Title: Performance of anti-pseudomonal beta lactams on the Accelerate PhenoTest® BC kit against a collection of *P. aeruginosa* isolates

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Phenotypic antimicrobial susceptibilities are particularly valuable for *P. aeruginosa* due to the complexity of resistance mechanisms this organism can harbor. The Accelerate PhenoTest® BC kit (AXDX) provides a fast phenotypic antimicrobial susceptibility testing (AST) method for testing *P. aeruginosa* directly from positive blood culture. This study evaluates updates to the Accelerate PhenoTest® BC kit made in order to improve the performance of beta-lactams when tested against *P. aeruginosa*.(1, 2)

144 *P. aeruginosa* isolates were spiked into a blood culture bottle containing healthy donor blood and incubated until positivity. Aliquots of positive blood culture were tested on the Accelerate Pheno® system (software 1.4.1.25) as previously described.(3) AST was also performed in triplicate by CLSI reference broth microdilution (BMD) using isolated colonies.(4) MIC results were compared to BMD to calculate essential agreement (EA), categorical agreement (CA), very major (vmj, susceptible by AXDX, resistant by reference), major (maj, resistant by AXDX, susceptible by reference), and minor (min, intermediate by one AST method, susceptible or resistant by the other method) error rates.(5) For EA, BMD results were truncated to the same range as those reported by the Accelerate Pheno® system. FDA and CLSI breakpoints were applied (Table 1). (6, 7)

Table 2 provides the EA, CA, and error rates for the isolates tested on both the updated and previous assays. With respect to the updated assay and when interpreted with FDA breakpoints, nine of eleven errors observed for cefepime were within EA, including the single vmj error. Cefepime and ceftazidime do not have intermediate interpretation by FDA breakpoints; therefore, all errors can only be classified as maj or vmj for these antimicrobials.(6) When interpreted with CLSI breakpoints, all cefepime errors were min and 17/21 errors were

within EA. Bias towards a more resistant MIC for cefepime was observed by AXDX(Table 3). High cefepime min error rates with *P. aeruginosa* have been observed in various studies with other automated platforms such as Vitek®2 (9-18%), MicroScan WalkAway (32%-48%) and BD Phoenix™ (18%).(8–11) When interpreted by FDA breakpoints, a total of five errors were observed with ceftazidime and 2 of the 3 vmj errors were within EA; a good case example demonstrating the challenges with errors when an intermediate breakpoint does not exist. When interpreted with CLSI breakpoints, 1 maj and 1 vmj error remained for ceftazidime, with EA and CA above 90%. Fifteen min errors (10.4%) were observed with meropenem (Table 2), among which 9 were within EA. Eleven of the min errors were due to MIC interpreted as resistant by AXDX but intermediate by BMD.

Overall, the most notable improvements with the updated assay are within the maj and min error rates. In the original clinical trial data set for Accelerate Pheno® system, a total of 43 maj errors were observed amongst the Gram-negative organisms, with 26% for beta lactams tested against *P. aeruginosa*. This resulted in major error limitations imposed by the FDA and the aim for the updates to the assay described herein. (1) The data presented here are from a different population of isolates than those used in the original clinical trial. Specifically, the current data set was enriched to include approximately 20% of isolates with MICs at the breakpoint, allowing for a robust evaluation of performance post-improvement. Furthermore, the population described here is approximately 10% less susceptible than what is likely to be observed in clinical laboratories based on US surveillance of *P. aeruginosa* bloodstream infections.(12) This is important as differences in MIC distributions impact the propensity of errors. Therefore, direct comparisons between two different isolate sets, such as the present

data and that published in Pancholi et al, cannot be directly made. Nonetheless, the improvements described herein led to the removal of maj error limitations for piperacillin/tazobactam, meropenem, ceftazidime, and cefepime.

P. aeruginosa susceptibility testing is known to be challenging.(8–11) As technologies for susceptibility testing advance, assay development of these difficult-to-test organisms is prudent and likely an ongoing necessity. Moreover, clinical microbiology labs should seek to understand their local epidemiology when evaluating an assay as performance can vary amongst different populations of isolates. These data demonstrate markedly improved performance, particularly with respect to maj, of beta lactams against *P. aeruginosa* on the Accelerate Pheno® system compared with previous versions of the assay.

