



Evaluation of the Accelerate Pheno System for Identification of *Acinetobacter* Clinical Isolates and Minocycline Susceptibility Testing

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ABSTRACT The Accelerate Pheno system (AXDX) is a rapid phenotypic bacterial identification and susceptibility testing system which is approved for use with positive blood cultures. *Acinetobacter baumannii* is a nosocomial pathogen for which the limited treatment options include minocycline in the case of multidrug resistance. Here, we studied the performance of *A. baumannii* identification and minocycline susceptibility testing by AXDX using 101 contemporary *Acinetobacter* sp. clinical isolates. Overall, the sensitivity for *A. baumannii* and *A. baumannii* complex identification was 100% (73/73) and 97.6% (82/84), respectively. Specificity for *A. baumannii* complex identification was 86.6% (13/15). The essential agreement of minocycline susceptibility results ($\pm 1 \log_2$ MIC agreement) of AXDX MICs with reference broth microdilution was 98.0% (96/98). There were no very major errors or major errors. Overall, 24.5% (24/98) of results yielded minor errors. AXDX reliably identified *A. baumannii* and predicted minocycline susceptibility results, which should help guide treatment choices in a timely manner for infections where options are limited.

KEYWORDS fluorescence *in situ* hybridization, morphokinetic cellular analysis, rapid diagnostics, susceptibility testing

A *Acinetobacter baumannii* is a major causative organism of bacteremia, pneumonia, and wound infection. Healthcare-associated *A. baumannii* infections are associated with high mortality rates, due to multidrug resistance (1). *A. baumannii* is one of four major genomospecies which constitute the *A. baumannii* complex, which also includes *Acinetobacter nosocomialis*, *Acinetobacter pittii*, and *Acinetobacter calcoaceticus*. The latter three genomospecies are collectively less pathogenic in humans than *A. baumannii*.

Minocycline is one of the few agents that retains activity against multidrug-resistant *A. baumannii*, including against carbapenem-resistant strains (2), and may have clinical utility in the treatment of infections caused by such strains (3). Early identification and antimicrobial susceptibility testing (AST) of *A. baumannii* in positive blood and respiratory cultures is key to early administration of appropriate therapy and may improve the clinical outcome (4). The Accelerate Pheno system (AXDX) is a rapid bacterial identification and AST device which is currently cleared for use with positive blood cultures by the U.S. Food and Drug Administration (FDA) and under development for bronchoalveolar lavage (BAL) and mini-BAL fluid specimens (5). AXDX minocycline results are currently available as research use only (RUO), due to an insufficient number

Citation Bowler SL, Towne JW, Humphries RM, Doi Y. 2019. Evaluation of the Accelerate Pheno system for identification of *Acinetobacter* clinical isolates and minocycline susceptibility testing. J Clin Microbiol 57:e01711-18. <https://doi.org/10.1128/JCM.01711-18>.

Editor Nathan A. Ledebor, Medical College of Wisconsin

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Received 22 October 2018

Returned for modification 19 November 2018

Accepted 7 January 2019

Accepted manuscript posted online 16 January 2019

Published 27 February 2019

of *Acinetobacter* spp. encountered in the original clinical trial to allow FDA clearance (6). The aims of this study were to (i) determine the spectra of *Acinetobacter* species identified by the *A. baumannii* probe used in the Accelerate Pheno system and (ii) test the performance of the Accelerate Pheno system minocycline AST compared to reference broth microdilution (BMD) for *Acinetobacter* species.

MATERIALS AND METHODS

Bacterial isolates and identification. A total of 101 *Acinetobacter* clinical isolates recovered from unique patients at the University of Pittsburgh Medical Center between 2013 and 2017 were evaluated in this study. Based on the initial species identification at the clinical microbiology laboratory using a Bruker matrix-assisted laser desorption ionization (MALDI) Biotyper (Billerica, MA), these included 71 *A. baumannii* isolates and 30 belonging to other *Acinetobacter* genomospecies. For *A. baumannii*, strains that were nonsusceptible to minocycline were preferentially included for the purpose of AST performance evaluation. Among the isolates evaluated, 60 met the criteria for multidrug resistance (i.e., not susceptible to at least 1 agent in ≥ 3 antimicrobial categories appropriate for *Acinetobacter* spp.) and 42 met criteria for extensive drug resistance (i.e., not susceptible to at least 1 agent in all but ≤ 2 antimicrobial categories appropriate for *Acinetobacter* spp.) as defined by Magiorakos and colleagues (7).

