Confirming the Impact of Iron Limitation on *Acinetobacter baumannii* Growth

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ABSTRACT

Background: The CDC/WHO have labeled *A. baumannii* as the highest threat level in their ranking system for antibiotic-resistant pathogens, and no optimal treatments exist. Previously we have shown that iron limitation creates growth inhibition. In this study we verify this claim with iron supplementation.

Methods: Nine clinical and one lab isolate of *A. baumannii* strain were tested. Deferiprone (2mM) was used to create growth. To confirm the impact of iron availability on growth inhibition iron was supplemented as a concentration gradient. Bacterial burden was monitored over 400 minutes starting at approximately 6 log CFU/mL.

Results: An increase in iron concentration consistently resulted in a reversal of growth of inhibition.

Conclusions: Our data verify the impact of iron limitation on growth of *A. baumannii*. Manipulation of iron concentration has the potential to be used to treat antimicrobial resistant *A. baumannii* infections.

BACKGROUND

- *Acinetobacter baumannii* is a pathogenic bacteria that has developed antimicrobial-resistant properties causing significant hospitalizations and deaths.
- Treatment options are urgently needed to mitigate its spread and associated mortality
- Nutrient availability, such as iron, plays an essential role in the survival of *A. baumannii*.
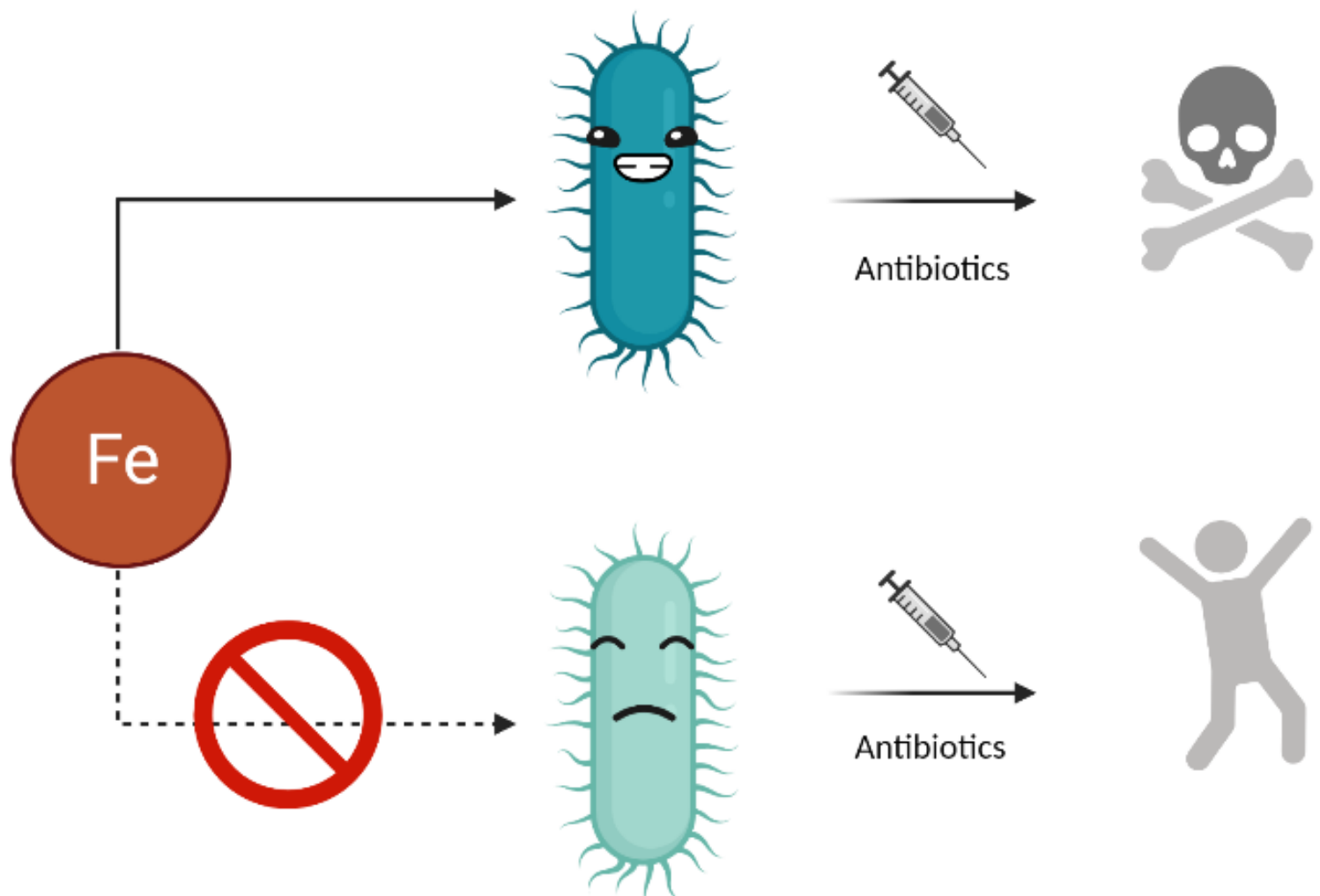- We have previously demonstrated growth inhibition using deferiprone (a metal chelator).

