# Impact of an iron chelator on *in vitro and in vivo* growth of *Acinetobacter baumannii*

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## REVISED ABSTRACT

**Background:** *Acinetobacter baumannii* (AB) has become multidrug-resistant in recent years and currently there is no consensus on the optimal treatment for these infections. To combat this urgent threat, novel treatment options are needed to minimize spread and lower mortality rates. Nutrient metals (e.g., iron) are essential to the metabolic functions of AB. If the bioavailability of essential nutrients is restricted, this can cause a bacterial stress response and alter their ability to multiply.

**Methods:** The objective of the study was to examine the impact of iron chelation on the growth of AB in vitro and in vivo. MDR AB bloodstream isolates (n=5) were recovered from unique patients between 2011 and 2018. Clonal diversity was ascertained by Fourier Transform Infrared Spectroscopy. In vitro bacterial densities were measured longitudinally over 20-hours to determine the growth profile. Isolates were started at a baseline concentration of approximately 5.5 log CFU/mL in tryptic soy broth. Variable amounts of an iron chelating agent (deferiprone, 0.2-2mM) were added to create a concentration gradient. Galleria mellonella larvae (100-150mg) were inoculated with approximately 5 log CFU of an isolate, with and without deferiprone (10mM). Quantitative culture was used to ascertain the bacterial burden of aggregate larvae (n=10) immediately and 4h post infection. Additional infected larvae (n=20 in each group) were incubated at 35°C and monitored hourly for 24h.

**Results:** Time to mortality was compared using Kaplan-Meier survival analysis and log-rank test. Increasing concentrations of the chelating agent caused a transient and concentration-dependent hindrance of in vitro bacterial growth, compared to the no-treatment group. Bacterial burden immediately post-infection of the control and treatment group were comparable at 6.4 log CFU/g. After 4 hours the bacterial burden was much higher in the control group than the treatment group, 8.9 and 7.0 log CFU/g respectively. A trend of delayed mortality was shown in the iron chelator group (median survival 6.5 vs 10 hours, p=0.15).

**Conclusions:** These results support that micro-nutrient limitation has the potential to be a novel approach for treating high-risk infections due to MDR AB. Future studies are warranted to further define its optimal place in therapy.

## BACKGROUND

- *A. baumannii* is a pathogenic bacterium requiring solutions due to mounting antimicrobial resistance
- Innovative treatment strategies are needed

## OBJECTIVE

- Examine the impact of iron chelation on the growth of AB *in vitro* and *in vivo*

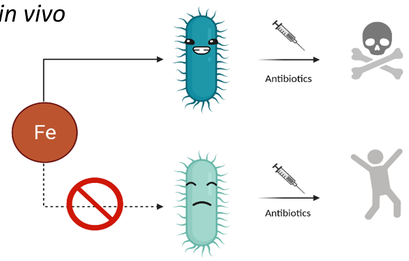



***Figure 1. Conceptual Hypothesis***

## BACTERIAL ISOLATES

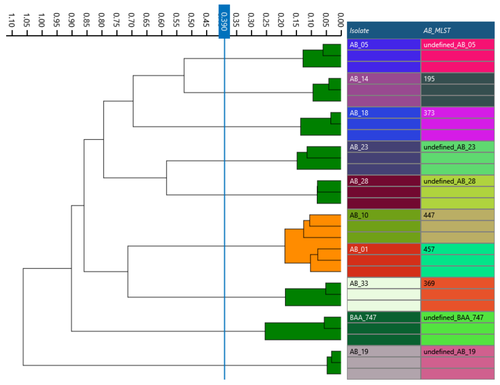
***Table 1. Clinical Bloodstream Isolates Examined***

| 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

- Control – Laboratory Strain: ATCC BAA 747
- Recovered from unique patients between 2011-2018

***Figure 2. Clonal uniqueness of isolates***




## METHODS

***In vitro* growth**

- BacterioScan 216Dx; optical signals are converted to CFU/mL through a validated algorithm
- Measurements taken every 10 minutes over 20 hours; initial concentration of 5.5 log CFU/mL in TSB
- Concentration gradients of additives:
  - Deferiprone, DFP
  - Iron ions, Fe
  - Copper ions, Cu

