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Performance of Colistin MIC Determination of the Accelerate PhenoTest™ BC Kit for Research Use Only

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INTRODUCTION

Antimicrobial susceptibility testing of colistin has been a challenge for laboratories due to several technical issues, including variability of stock powders and solutions, the propensity for colistin to adsorb to plastics, and the absence of U.S. Food and Drug Administration-endorsed clinical breakpoints by which to interpret colistin minimum inhibitory concentrations (MIC). This study compared MIC agreement of the Accelerate PhenoTest™ BC kit (AXDX) research-use-only (RUO) colistin results to those of reference broth microdilution (rBMD). CLSI colistin breakpoints and epidemiological cut-off values (ECVs) were utilized to interpret both rBMD and AXDX MICs.

METHODS

A challenge set of gram-negative bacteria that was previously used by the CLSI to evaluate colistin was enriched for colistin-resistant phenotypes (n=38). Four isolates did not produce results and were excluded from subsequent analyses. The remaining 34 isolates included 5 *Acinetobacter baumannii* (ABA; 1 resistant), 10 *Pseudomonas aeruginosa* (PAE; 2 resistant) and 19 Enterobacteriaceae (10 not-wild-type by CLSI ECV, 6 with *mcr-1* resistance gene).

Isolates were seeded into BD BACTEC™ Aerobic Plus bottles containing 10 mL of human blood at a concentration of 30-50 bacterial clones per mL, and were incubated on the BD BACTEC™ FX until they flagged positive. Isolates were assessed with the Accelerate PhenoTest™ BC kit and the Accelerate Pheno™ system and compared to rBMD according to the CLSI M07-A4 standard.

Antimicrobial susceptibility testing of the Accelerate PhenoTest™ BC kit using the Accelerate Pheno™ system utilizes morphokinetic cellular analysis (MCA), which optically records bacteria growing in the presence of an antibiotic. This study used a single concentration of colistin of 2 µg/mL. Bacterial cell response profiles are generated from time-lapse images and compared to algorithm models to report out MIC values. The AXDX software utilized v1.3.2 image analysis.

Essential agreement (EA; i.e., MIC +/- 1 $\log_2$ dilution) and categorical agreement (CA) with CLSI breakpoints/ECVs were assessed. Additionally, two features of the algorithm used to calculate MIC values for AXDX were examined (i.e., number of growing clones and division rate of growing clones).

RESULTS

Figure 1 shows time-lapse images of *Escherichia coli* clones growing in the presence of colistin at time 0, 1, 2, 3, and 4 hours. **Figure 2** shows growth curves of clones susceptible to colistin (top) and resistant to colistin (bottom).

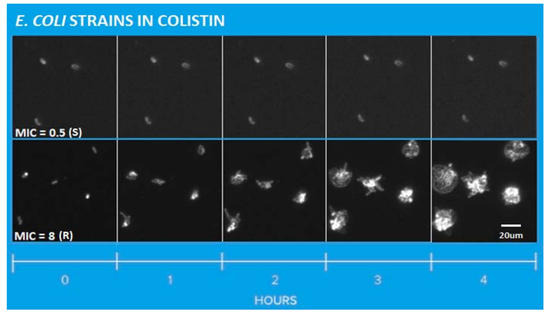

Figure 1. (above): Time-lapse images of *E. coli* strains with AXDX MICs of 0.5 µg/mL (top panels) and 8 µg/mL (bottom panels). Clones with MIC of 0.5 µg/mL (susceptible) arrest or lyse while clones with MIC of 8 µg/mL (resistant) continue to grow. Images zoomed in to view individual bacterial clones.

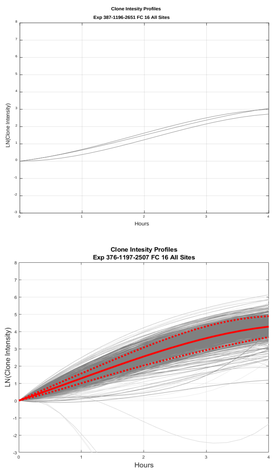

Figure 2. Bacterial response profiles for clones used in this study that are susceptible (right top) and resistant (right bottom). Each gray line is the growth curve for an individual clone. The solid red line is the mean and dotted red lines are two standard deviations from the mean.

Table 1 shows concordance between rBMD MIC and AXDX MIC. CA is 94.1% (32/34) and EA is 91.2% (31/34). One ABA and one *E. coli* isolate were out of both EA and CA (circled). When tested previously, the ABA isolate had MICs ranging from 1 to 2 µg/mL. Interestingly, upon sub-culture from frozen, 2 ABA and 1 PAE were found to no longer be CST resistant.

AXDX MIC \ Reference Broth Microdilution (rBMD) MIC	0.5	1	2	4	8
0.5	11	4	0	0	0
1	0	4	0	0	0
2	1	0	0	0	0
4	1	0	0	1	0
8	0	0	1	2	9

Table 1: Study results (N=34). Categorical Agreement (CA) = 94.1%, Essential Agreement (EA) = 91.2%. 2018 CLSI breakpoint in red.

RESULTS

Figure 3 shows the relationship between rBMD MIC and two measures of bacterial growth used to determine MIC for the AXDX system (i.e., growing clone number ratio and growing clone division ratio). As would be expected given the high CA and EA observed for these data, this analysis shows a clear separation between susceptible clones (blue dots) and resistant clones (red dots). The two isolates that were out of CA and EA (see Table 1) are indicated with arrows.

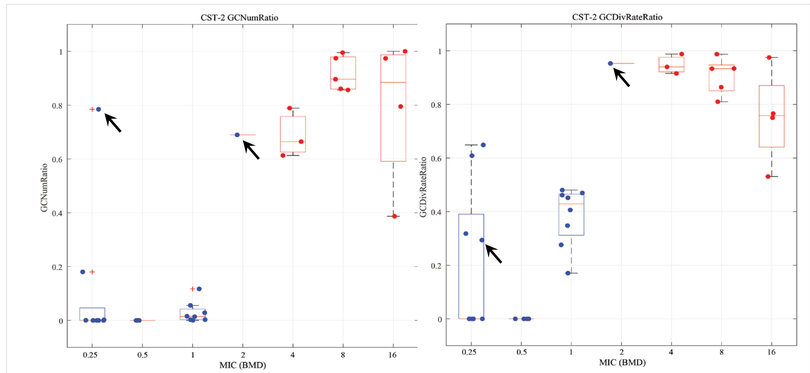

Figure 3. (Left): Box plot of ratio of number of growing test clones relative to number of growing control clones. (Right): Box plot of ratio of division rate of growing test clones relative to division rate of growing control clones. Blue dots represent susceptible clones and red dots represent resistant clones as defined by rBMD. Isolates out of EA/CA indicated by arrows.

CONCLUSIONS

- The results of this study demonstrated the RUO colistin test performed well when compared to rBMD for *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae.
- Further studies are needed to assess the performance of this test, which may be useful for research applications.

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