Figure 1. Multidrug resistant bacteria with metal availability results in fatal infections despite antibiotics (top). Bacteria with limited metal availability, are susceptible to antibiotics, resulting in lower mortality rates (bottom).

OBJECTIVE

Verify the impact of iron limitation on *A. baumannii* growth with iron supplementation.

METHODS

- Nine bloodstream isolates from unique patients between 2011 and 2018 were used (Table 1).
- Bacteria was grown in TSB with 2mM of deferiprone and increasing concentration of iron over 400 minutes.
- BacterioScan 216Dx: measures bacterial density through optical signals and converts them to log CFU/mL by a validated algorithm integrating both absorbance and forward light scattering.

Table 1. Isolate characterization and antimicrobial susceptibility testing was performed using VITEK®2 system. Laboratory strain BAA 747, not typed and not MDR.

Isolate #	Gender	Age	Source	MLST[1]	MDR[2]
01	M	39	Respiratory	457	Yes
05	M	80	Respiratory	NT	Yes
10	M	81	Urinary	447	Yes
14	M	50	Abdominal	195	No
18	M	76	Biliary	373	No
19	M	76	Line	NT	No
23	M	74	Respiratory	NT	No
28	M	80	Respiratory	NT	Yes
33	F	65	Respiratory	369	Yes

[1] MLST – Multilocus sequence typing via Oxford Scheme; NT – non-typable
[2] MDR – Multi-drug resistance multidrug resistance, as nonsusceptibility to ≥1 agent in ≥3 antimicrobial categories

