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LETTER TO THE EDITOR

# Potential of Inaccurate Cefiderocol Susceptibility Results: a CLSI AST Subcommittee Advisory

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Cefiderocol (Shionogi, Inc.) is a novel siderophore-conjugate cephalosporin with activity against aerobic, Gram-negative bacteria, including multidrug-resistant strains (1). The mode of action (MOA) for cefiderocol is unique as it exploits active iron transport, binding iron to the siderophore moiety of the antimicrobial and entering the periplasmic space of Gram-negative bacteria via active iron transport mechanisms (2). The cephalosporin moiety then targets penicillin-binding protein 3, inhibiting peptidoglycan synthesis. This unique MOA makes susceptibility testing difficult using Clinical and Laboratory Standards Institute (CLSI) reference methods, as bacterial iron transporters are upregulated under *in vivo* iron-depleted conditions, but not in the iron concentrations found in cation-adjusted Mueller-Hinton broth used in the CLSI reference broth microdilution (BMD) method (3). A modification of the reference BMD was published by CLSI for testing of cefiderocol, which uses cation-adjusted Mueller-Hinton broth (CA-MHB) depleted of iron to a final iron concentration of ≤0.03 $\mu$g/mL; See Appendix I in reference (4). Unlike BMD, disk diffusion (DD) methods were developed to work with standard Mueller-Hinton agar and do not require iron depletion.

Shionogi has been working with the CLSI AST subcommittee to develop and optimize methods and interpretations for cefiderocol AST. In 2021, CLSI approved breakpoints for the Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex, and *Stenotrophomonas maltophilia* (5, 6, 7). At this time, it was noted that DD zone diameters of ≤14 mm for *A. baumannii* complex were associated with susceptible, intermediate and resistant MIC results when BMD was performed in parallel. To address this challenge, CLSI published a susceptible-only disk breakpoint (≥15mm disk zone diameter), and a warning in M100, 32nd Edition encouraged laboratories to confirm disk zone diameters ≤14 mm by an MIC method (4, 6). In parallel, CLSI requested that Shionogi perform additional studies, to resolve the discrepancies between DD and MIC results for *A. baumannii* complex. The findings of these studies were presented to the CLSI AST subcommittee in 2022, which raised concerns regarding the reproducibility and accuracy of both cefiderocol BMD and DD methods, despite passing routine quality control. The data demonstrated: (i) poor reproducibility of BMD and DD results performed at two laboratories for *A. baumannii* complex isolates with initial cefiderocol

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MICs >2 $\mu$g/mL, with isolates testing highly susceptible upon repeat evaluation; (ii) difficult-to-interpret MIC and DD endpoints, due to trailing by BMD or colonies within predominant zones of growth inhibition by DD; and 3) minor variability in the inoculum preparation (within the acceptable CLSI range of $2\times 10^5$ to $8\times 10^5$ CFU/mL) resulting in major differences in cefiderocol MIC values (8, 9). The data presented by Shionogi can be found in the CLSI AST meeting file resources for the January and June 2022 Subcommittee meetings (8, 9).

The CLSI AST Subcommittee is working closely with Shionogi to resolve these issues and provide further guidance to laboratories. A warning will be published in the M100, 33rd edition in 2023 to raise awareness of the issues described herein and published elsewhere (9–11). In the meantime, laboratories that perform cefiderocol testing in-house should discuss the limitations of the test's accuracy and reproducibility with their antimicrobial stewardship team, infectious disease physicians and/or a clinical champion to determine the best approach at an institutional level. For laboratories that prepare iron depleted CA-MHB for cefiderocol susceptibility testing, it is strongly recommended that residual iron concentrations be measured after chelation, as the CLSI method for preparation may not reduce iron levels to the acceptable range of $\leq$0.03 $\mu$g/mL (4). If AST is performed in-house, laboratories should pay close attention to QC data and investigate new trends (e.g., results running on the low or high end of the QC range), which might indicate media variability or inoculum effects (12). Laboratories should use an automated nephelometer and/or routinely perform colony counts to aid with creating a standardized inoculum preparation when performing testing with cefiderocol. Repeat testing on subsequent isolates recovered from the same patient is also indicated. Further studies are ongoing to elucidate the problems surrounding cefiderocol AST to help provide additional guidance to laboratories. At this point, laboratorians must be aware that significant inaccuracy and lack of reproducibility has the potential to hamper the value of testing cefiderocol, by both MIC and DD methods, particularly for isolates of *A. baumannii* complex.

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