

Calculating the Minimum Inhibitory Concentration (MIC) of Gentamycin on *Staphylococcus epidermidis* Grown Under Simulated Microgravity

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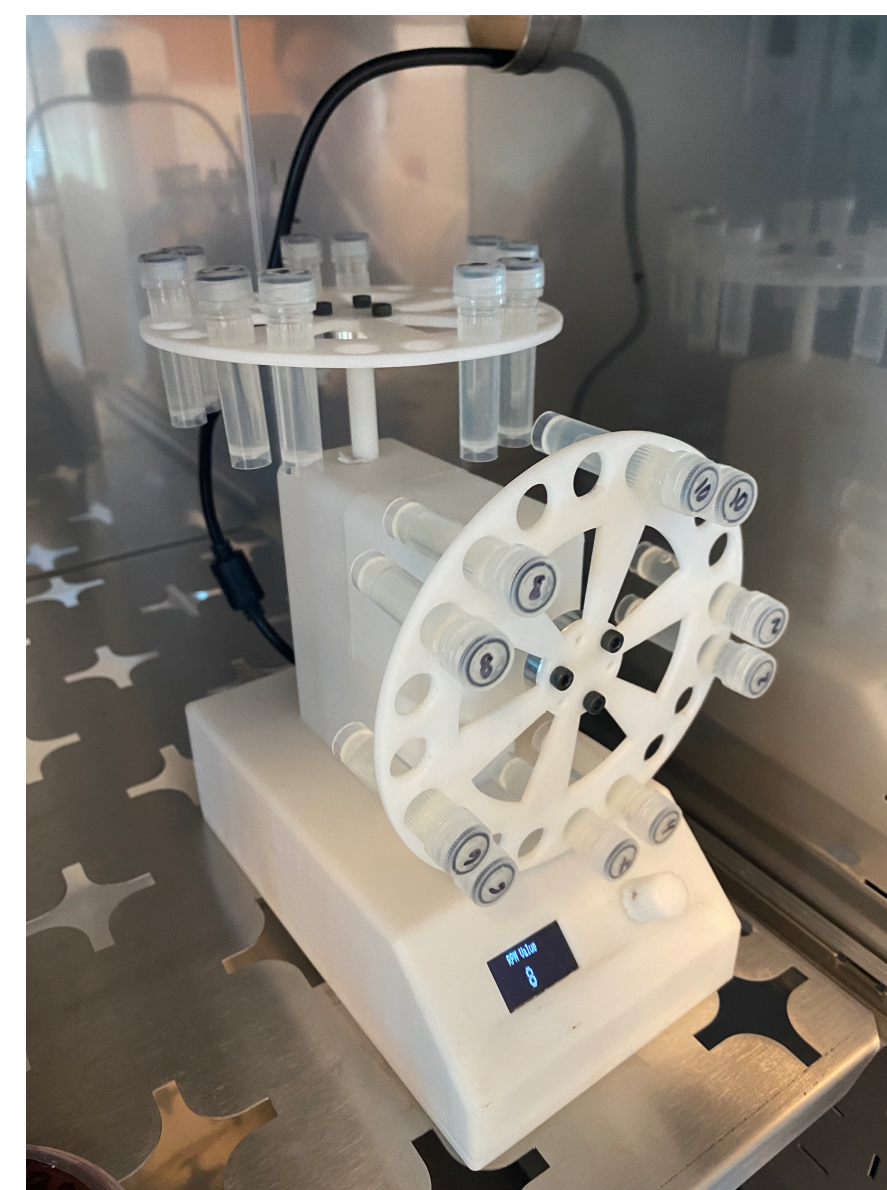


Introduction

- With space travel becoming more prominent, scientists have been searching for ways to allow for a thriving environment while on long-duration space missions.
- One of the ways researchers are investigating ways to maintain optimal health in astronauts is through antibiotic resistance.
- An astronaut's immune system functions differently in microgravity than it does on Earth due to metabolic changes.
- Therefore, a certain dosage of an antibiotic may be used to treat a certain infection may be affected in these conditions.
- This study aims to determine the MIC (minimum inhibitory concentration) of Gentamicin and measure its effect on *S. epidermidis* under simulated microgravity.

