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Increasing pH of the Test Medium Suppresses Trailing Phenomenon in Cefiderocol MIC Determinations for *Acinetobacter baumannii*

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Introduction

- Cefiderocol is a siderophore cephalosporin with activity against a wide variety of multidrug resistant Gram-negative bacteria, including carbapenemase-producing isolates [1].
- Incomplete growth inhibition compared to growth control over several wells (trailing) has been observed with some strains of *Acinetobacter baumannii* when minimum inhibitory concentration (MIC) is determined by the broth microdilution method.
- When trailing is observed, the MIC for cefiderocol should be read as the lowest concentration at which a significant reduction of the growth is observed. Despite guidance from Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) on how to read MIC endpoints ambiguity remains. Suppressing trailing, resulting in a clear MIC endpoint, would simplify reading of cefiderocol MIC and improve the reproducibility within and across laboratories.
- In this study, the effect of pH and other media conditions, such as medium supplementations, to suppress trailing were investigated.

Methods

- Two *A. baumannii* clinical strains which showed trailing were used.

Strain No.	β-lactamase content	MIC (μg/mL)
AB127	bla_{TEM} and $bla_{OXA\text{-}23\text{-}type}$	1
AB143	$bla_{OXA\text{-}72}$	0.5

- MICs of cefiderocol were determined by broth microdilution according to CLSI guidelines using iron-depleted cation adjusted Mueller Hinton broth (ID-CAMHB). Modification to ID-CAMHB included addition of cations, saccharides, and $NaHCO_3$, and changes in pH, and atmospheric incubation conditions.
- Morphological changes of AB127 were studied by microscope in ID-CAMHB at pH 7.2 or 8.0 after 24-hour exposure to cefiderocol at 2 × MIC (2 μg/mL).
- Bactericidal activity against AB127 was measured through time-kill studies at 16 × MIC (16 μg/mL) in ID-CAMHB at pH 7.2 or 8.0.

Results

- Suppression of trailing for AB127 and AB143 was observed by the addition of 6.25 mM $NaHCO_3$ to ID-CAMHB, or by adjusting the pH of the medium to 8.0 with NaOH **(Figure 1)**. Suppression of trailing was not yet observed at pH 7.5 **(Figure 2)**. Suppression of trailing by $NaHCO_3$ is likely been caused by increasing the pH of the medium.
- Addition of cations (30 mM NaCl, $MgCl_2$), or saccharides (10 mM glucose, galactose, arabinose, sucrose), as well as incubation under 5% CO_2 did not affect trailing.
- Extensive filamentous growth (indicative of inhibition of PBP3) was observed in wells that showed trailing in ID-CAMHB at pH 7.2, but not at pH 8.2 **(Figure 3)**.
- Time-killing tests showed static killing in ID-CAMHB at pH 7.2, and restoration of bactericidal activity of cefiderocol at pH 8.0 **(Figure 4)**.

Figure 1. Suppression of trailing in *Acinetobacter baumannii* isolates.

