94.5	0.0	0.0	5.5
<i>Proteus mirabilis</i>	1089	99.5	96.3	0.0	0.0	3.7
<i>Proteus vulgaris/penneri</i>	181	98.3	97.8	0.0	0.0	2.2
<i>Providencia stuartii</i>	320	99.4	95.6	0.0	0.0	4.4
<i>Providencia</i> species	111	100.0	99.1	0.0	0.0	0.9
<i>Pseudomonas aeruginosa</i>	2595	98.1	93.1	0.5	0.3	6.5
<i>Pseudomonas</i> species	62	95.2	93.5	0.0	0.0	6.5
<i>Salmonella typhi</i>	21	100.0	100.0	0.0	0.0	0.0
<i>Salmonella</i> species	205	100.0	100.0	0.0	0.0	0.0
<i>Serratia marcescens</i>	995	94.4	87.7	7.7	0.0	11.3
<i>Serratia</i> species	146	95.9	98.6	0.0	0.0	1.4
<i>Shigella sonnei</i>	56	100.0	100.0	0.0	0.0	0.0
<i>Shigella</i> species	14	100.0	100.0	0.0	0.0	0.0
<i>Stenotrophomonas maltophilia</i>	400	93.0	78.5	1.6	2.2	20.0
<i>Yersinia enterocolitica</i>	22	100.0	100.0	0.0	0.0	0.0

Table 2
Performance of the BD Phoenix™ Automated Microbiology System for fluoroquinolone susceptibility testing of approximately 3000 clinical isolates of gram-negative bacilli.

Gram-Negative Bacillus Organism Group	n	Fluoroquinolone	Categorical Agreement (%)	Minor Error Rate (%)
<i>Serratia marcescens</i>	143	ciprofloxacin	83.9	16.1
		gatifloxacin	88.1	9.1
		levofloxacin	88.1	11.2
		lomefloxacin	88.7 ^a	9.9 ^a
		norfloxacin	89.5	9.8
		ofloxacin	84.5 ^a	14.8 ^a
<i>Enterobacter</i> species	22	ciprofloxacin	77.3	22.7
		gatifloxacin	86.4	13.6
		norfloxacin	86.4	13.6
		ofloxacin	86.4	13.6
<i>Burkholderia cepacia</i>	15	levofloxacin	86.7	13.3
		norfloxacin	73.3	26.7
		ofloxacin	86.7	13.3
<i>Stenotrophomonas maltophilia</i>	100	ciprofloxacin	77.0	22.0
		norfloxacin	70.0	30.0
		ofloxacin	77.0	20.0
Miscellaneous non-enteric	22	ciprofloxacin	86.4	13.6
		levofloxacin	81.8	13.6
		norfloxacin	81.8	18.2
<i>Aeromonas</i> species	6	levofloxacin	83.3	16.7
<i>Pantoea agglomerans</i>	16	ofloxacin	87.5	12.5
<i>Providencia stuartii</i>	44	gatifloxacin	86.4	13.6
<i>Pseudomonas</i> species	16	ciprofloxacin	87.5	12.5

^aPercentage based on 142 isolates

Table 3
Combinations of fluoroquinolones and gram-negative bacilli demonstrating less than 90% categorical agreement when reference NCCLS broth microdilution results were compared to those obtained with the BD Phoenix™ Automated Microbiology System.

Table 4
 Combinations of fluoroquinolones and gram-negative bacilli yielding very major error rates of greater than 5% when reference NCCLS broth microdilution results were compared to those obtained with the BD Phoenix™ Automated Microbiology System.

Gram-Negative Bacillus Organism Group	Number of Resistant Strains	Fluoroquinolone	Very Major Error Rate (%)
<i>Serratia marcescens</i>	20	gatifloxacin	20.0
	8	levofloxacin	12.5
	27	lomefloxacin	7.4
	19	moxifloxacin	5.3
	12	norfloxacin	8.3
<i>Stenotrophomonas maltophilia</i>	19	ofloxacin	5.3

CONCLUSIONS

■ The BD Phoenix™ Automated Microbiology System was characterized by greater than 95% essential accord and categorical agreement in comparison to a reference broth microdilution method for fluoroquinolone susceptibility testing of gram-negative bacilli.

■ Overall rates of very major and major errors generated by this system when used for fluoroquinolone susceptibility testing of gram-negative bacilli were low (less than 0.6%).

■ Categorical agreement for *B. cepacia*, *Enterobacter* species other than *E. aerogenes* and *E. cloacae*, miscellaneous non-enteric gram-negative bacilli, *S. marcescens* and *S. maltophilia* was <90% for at least three fluoroquinolones, due to minor errors which were largely within EA. However, the numbers of isolates of these species examined in this study were low. Further study may be required.

■ In general, the BD Phoenix™ Automated Microbiology System was easy to use and performed well in generating rapid and accurate fluoroquinolone susceptibility test results with enteric and non-enteric gram-negative bacilli.

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