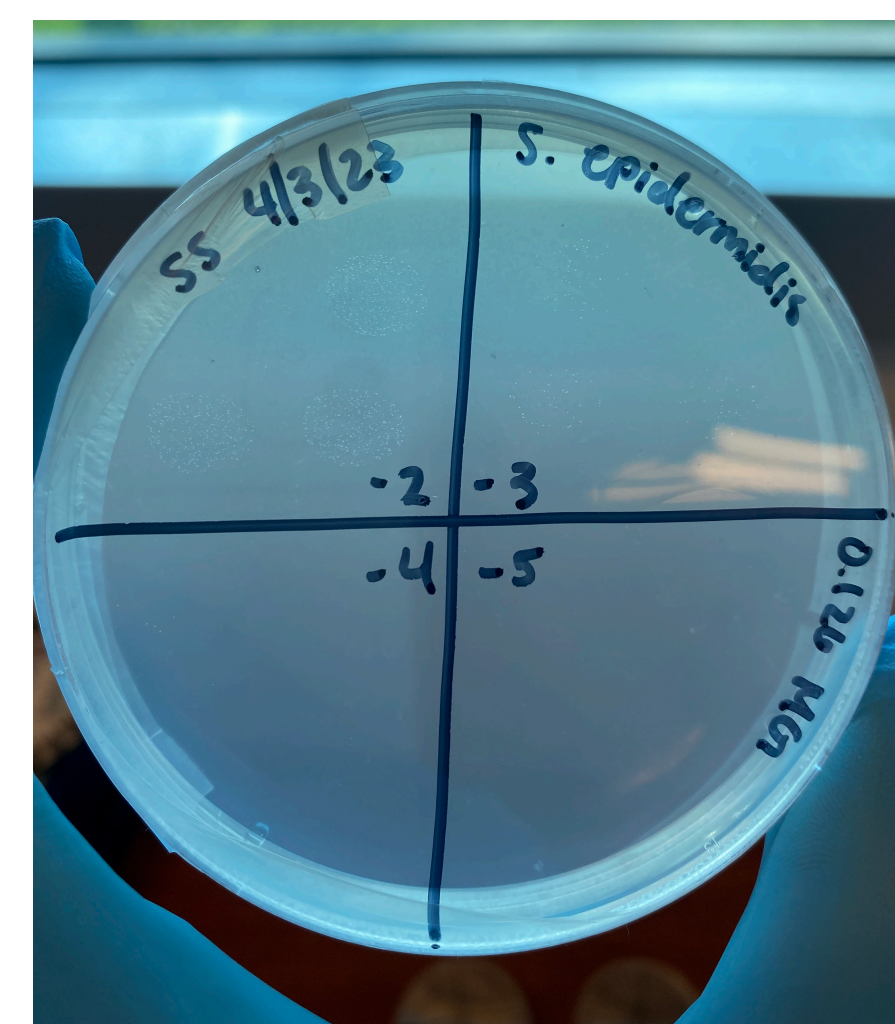
Experimental Design

- S. epidermidis* was cultured from glycerol stocks for short term exposure experiments.
- S. epidermidis* was cultured in Nutrient Broth (NB) media overnight.
- These cultures were then grown in epitubes on the EAGLESTAT within different antibiotic concentrations.
- After growth occurred, the cultures were transferred to a 96-well plate and their optical density was analyzed. Drop plates were also made to count colonies.
- Data from this growth was analyzed and used to decide which antibiotic concentration was significant enough to use for the growth curve.