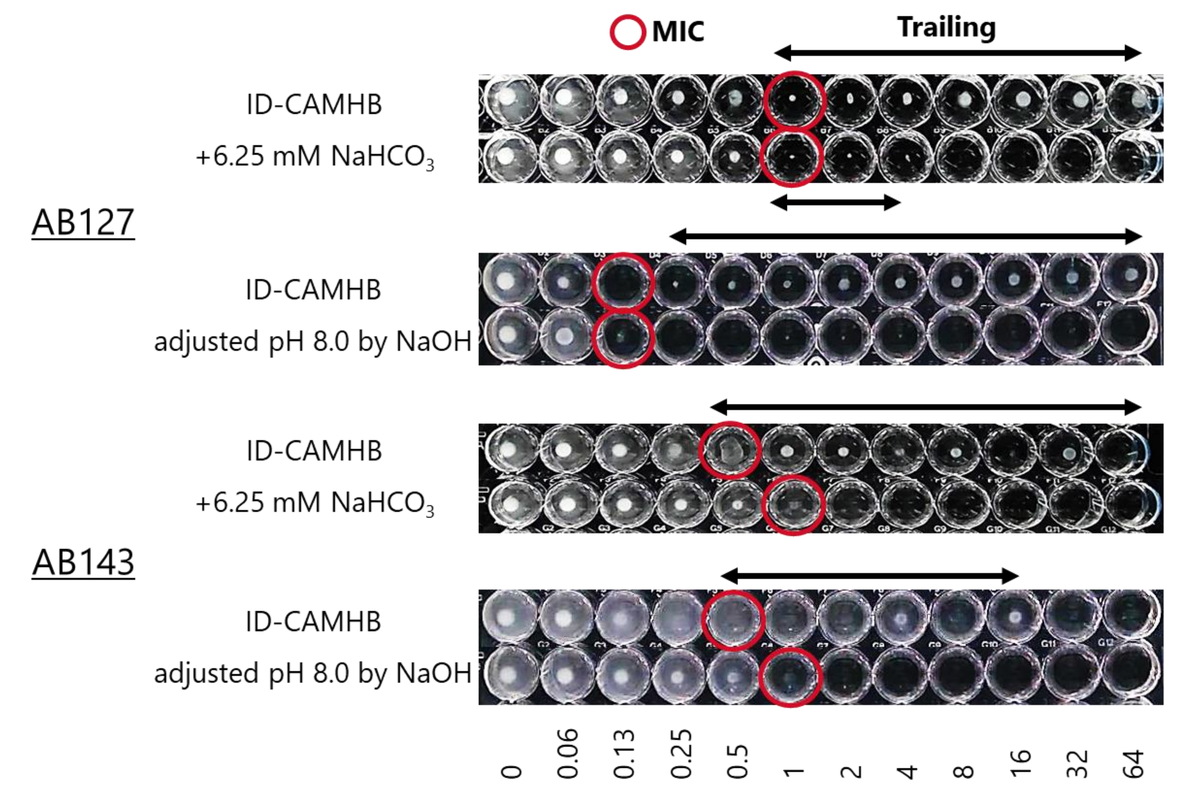


Figure 2. Suppression of trailing by addition of $NaHCO_3$ or increase of pH by NaOH

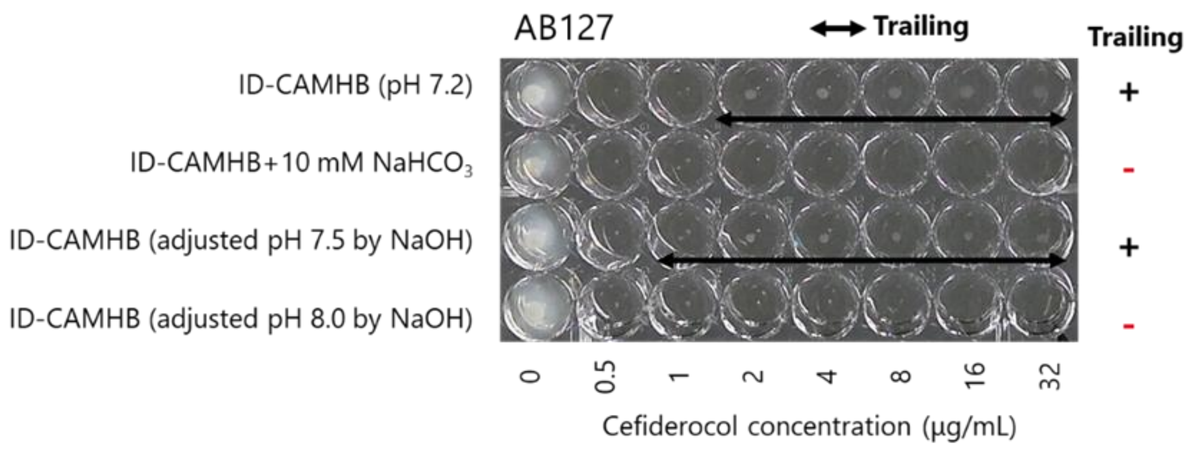


Figure 3. Morphology of AB127 after 24-hour incubation in presence of 2 μg/mL (2 × MIC) of cefiderocol