Species identification. The species were identified by a Bruker MALDI Biotyper system (MALDI-TOF) Biotyper in the clinical microbiology laboratory at the time of isolation, which was then repeated with the same instrument at the research laboratory. In addition, 14 non-*baumannii* *A. baumannii* complex isolates and 5 non-*A. baumannii* complex isolates that did not yield a consensus identification between the two MALDI-TOF identification runs were subjected to *rpoB* sequencing for definitive species identification (8).

Antimicrobial susceptibility testing. AST was performed on each isolate by Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD), using panels prepared by Accelerate Diagnostics, Inc. For BMD, the minocycline concentrations tested ranged from 0.125 to 32 $\mu\text{g/ml}$, and minocycline was obtained from Sigma. Mueller-Hinton broth (Difco, BD, Sparks, MD) was cation adjusted in-house. BMD for minocycline was performed in triplicates using the same inoculum of bacteria, and the modal MIC was used for evaluation (6, 9). If no modal MIC was obtained, BMD testing was repeated in triplicates for the isolate, and a modal MIC was obtained from the results of the six replicate MICs. Samples that did not produce a modal MIC after six replicate BMD tests were excluded from analysis.

Species identification and AST on AXDX. Using the same source plate used to select colonies for BMD, isolates were subcultured into human blood at a concentration of 10 to 100 CFU/ml, which was then inoculated into a Bactec Plus aerobic blood culture bottle and incubated on a Bactec FX blood culture system (BD, Sparks, MD). Species identification and AST were performed using the Accelerate PhenoTest BC kit run on the Accelerate Pheno system according to the manufacturer's instructions (10). The range of minocycline MICs available on AXDX is 1 $\mu\text{g/ml}$ to 32 $\mu\text{g/ml}$.

Statistics. Sensitivity and specificity were calculated for each identification (ID) target using MALDI-TOF mass spectrometry (MS; or *rpoB* sequencing, if performed) as the comparator. Essential and categorical agreement values were calculated for minocycline for Accelerate Pheno system-based MICs using the BMD modal MIC as the gold standard. Susceptibility results were interpreted using current CLSI breakpoints (11).

RESULTS AND DISCUSSION

Identification of *A. baumannii* complex. Among the 101 isolates evaluated, 30 were initially identified as non-*baumannii* species by the clinical microbiology laboratory and were examined further by *rpoB* sequencing. These were identified as *A. pittii* ($n = 5$ isolates), *Acinetobacter ursingii* ($n = 5$), *A. calcoaceticus* ($n = 4$), *A. nosocomialis* ($n = 3$), *Acinetobacter radioresistens* ($n = 3$), *A. baumannii* ($n = 3$), *Acinetobacter lwoffii* ($n = 2$), *Acinetobacter johnsonii* ($n = 2$), *Acinetobacter baylyi* ($n = 1$), and *Acinetobacter bereziniae* ($n = 1$). The species of one isolate, initially identified as *Acinetobacter haemolyticus*, could not be identified definitively by *rpoB* sequencing. In summary, there were a total of 74 *A. baumannii* isolates and 27 non-*baumannii* *Acinetobacter* isolates.

Accelerate pheno system-based species identification. Two isolates, one *A. baumannii* and one *A. ursingii*, could not be identified due to an insufficient number of cells upon testing; this result was repeated when the isolates were tested a second time. For these two isolates, AXDX yielded an error code, signaling the laboratory would need to test by an alternative method. Of the remaining 73 *A. baumannii* isolates, all were identified as *A. baumannii* by AXDX. Among the 11 non-*baumannii* *A. baumannii* complex isolates, 5 of 5 *A. pittii*, 3 of 3 *A. nosocomialis*, and 2 of 4 *A. calcoaceticus* isolates resulted in a positive identification by the *A. baumannii* probe in the Accelerate Pheno