RESULTS

- No growth was observed in the presence of deferiprone without iron supplementation.
- Increased concentrations of iron resulted in a reversal of growth inhibition
- All isolates display a growth profile difference when comparing the control group at 0uM (orange) and 320uM (green)
- Although all isolates followed the same trend the degree of change varied for 80uM (red) and 320 uM (green) of iron
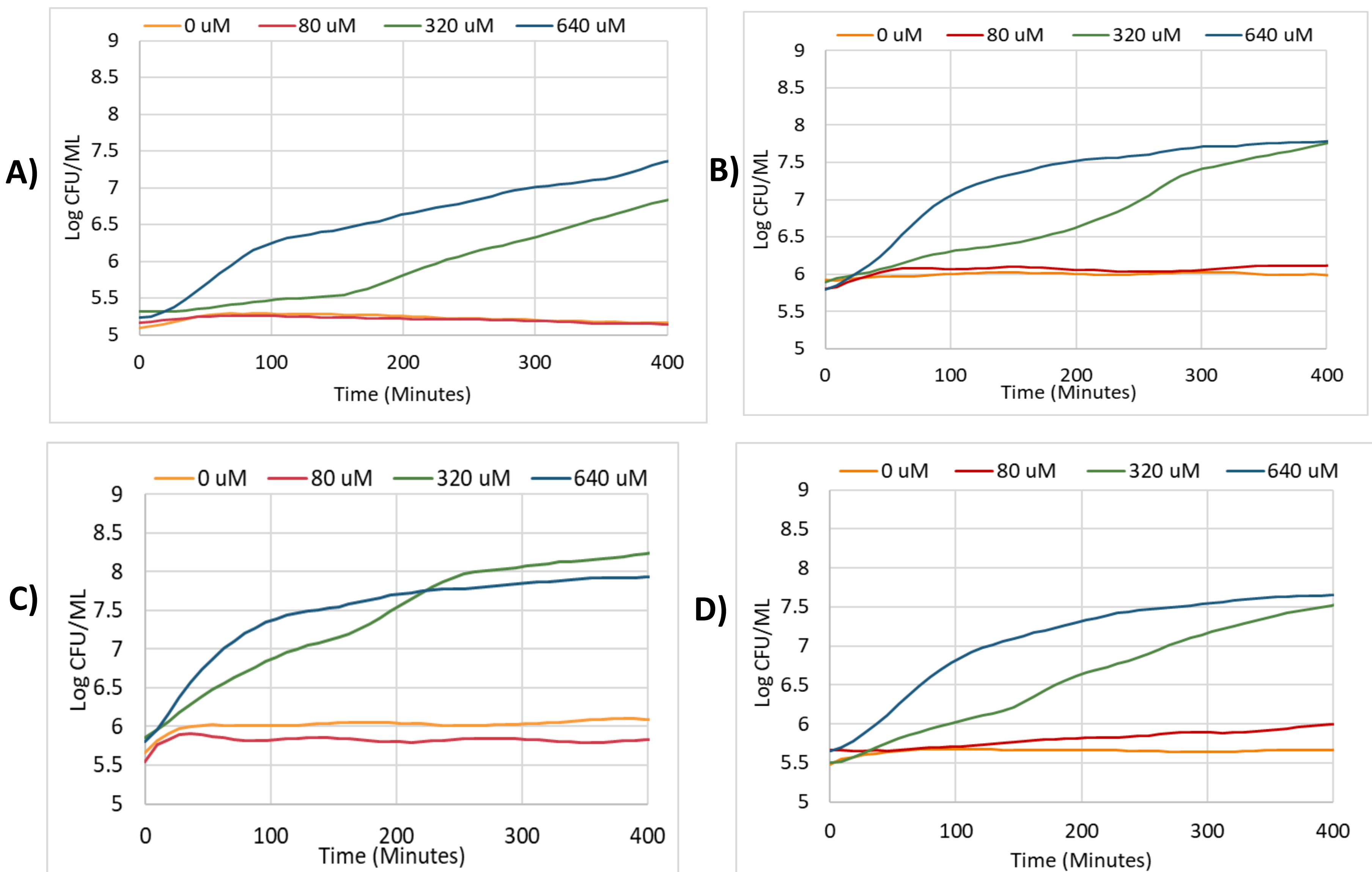
Figure 2. Representative graphic representation of bacterial growth in log CFU/mL displayed over 400 mins. All samples included 2mM of DFP in addition to iron supplementation at 0uM (orange), 80uM (red), 320uM (green), and 640uM (blue). Clinical isolates displayed are #28 (A), #33 (B), #10 (C), and #01 (D).

CONCLUSIONS

- Our data verify iron depletion was associated with bacterial growth inhibition.
- Manipulation of iron concentration has the potential to treat antimicrobial resistant *A. baumannii* strains.

FUTURE DIRECTIONS

- Test other metals that bind to deferiprone to determine any changes in growth inhibition with the anticipation that they will not.
- Further explore the potential of iron depletion to be used as a therapeutic approach in the treatment of antibiotic resistant infections.

ACKNOWLEDGEMENTS