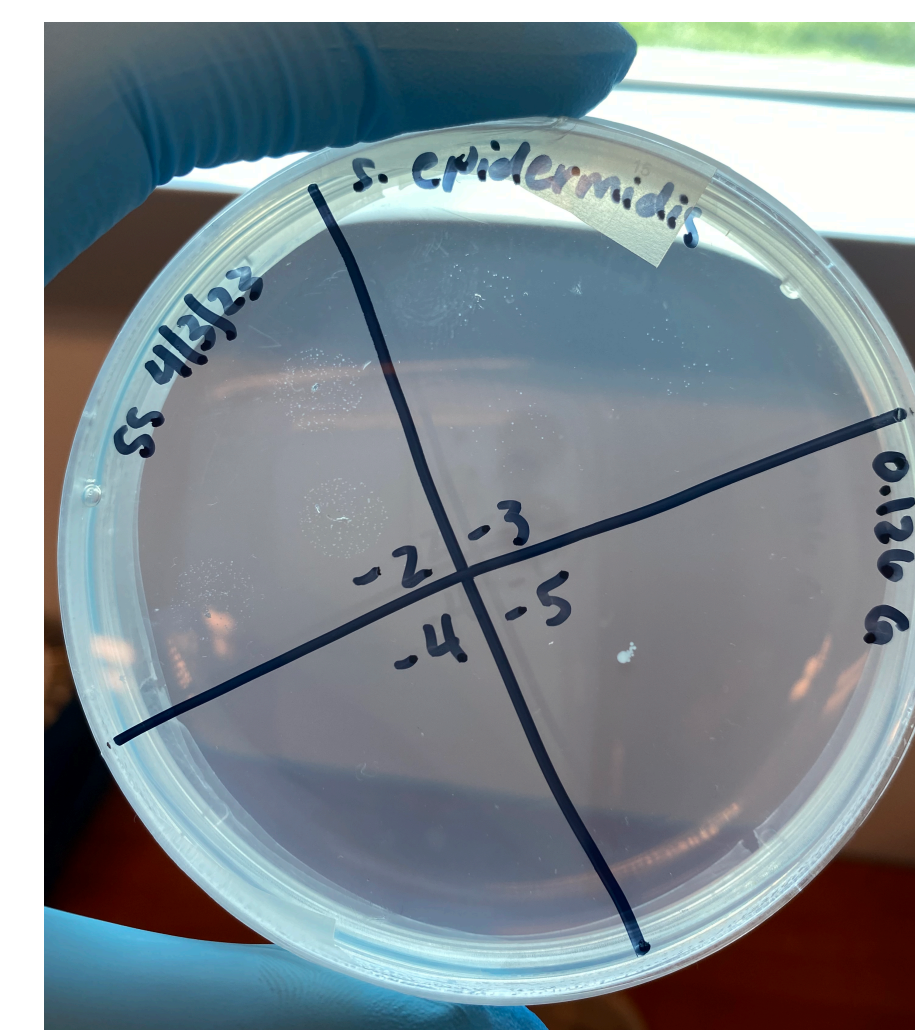


Growth Curve Procedure

- S. epidermidis* was grown in a 24 well plate with concentrations of Gentamicin ranging from 0.5 to 0.126 µg/ml.
- Plate was placed on a shaker and incubated at 30°C.
- Absorbance was read on a spectrophotometer to observe the changes in biomass over a period of 8 hours.
- Dilutions were made to measure numbers of colonies at a countable quantity that were able to grow after being exposed to the antibiotic and microgravity.
- Colony-forming units were obtained by counting the number of colonies that were grown on the plates.