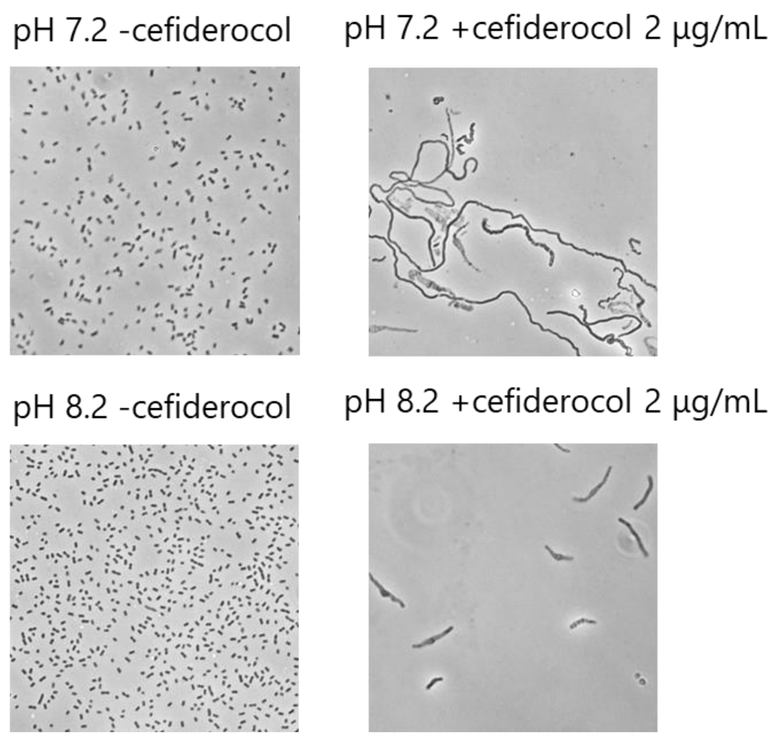
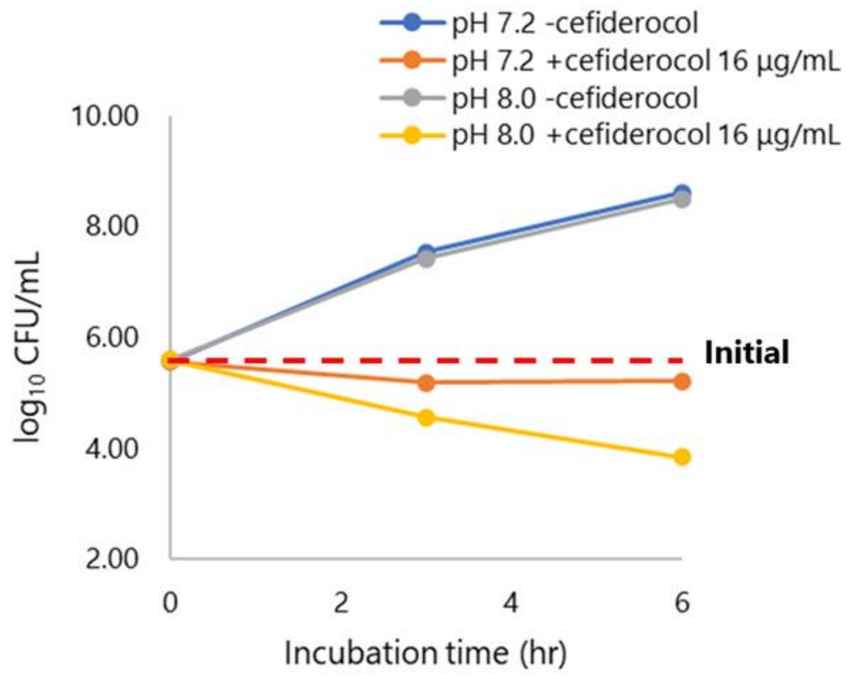


Figure 4. Bactericidal activity of cefiderocol against AB127 in ID-CAMHB at pH 7.2 and pH 8.0



Conclusion

Trailing observed for some *A. baumannii* strains when determining the cefiderocol MIC can be suppressed by elevating the pH of ID-CAMHB to 8.0. Reduction of trailing simplifies MIC determinations for cefiderocol and should lead to increased reproducibility. Further exploration of these findings is warranted.

References

1. Y. Yamano, Clin Infect Dis, 69:S544-S551, 2019