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Short Communication

Antimicrobial susceptibility testing performed *via* BD Phoenix™, broth microdilution, and disk diffusion for *Pseudomonas aeruginosa*

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ABSTRACT

Antimicrobial susceptibility testing is often used to guide antibiotic selection for cystic fibrosis (CF) exacerbations; however, there is concern regarding the accuracy of automated systems, particularly for *P. aeruginosa.* The primary objective of this study was to evaluate the accuracy of the BD Phoenix™ Automated Microbiology System in determining susceptibility of *P. aeruginosa.* Clinical isolates form CF respiratory specimens were included. Susceptibility testing was performed *via* broth microdilution (BMD), disk diffusion (DD), and the BD Phoenix™. Minor, major, and very major errors were compared between the three methods. Forty-seven *P. aeruginosa* isolates were included; categorical agreement was 87.8% for the BD Phoenix™ and 87.9% for DD, compared to BMD. Both methods were found to have higher than acceptable error rates.

KEYWORDS: antimicrobial susceptibility testing methods, *Pseudomonas aeruginosa*, Phoenix.

INTRODUCTION

Automated antimicrobial susceptibility testing (AST) increases microbiology laboratory efficiency and standardization of testing and reporting. However, there is concern regarding the accuracy of these systems, specifically for *Pseudomonas aeruginosa*, as some studies have shown unacceptable error rates compared to standard testing methods [1-3]. Our institution utilizes the BD Phoenix™ Automated Microbiology System (Becton, Dickinson, and Company, Franklin Lakes, NJ) to perform susceptibilities on clinical isolates, but due to concerns for possible inaccuracy, disk diffusion (DD) is used to perform susceptibilities for all *P. aeruginosa* isolates. The objective of this study was to determine the accuracy of the BD Phoenix™ and DD for *P. aeruginosa* compared to broth microdilution (BMD) susceptibility testing.

MATERIALS AND METHODS

Non-mucoid *P. aeruginosa* from cystic fibrosis respiratory specimens were included in this study. Isolates were obtained from specimens at a single, academic medical center in Charleston, South Carolina; only one isolate per patient was included. Isolates were identified *via* matrix-assisted laser ionization-time of flight mass spectrometry (MALDI-TOF-MS) and then frozen at -70 °C until AST was performed. AST was performed *via* BMD using custom panels obtained from Remel Microbiology Products (Thermo Scientific, Lenexa, KS). Susceptibilities were also performed *via* DD and the BD Phoenix™ Automated Microbiology

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System (NMIC 300 panel). All procedures were performed in accordance with the methodology established by the Clinical Laboratory Standards Institute (CLSI) or manufacturer labeling. *Escherichia coli* ATCC 35218 and *P. aeruginosa* ATCC 27853 were used for quality control with each batch. AST was only performed once per isolate. CLSI-established breakpoints or interpretive categories based on zone diameters were used to determine interpretive category: susceptible (S), intermediate (I), or resistant (R).

BMD was used as the reference AST method. The following definitions were utilized: minor error (test method is I; BMD is S or R; or vice versa), major error (test method is R; BMD is S), very major error (test method is S; BMD is R), essential agreement (test method MIC result equals BMD MIC ±1 dilution), and categorical agreement (test method interpretive category same as BMD). Major error rate calculations included susceptible strains only, while very major errors rate calculations included resistant strains only [4]. The minimal performance requirement was set at 90% overall categorical agreement between test method and reference method with less than 3% major errors and less than 1.5% very major errors [4, 5].

RESULTS

There were 47 *P. aeruginosa* isolates included in the study. Seventeen isolates (36.2%) did not grow in the Phoenix™ system, likely due to the slow growth of antibiotic-damaged strains. Of those that did grow (n = 30), the overall categorical agreement and essential agreement between BMD and BD Phoenix™ was 87.8% and 85%, respectively (Table 1). All errors that occurred were minor errors. The highest error rates were seen with levofloxacin (30%) and amikacin (20%). For meropenem, there was a very high categorical agreement (96.7%), but essential agreement was very low (76.7%). For BMD versus DD, the overall categorical agreement was 87.9%; error rates are shown in Table 2. In contrast with the BD Phoenix™, four very major errors occurred.

DISCUSSION

Overall, it was found that the categorical and essential agreement between the BD Phoenix™ and BMD was below the recommended performance requirement. Additionally, for certain antibiotics, such as levofloxacin and amikacin, there was a high rate of minor errors. A similar study found a high percentage of categorical agreement (93.1%) and essential agreement (94.2%) when the Phoenix™

Table 1. Agreement between broth microdilution and BD Phoenix™ for *Pseudomonas aeruginosa* (n = 30).