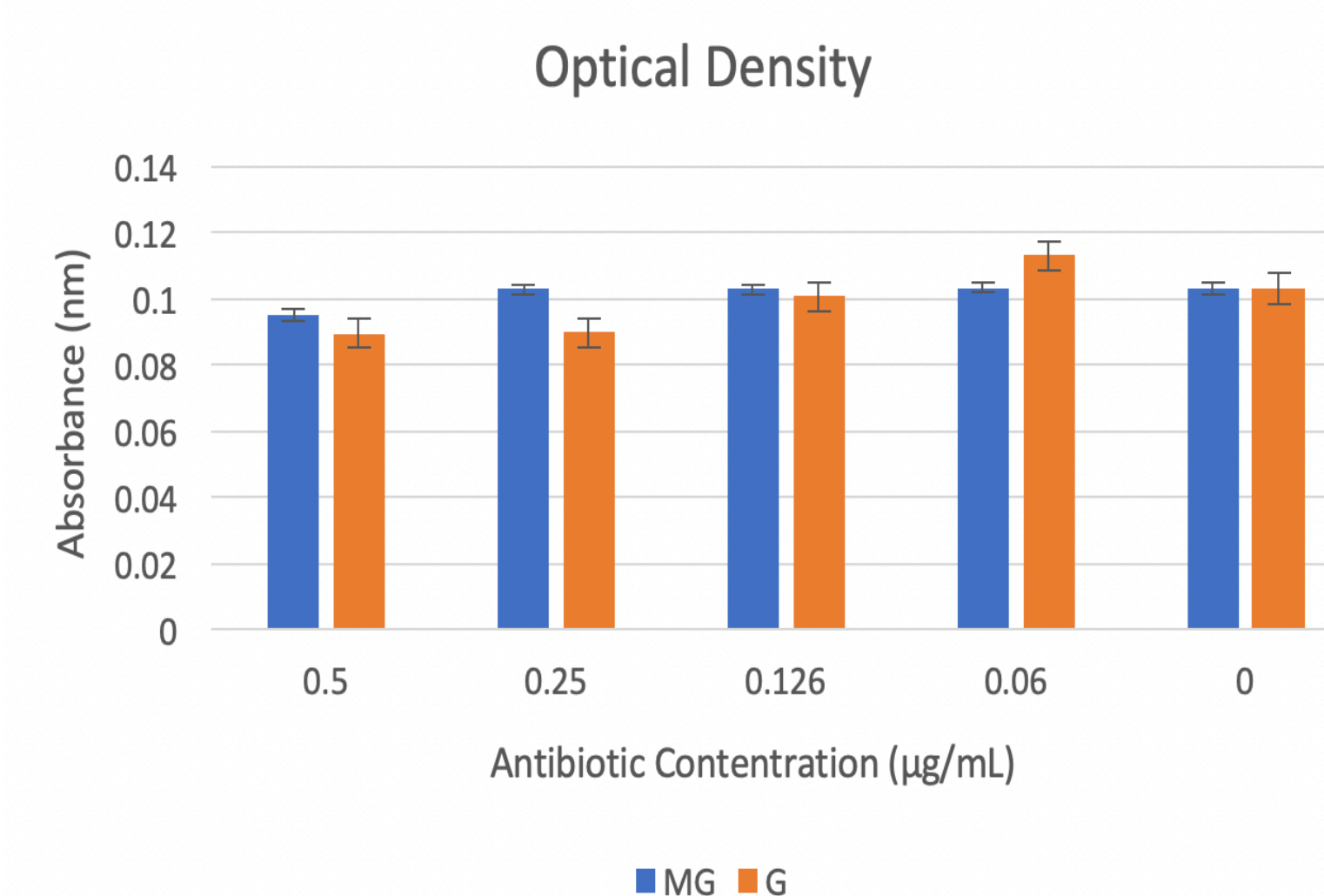
Colony-Forming Units: 0.126 µg/mL (Microgravity) 24 Hrs



