Bacterial Growth and Susceptibility to Antibiotics in Simulated Reduced Levels of Gravity

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Abstract

Changes in bacterial growth and in their susceptibility to antibiotics during spaceflight have been observed since the beginning of the U.S. and Soviet space programs. Regarding bacterial growth, decreased lag phase and increased final population counts are common results of spaceflight experiments. In terms of susceptibility to antibiotics, spaceflight experiments have needed up to four times the antibiotic concentration, relative to Earth, to inhibit bacterial growth. These two phenomena are being investigated in the laboratory with the help of clinostats to simulate the reduced gravity environment around the bacterial cell. A novel method of inclining clinostats at different degrees permit to simulate 0, 1/6 and 1/3 *g*. Cultures of *E. coli* are being grown, separately, in BioServe's Fluid Processing Apparatus (FPA) in these clinostats. Optical density measurements are taken throughout the cultures' growth to assess lag phase duration and final cell concentration. These same bacteria are placed in different concentrations of Colistin Sulfate to assess their susceptibility to the antibiotic in these gravitational environments. For each of these environments, the Minimum Inhibitory Concentrations (MIC) is identified and compared to 1 *g* controls. Although testing continues to achieve statistical significance, the first tests have shown that *E. coli* grow to 9% larger final cell density in the 0 *g* simulation relative to the 1 g control. The antibiotic assessment test is currently under way.

Background

Multiple spaceflight experiments have shown that, in space, bacteria can grow to increased final cell populations, compared to 1 *g* controls [1-12] and can proliferate in antibiotic concentrations that on Earth would kill them [1,13-19]. In a parallel fashion, a Drug-Resistant Bacteria problem here on Earth is taking the lives of over a hundred thousand people around the world, every year, and costing the U.S. Government alone over US$20B a year in excess health care cost[20]. One of this project's aims is to use the spaceflight bacterial phenomena to gain and translate knowledge to the problem on Earth.

Hypotheses

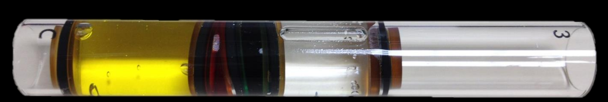
1. *E.coli* culture's final cell count is dependent on gravitational regime: as gravity decreases, the final cell count increases.
2. The Minimum Inhibitory Concentration (MIC) of antibiotic (Colistin Sulfate) required for *E.coli* increases as gravity decreases, i.e. the lower the gravity, the higher antibiotic concentration needed to inhibit bacterial growth.

Experiment Variables

- Bacterial species and strain: *Escherichia coli* ATCC® 4157™ (non-motile)
- Growth Medium: Medium E supplemented with 5 g/L Glucose [21]
- Incubation temperature: 30°C
- Definition of Minimum Inhibitory Concentration: Lowest antibiotic concentration that inhibits bacterial growth after being incubated for 32 hours at 30°C with a starting cell concentration of $5.0 \times 10^6 cell/mL$.
- Antibiotic: Colistin Sulfate

Test Procedure

1. A mother culture of *E. coli* is grown in Medium E supplemented with 5 g/L Glucose at 30°C until it reaches it exponential growth phase.
2. At that time, it is diluted to reach a $1.25 \times 10^6 cell/mL$ concentration.



BioServe's FPA

3. Several test tubes (BioServe's Fluid Processing Apparatus – FPA shown above) are filled and installed inside of BioServe's Group Processing Apparatus (GAP – shown below).

BioServe's GAP floating in ISS. Picture: NASA

4. The GAPs are placed in clinostats inside an incubator at 30°C. Three clinostats at different inclinations – to simulate different gravitational regimes – and horizontal ground controls are used.
5. Optical density measurements at 600 nm are taken with a spectrophotometer to assess bacterial growth. With that data, growth curves are produced for each gravitational environment.
6. For each gravitational regime, a sample is taken during exponential growth phase at around $5.0 \times 10^6 cell/mL$ and distributed throughout five FPAs, each with a different concentration of Colistin Sulfate.
7. This cultures are allowed to incubate and the MIC is identified and compared to that of the 1 *g* control.

Reduced Gravity Simulation

- Reduced gravity environments were simulated using clinostats, devices that continuously rotate cells in a fluid as a solid body, re-orienting the gravity vector.
- Clinostats don't *remove* the gravity vector but simulate the quiescent environment around a cell characteristic of reduced gravity.
- Clinostats have been used for gravitational microbiology experiments of different kinds[2, 5, 22-29].
- The clinostat rotational speed must be calculated based on multiple variables, including cell density and volume, medium density and viscosity, cell sedimentation velocity, supplement and byproducts diffusion coefficients, etc.
- The clinostat must rotate fast enough so the particle doesn't sediment off its *zone of depletion* and slow enough to ensure it is not centrifuged out of it [30, 31].
- Using a coordinate system *[x,z]* rotating at an angular velocity ω around a *y* axis, a summation of forces analysis including weight, buoyancy and centrifugal force yields two second order, non-homogeneous partial differential equations. The one for the *x* axis is:

$$\frac{\delta^2 x}{\delta t^2} + \frac{f}{m}\frac{\delta x}{\delta t} - \frac{m^*}{m}\omega^2 x = -\frac{m^* g}{m} sin\omega t$$