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117 **Table 1. Current FDA and CLSI designated breakpoints of anti-pseudomonal beta lactams**

Beta-lactam antibiotic	Susceptible	Intermediate	Resistant
Aztreonam (FDA & CLSI)	≤8	16	≥32
Cefepime (FDA)	≤8	-	≥16
Cefepime (CLSI)	≤8	16	≥32
Ceftazidime (FDA)	≤8	-	≥16
Ceftazidime (CLSI)	≤8	16	≥32
Meropenem (FDA & CLSI)	≤2	4	≥8
Piperacillin/tazobactam (FDA & CLSI)	≤16/4	32/4-64/4	≥128/4

118 MICs are represented in µg/mL.

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120 **Table 2.** Performance of anti-pseudomonal beta lactams tested against *P. aeruginosa* isolates on the Accelerate PhenoTest® BC
121 kitcompared with BMD.

Beta-lactam antibiotic	N	S	R	CA	EA	vmj	maj	min
***Aztreonam (FDA & CLSI)**	144	105	35	134 (93.1%)	135 (93.8%)	0 (0%)	1 (1.0%)	9 (6.2%)
Aztreonam (FDA & CLSI)	144	105	35	122 (84.7%)	124 (86.1%)	0 (0%)	1 (1.0%)	21 (14.6%)
***Cefepime (FDA)**	143	107	36	132 (92.3%)	136 (95.1%)	1 (2.8%)	10 (9.3%)	-
Cefepime (FDA)	144	108	36	84 (58.3%)	81 (56.2%)	0 (0%)	60 (55.6%)	-
***Cefepime (CLSI)**	143	107	29	122 (85.3%)	136 (95.1%)	0 (0%)	0 (0%)	21 (14.7%)
Cefepime (CLSI)	144	108	29	76 (52.8%)	81 (56.2%)	0 (0%)	2 (1.9%)	66 (45.8%)
***Ceftazidime (FDA)**	141	103	38	136 (96.5%)	136 (96.5%)	3 (7.9%)	2 (1.9%)	-
Ceftazidime (FDA)	144	104	40	46 (31.9%)	47 (32.6%)	0 (0%)	98 (94.2)	-
***Ceftazidime (CLSI)**	141	103	31	132 (93.6%)	136 (96.5%)	1 (3.2%)	1 (1.0%)	7 (5.0%)
Ceftazidime (CLSI)	144	104	33	40 (27.8%)	47 (32.6%)	0 (0%)	20 (19.2%)	84 (58.3%)
***Meropenem (CLSI & FDA)**	144	102	25	127 (88.2%)	136 (94.4%)	0 (0%)	2 (2.0%)	15 (10.4%)
Meropenem (CLSI & FDA)	144	102	25	98 (68.1%)	107 (74.3%)	0 (0%)	2 (2.0%)	44 (30.6%)
***Piperacillin/tazobactam (CLSI & FDA)**	138	101	30	130 (94.2%)	133 (96.4%)	0 (0%)	0 (0%)	8 (5.8%)
Piperacillin/tazobactam (CLSI & FDA)	144	106	31	45 (31.2%)	52 (36.1%)	0 (0%)	12 (11.3%)	52 (36.1%)

122 *Designates the performance of the improved software.
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126 **Table 3.** Error trends of beta lactam antibiotics tested against *P. aeruginosa* isolates on
127 Accelerate PhenoTest® BC kit compared with BMD.
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Beta-lactam antibiotic (n = errors)	More susceptible	More resistant	within EA
Aztreonam n=10	6	4	9
Cefepime n=11	1	10	10
Cefepime (CLSI) n=21	9	12	17
Ceftazidime n=5	3	2	2
Ceftazidime (CLSI) n=9	3	6	6
Meropenem n=17	1	16	9
Piperacillin/tazobactam n=8	1	7	6

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