TABLE 1 Number of isolates of each species identified by the AXDX *A. baumannii* probe

Species	No. detected/no. tested (%)
<i>A. baumannii</i> complex ^a	82/84 (97.6)
<i>A. baumannii</i>	73/73 (100)
<i>A. nosocomialis</i>	5/5 (100)
<i>A. pittii</i>	3/3 (100)
<i>A. calcoaceticus</i>	2/4 (50)
Non- <i>baumannii</i> complex	2/15 (13.3)
<i>A. ursingii</i> ^a	1/5 (20)
<i>A. radioresistens</i>	0/3 (0)
<i>A. lwoffii</i>	0/2 (0)
<i>A. johnsonii</i>	0/2 (0)
<i>A. baylyi</i>	1/1 (100)
<i>A. bereziniae</i>	0/1 (0)

^aOne isolate yielded a “no identification” result due to an insufficient number of cells in the system.

system. The 2 *A. calcoaceticus* isolates that were not identified by AXDX yielded nonidentification upon repeat testing. Among the 15 non-*A. baumannii* complex isolates, 2 (1 *A. radioresistens* and 1 *A. baylyi*) resulted in a positive identification by the *A. baumannii* probe in the Accelerate Pheno system, while 13 resulted in nonidentification by the *A. baumannii* target probe, with 11/13 producing an “off panel” result via signal from the universal bacterial fluorescence *in situ* hybridization (FISH) probe and 2/13 producing no result due to too few cells.

Table 1 summarizes the results. Overall, the sensitivity of identifying *A. baumannii* complex strains was 97.6% (82 of 84), and the specificity was 86.6% (13 of 15).

Few studies have assessed the performance of FDA-cleared rapid ID systems for testing blood cultures for their ability to correctly identify *Acinetobacter* spp. In the multicenter trial to evaluate AXDX, 69 of 70 isolates of *A. baumannii* complex were correctly identified (98.6% sensitive), and 3 specimens negative for *A. baumannii* among a total of 1,857 tested yielded a false-positive *A. baumannii* complex result (99.8% specificity) (8). The BioFire BCID panel (bioMérieux, Durham, NC), which was designed to identify *A. baumannii* alone, was shown in a multicenter evaluation to falsely identify 5 non-*baumannii* isolates among 23 (21.7%) tested as *A. baumannii*. These included all 4 *A. pittii* isolates and the 1 *A. junii* isolate evaluated in the study. In this study, all 51 *A. baumannii* isolates were correctly identified (12). The Verigene BC-GN test kit (Luminex, Austin, TX), in contrast, identified 62 isolates of *Acinetobacter* spp. in clinical trials, of which 2 were false positives (not *Acinetobacter* spp.). Additionally, during this study, one isolate of *A. baumannii* was not detected by the Verigene BC-GN test kit. Unfortunately, in this study, the species of *Acinetobacter* were not elucidated (13). As such, laboratorians and physicians should be aware that a result of *A. baumannii* in blood from these rapid identification systems may in fact be *A. baumannii*, *A. baumannii* complex, or in some cases, non-*baumannii* complex species. Importantly, for *A. baumannii* complex species which are considered to be of clinical significance, AXDX positively identified all strains of *A. baumannii*, *A. pittii*, and *A. nosocomialis* and two of four isolates of *A. calcoaceticus*.

Accelerate Pheno system-based AST of minocycline. Minocycline is active against the majority of *A. baumannii* strains and considered one of the few treatment options available for infections caused by extensively drug-resistant *A. baumannii* (1). The susceptibility breakpoint currently approved by the CLSI is an MIC of $\leq 4 \mu\text{g/ml}$, with an MIC of $8 \mu\text{g/ml}$ considered intermediate and $\geq 16 \mu\text{g/ml}$ resistant. By the standard BMD, MICs were reliably determined for 100 of 101 tested isolates. One isolate did not grow sufficiently in the BMD panels to accurately determine the MIC. Of the 100 isolates, 85 were susceptible, 14 intermediate, and 1 resistant by BMD. By AXDX, MICs were determined for 98 isolates, where 69 were susceptible, 24 were intermediate, and 5 were resistant (Fig. 1). The isolate that did not grow in BMD panels also did not yield an MIC on AXDX.