***In vivo* growth**

- *Galleria mellonella*, 100-150mg; inoculated with approximately 5 log CFU +/- DFP
- Bacterial burden ascertained using aggregate n=10 larvae and quantitative culture
- Survival monitored hourly over 24 hours at 35°C

## RESULTS – *in vitro*

**Figure 3:**

- Deferiprone caused a concentration dependent inhibition of *A. baumannii* growth
- Representative trend shown for all isolates studied
- No growth shown with 2000uM DFP for any isolate

**Figure 4:**

- Deferiprone included in all samples at 2000uM
- Incremental iron supplementation resulted in a reversal of the growth inhibition profile
- Copper supplementation had no effect despite deferiprone's affinity for the ion

***Figure 3. Deferiprone concentration gradient***

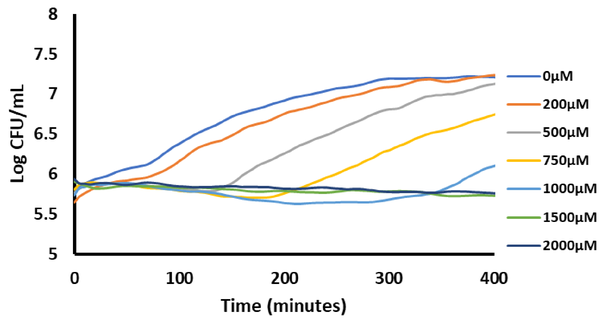



***Figure 4A. Iron Supplementation with Deferiprone***

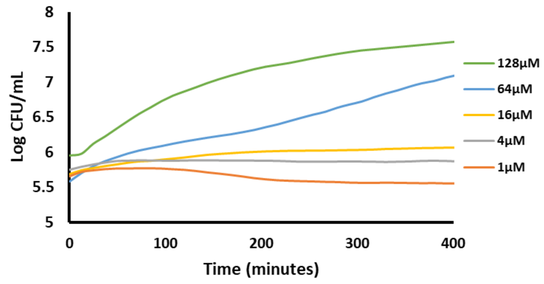
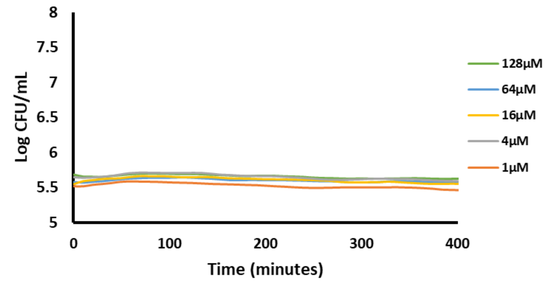



***Figure 4B. Copper Supplementation with Deferiprone***




## RESULTS – *in vivo*

**Figure 5:**

- Baseline comparable, 4 hours post infection showed higher CFU for the untreated group; 8.9 vs 7.0 log CFU/g

**Figure 6:**

- A trend in extended time-to-death observed in DFP treated larvae; 6.5 vs 10 hours, p=0.15

***Figure 5. Bacterial Burden***

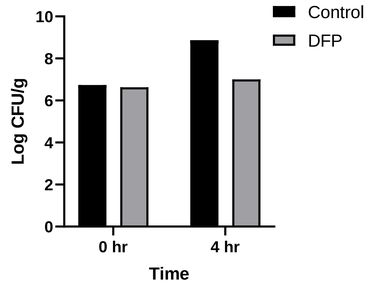
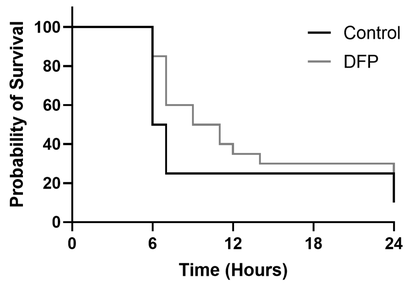



***Figure 6. Survival Analysis***




## CONCLUSIONS

- Limitation of iron showed a hinderance of growth under different *in vitro* and *in vivo* conditions
- Iron limitation has the potential to be a novel approach for treating high-risk infections such as MDR *A. baumannii*

## ACKNOWLEDGEMENTS

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