Antimicrobial agent	Broth microdilution			Phoenix™				
	Susceptibility results			Categorical agreement	Essential agreement	Minor errors	Major errors[1]	Very major errors[2]
	S	I	R	n, (%)	n, (%)	n, (%)	n, (%)	n, (%)
Piperacillin/Tazobactam	28	0	2	28 (93.3)	20 (66.7)	2 (6.7)	0 (0)	0 (0)
Cefepime	28	1	1	28 (93.3)	28 (93.3)	2 (6.7)	0 (0)	0 (0)
Meropenem	26	2	2	29 (96.7)	23 (76.7)	1 (3.3)	0 (0)	0 (0)
Tobramycin	27	2	1	28 (93.3)	28 (93.3)	2 (6.7)	0 (0)	0 (0)
Amikacin	24	3	3	24 (80)	28 (93.3)	6 (20)	0 (0)	0 (0)
Levofloxacin	19	5	6	21 (70)	26 (86.7)	9 (30)	0 (0)	0 (0)
Overall percentage				**87.8**	**85**	**12.2**	**0**	**0**

[1]Percent major error: only included susceptible isolates.
[2]Percent very major error: only included resistant isolates.

Table 2. Agreement between broth microdilution and disk diffusion for *Pseudomonas aeruginosa* (n = 47).

Antimicrobial agent	Broth microdilution			Disk diffusion			
	Susceptibility results			Categorical agreement	Minor errors	Major errors[1]	Very major errors[2]
	S	I	R	n, (%)	n, (%)	n, (%)	n, (%)
Piperacillin/Tazobactam	42	0	5	43 (91.5)	1 (2.1)	1 (2.4)	2 (40)
Cefepime	37	5	5	39 (83)	6 (12.8)	1 (2.7)	1 (20)
Meropenem	36	3	8	42 (89.4)	3 (6.4)	1 (2.8)	1 (12.5)
Tobramycin	38	4	5	43 (91.5)	3 (6.4)	1 (2.6)	0 (0)
Amikacin	32	5	10	43 (91.5)	3 (6.4)	1 (3.1)	0 (0)
Levofloxacin	24	7	16	38 (80.9)	8 (17)	1 (4.2)	0 (0)
Overall percentage				**87.9**	**8.5**	**2.9**	**8.2**

[1]Percent major error: only included susceptible isolates.
[2]Percent very major error: only included resistant isolates.

was compared to BMD for non-fermenting Gram-negative bacteria (included 55 strains of *P. aeruginosa*); however, the major error rate was 5.2% [2]. The highest error rates were seen with β-lactam antibiotics, specifically cefepime (10.3% minor errors and 15.8% major errors), and unlike our study, aminoglycosides were found to have low error rates. Another study also found unacceptable error rates with the BD Phoenix™ for *Pseudomonas* compared with BMD (n = 60) [3]. The highest rates of error were seen with aztreonam (33.3% minor error and 1.7% major error), cefepime (18.3% minor and 1.7% major errors), and ceftazidime (18.3% minor and 1.7% very major errors). An additional study determined that there was 92% categorical agreement overall between the Phoenix™ and agar dilution testing for *P. aeruginosa* (n = 100); however, imipenem and cefepime had overall agreement rates <90% [6]. In our study, it is also important to note that approximately one third (34.3%) of the *P. aeruginosa* isolates that were set up in the automated system did not grow, which leads to delays in reporting of results since manual susceptibilities must then be performed.

It was determined that there was 87.9% overall categorical agreement (8.5% minor errors, 2.9% major errors, and 8.2% very major errors) between BMD and DD. In a similar study, comparing BMD to DD for *P. aeruginosa* isolates (n = 597) from patients with CF, it was found that the overall categorical agreement between the two methods was 87.1% with 11.3% minor errors, 1.1% major errors, and 0.4% very major errors [7]. The highest rate of errors was seen with ciprofloxacin and amikacin. Our study found the highest error rates with levofloxacin (8 minor errors and 1 major error) and cefepime (6 minor errors, 1 major error, and 1 very major error). It has been hypothesized that liquid medium, such as with BMD, may lead to better detection of resistance that is mediated through efflux pumps when compared to DD, therefore more very major errors (false susceptibility) may occur [8]. Four very major errors did occur with DD in our study, while there were no very major errors by the BD Phoenix™.

It is recognized that our study has several limitations. These include that AST was only performed once on each isolate, therefore accuracy could not be guaranteed. Additionally, AST and interpretation of the results were performed by multiple study personnel, which may have led to slight variation in results. Also, despite being clinical isolates from cystic fibrosis specimens, there was only a small number of highly resistant isolates included in the study.

CONCLUSION

In conclusion, when compared to BMD, both the BD Phoenix™ and DD were found to have unacceptably high error rates in determining

susceptibilities for *P. aeruginosa*; however, very major errors only occurred with DD.

CONFLICT OF INTEREST STATEMENT

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