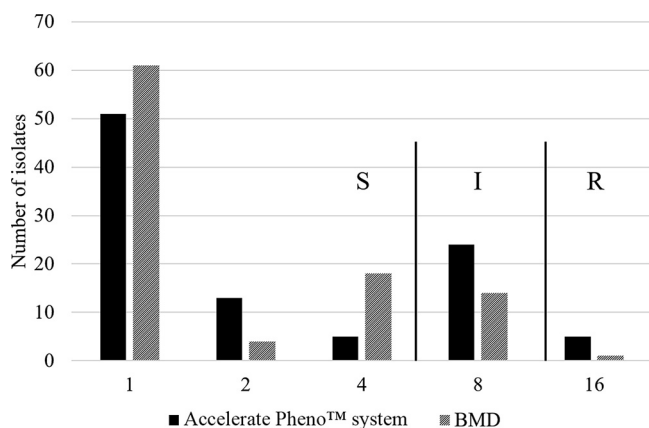


FIG 1 Minocycline MIC ($\mu\text{g/ml}$) distributions of *Acinetobacter* isolates by AXDX. S, susceptible; I, intermediate; R, resistant.

The essential agreement (± 1 log₂ MIC agreement) of AXDX-based MICs with BMD was 98.0% (96/98). The categorical agreement was 75.5% (74/98); all errors were minor errors, i.e., cases in which one AST method yielded an intermediate interpretation and the other yielded either a susceptible or resistant interpretation. There were no very major errors (i.e., susceptible by AXDX, resistant by BMD) or major errors (resistant by AXDX, susceptible by BMD). The majority of minor errors (20/24) involved AXDX reporting an MIC one dilution higher than the modal BMD MIC (16 $\mu\text{g/ml}$ versus 8 $\mu\text{g/ml}$ in 5 isolates, 8 $\mu\text{g/ml}$ versus 4 $\mu\text{g/ml}$ in 15 isolates). As seen in Fig. 1, the relatively high rate of minor errors was caused by the MIC distribution for minocycline, which bisected the susceptibility breakpoint. Nonetheless, the bimodal distribution of the MICs and the excellent essential agreement suggests that AXDX will rapidly identify the majority of *Acinetobacter* isolates with low MICs, for which minocycline may be considered a treatment option. A previous study demonstrated similar performance for minocycline MIC determination for *Acinetobacter* spp. by AXDX. Among 224 isolates evaluated, essential agreement was 97.4% and categorical agreement was 92.1%, and 17 minor errors and 1 major error were observed (8). Previous studies have demonstrated that other commercial AST instruments have similarly yielded a high rate of minor errors when testing *A. baumannii* against minocycline. In one study, which evaluated the performance of Spanish laboratories to accurately determine *A. baumannii* AST results, minor errors of 88.6% were observed across all systems evaluated (14). A second study demonstrated minor errors of 15% to 40% across different disk diffusion, Sensititre, and Etest methods (15).

Conclusion. AXDX was able to identify *A. baumannii* complex species of clinical significance, including *A. baumannii*, *A. pittii*, and *A. nosocomialis*, with 100% sensitivity. Two non-*A. baumannii* complex strains were identified as *A. baumannii* (1 *A. radiore-sistens* and 1 *A. baylyi*). Minocycline MIC AXDX results showed excellent essential agreement of 98.0% with BMD. One limitation of this study was that the majority of isolates tested were susceptible or intermediate to minocycline. Overall, the findings support the ability of AXDX to reliably predict the susceptibility of *A. baumannii* complex to minocycline, which should help guide treatment choices for infections where options are limited.

ACKNOWLEDGMENTS

This study was funded by Accelerate Diagnostics, Inc. The effort of Y.D. was supported by NIH grants R01AI104895, R21AI123747, and R21AI135522.

J.W.T. and R.M.H. are employees of Accelerate Diagnostics, Inc. Y.D. has consulted for Roche, Tetrphase, Geom, and Recida and received speaking fees from Merck and Astellas.