Colony-Forming Units: 0.126 µg/mL (Gravity) 24 Hrs

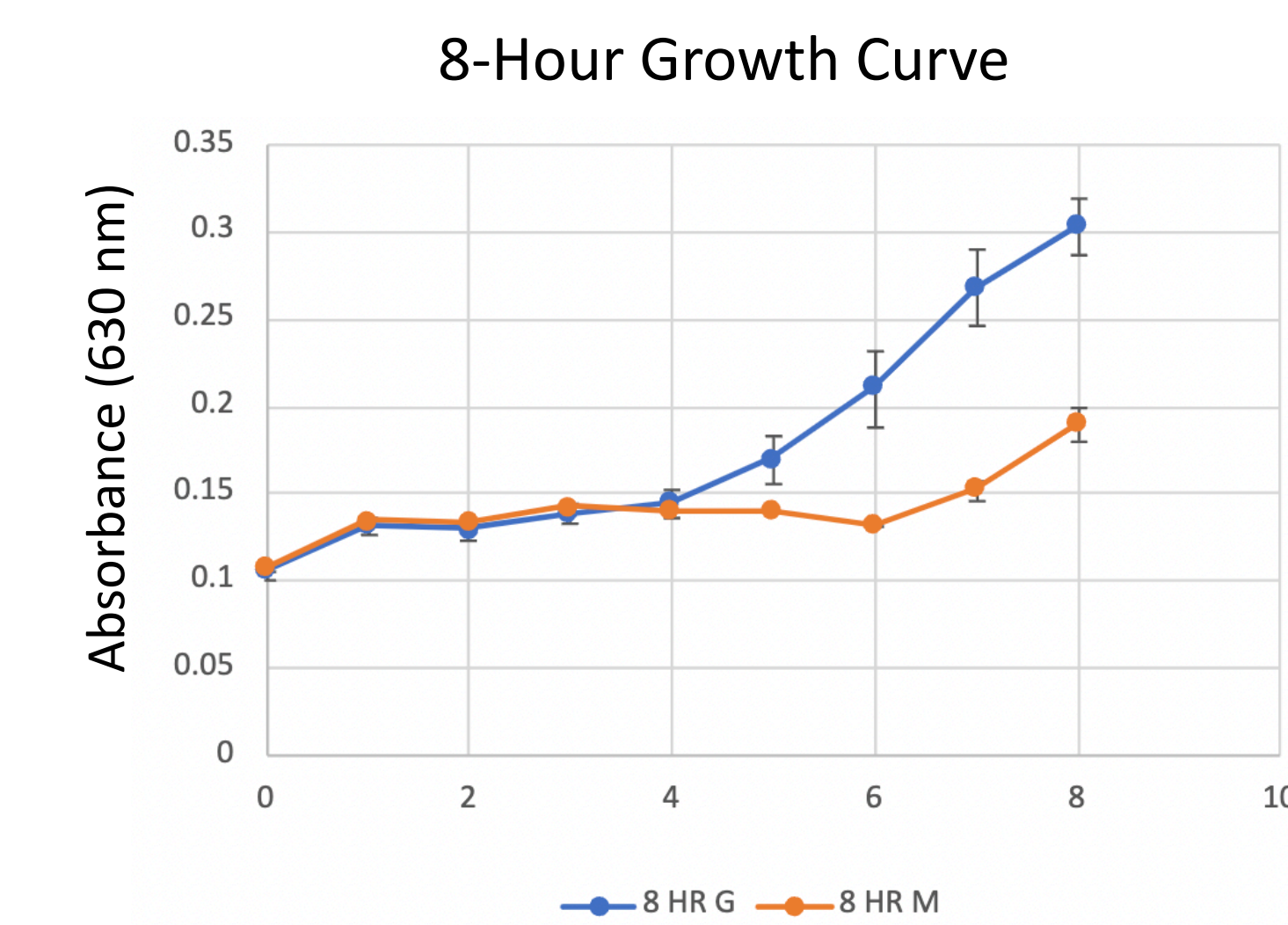
Optical Density

- S. epidermidis* was grown in 2 mL epitubes on the EAGLESTAT and incubated for 30 hours.
- Epitubes had range of antibiotic concentrations.
- After the 30-hr incubation period, absorbance was read at 630 nm on the spectrophotometer to determine biomass.



Growth Curve

- Samples were collected to test their viability at hourly time points.
- These samples were incubated for 8 hours and their absorbance was measured every hour.
- Results indicate a major difference between the two time points, with gravity being more viable at 8 hours and microgravity being more viable at 24 hours.



Future Research

- Expand screening of antibiotics and their concentrations.
- Expand the time period of the growth curve.
- Observe growth of post-antibiotic exposure.
- Observe antibiotic mechanisms of action.
- Study changes in gene expression of cultures.

References:
 Tirumalai, M. R., Karouia, F., Tran, Q., Stepanov, V. G., Bruce, R. J., Ott, C. M., ... & Fox, G. E. (2019). Evaluation of acquired antibiotic resistance in *Escherichia coli* exposed to long-term low-shear modeled microgravity and background antibiotic exposure. *Mbio*, 10(1), e02637-18.
 Cira, N. J., Ho, J. Y., Dueck, M. E., & Weibel, D. B. (2012). A self-loading microfluidic device for determining the minimum inhibitory concentration of antibiotics. *Lab on a Chip*, 12(6), 1052-1059.
 Credito, K., Lin, G., & Appelbaum, P. C. (2007). Activity of daptomycin alone and in combination with rifampin and gentamicin against *Staphylococcus aureus* assessed by time-kill methodology. *Antimicrobial agents and chemotherapy*, 51(4), 1504-1507.