REFERENCES

- Doi Y, Murray GL, Peleg AY. 2015. *Acinetobacter baumannii*: evolution of antimicrobial resistance-treatment options. *Semin Respir Crit Care Med* 36:85–98. <https://doi.org/10.1055/s-0034-1398388>.
- Flamm RK, Castanheira M, Streit JM, Jones RN. 2016. Minocycline activity tested against *Acinetobacter baumannii* complex, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia* species complex isolates from a global surveillance program (2013). *Diagn Microbiol Infect Dis* 85: 352–355. <https://doi.org/10.1016/j.diagmicrobio.2016.03.019>.
- Ritchie DJ, Garavaglia-Wilson A. 2014. A review of intravenous minocycline for treatment of multidrug-resistant *Acinetobacter* infections. *Clin Infect Dis* 59 Suppl 6:S374–S380. <https://doi.org/10.1093/cid/ciu613>.
- Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. 2010. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob Agents Chemother* 54:4851–4863. <https://doi.org/10.1128/AAC.00627-10>.
- Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S. 2017. Evaluation of the Accelerate Pheno system for fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by Gram-negative pathogens. *J Clin Microbiol* 55:2116–2126. <https://doi.org/10.1128/JCM.00181-17>.
- Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, Granato PA, Harrington AT, Hernandez DR, Humphries RM, Jindra MR, Ledebouer NA, Miller SA, Mochon AB, Morgan MA, Patel R, Schreckenberger PC, Stamper PD, Simner PJ, Tucci NE, Zimmerman C, Wolk DM. 2018. Multicenter evaluation of the Accelerate Phentest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. *J Clin Microbiol* 56:e01329-17. <https://doi.org/10.1128/JCM.01329-17>.
- Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18:268–281. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>.
- La Scola B, Gundi VA, Khamis A, Raoult D. 2006. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol* 44:827–832. <https://doi.org/10.1128/JCM.44.3.827-832.2006>.
- Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 10th ed (M07-A10). Clinical and Laboratory Standards Institute, Wayne, PA.
- Accelerate Diagnostics. 2018. Accelerate PhenoTest BC kit instructions for use. Accelerate Diagnostics, Tucson, AZ.
- Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing, 28th ed (M100-S28). Clinical and Laboratory Standards Institute, Wayne, PA.
- Salimnia H, Fairfax MR, Lephart PR, Schreckenberger P, DesJarlais SM, Johnson JK, Robinson G, Carroll KC, Greer A, Morgan M, Chan R, Loeffelholz M, Valencia-Shelton F, Jenkins S, Schuetz AN, Daly JA, Barney T, Hemmert A, Kanack KJ. 2016. Evaluation of the FilmArray blood culture identification panel: results of a multicenter controlled trial. *J Clin Microbiol* 54:687–698. <https://doi.org/10.1128/JCM.01679-15>.
- Ledebouer NA, Lopanski BK, Dhiman N, Cavagnolo R, Carroll KC, Granato P, Thomson R, Jr, Butler-Wu SM, Berger H, Samuel L, Pancholi P, Swyers L, Hansen GT, Tran NK, Polage CR, Thomson KS, Hanson ND, Winegar R, Buchan BW. 2015. Identification of Gram-negative bacteria and genetic resistance determinants from positive blood culture broths by use of the Verigene Gram-negative blood culture multiplex microarray-based molecular assay. *J Clin Microbiol* 53:2460–2472. <https://doi.org/10.1128/JCM.00581-15>.
- Fernandez-Cuenca F, Tomas M, Caballero-Moyano FJ, Bou G, Pascual A, Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC). 2017. Reporting antimicrobial susceptibilities and resistance phenotypes in *Acinetobacter* spp: a nationwide proficiency study. *J Antimicrob Chemother* 73:692–697. <https://doi.org/10.1093/jac/dkx464>.
- Wang P, Bowler SL, Kantz SF, Mettus RT, Guo Y, McElheny CL, Doi Y. 2016. Comparison of minocycline susceptibility testing methods for carbapenem-resistant *Acinetobacter baumannii*. *J Clin Microbiol* 54:2937–2941. <https://doi.org/10.1128/JCM.01810-16>.