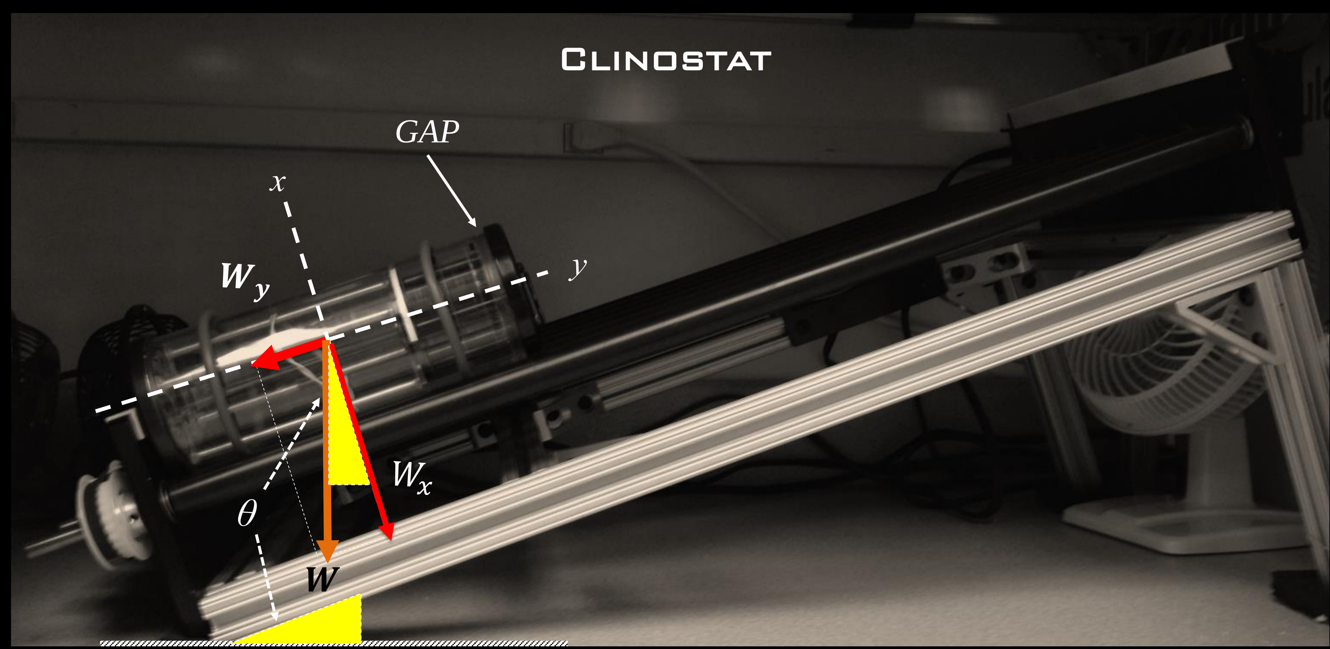
where *f* is the cell's Stoke's radius, *m* is its mass and *m** is its buoyancy-corrected mass.

- The solution to these equations describes a particle circular motion with radius *q*:

$$q = \frac{v_{sed}}{\omega} = \frac{2ga^2\Delta\rho}{9\mu\omega}$$

here, v_{sed} is the cell's sedimentation velocity and $\Delta\rho$ is the density difference between the cell and fluid.

- This bacterial cell's circular motion has to have a smaller radius than that of the sphere described by the particle's Brownian motion [32].
- Partial gravity, *i.e.* 1/3 and 1/6 *g* are attained by inclining the clinostat at an angle *theta*, where the value of *theta* is between 0° and 90°. One third *g*, or Martian gravity, is generated when the clinostat is inclined at a 19.5° angle; one sixth, or lunar gravity, when it is at a 9.6° angle. Details and validation of the system is reported in Ref 33.

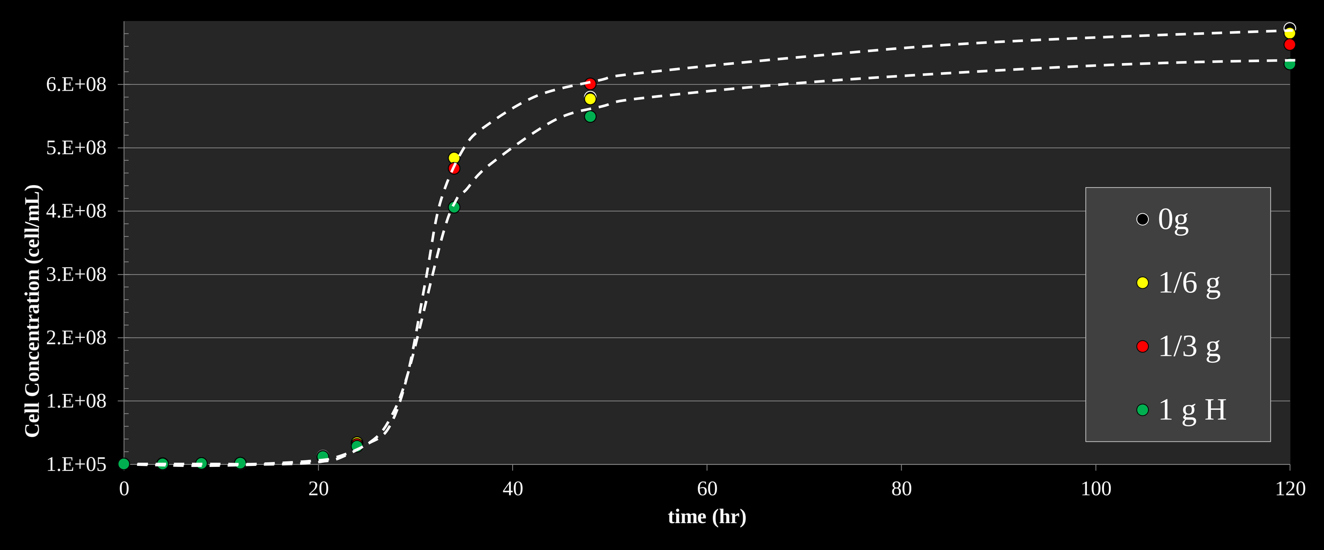


References

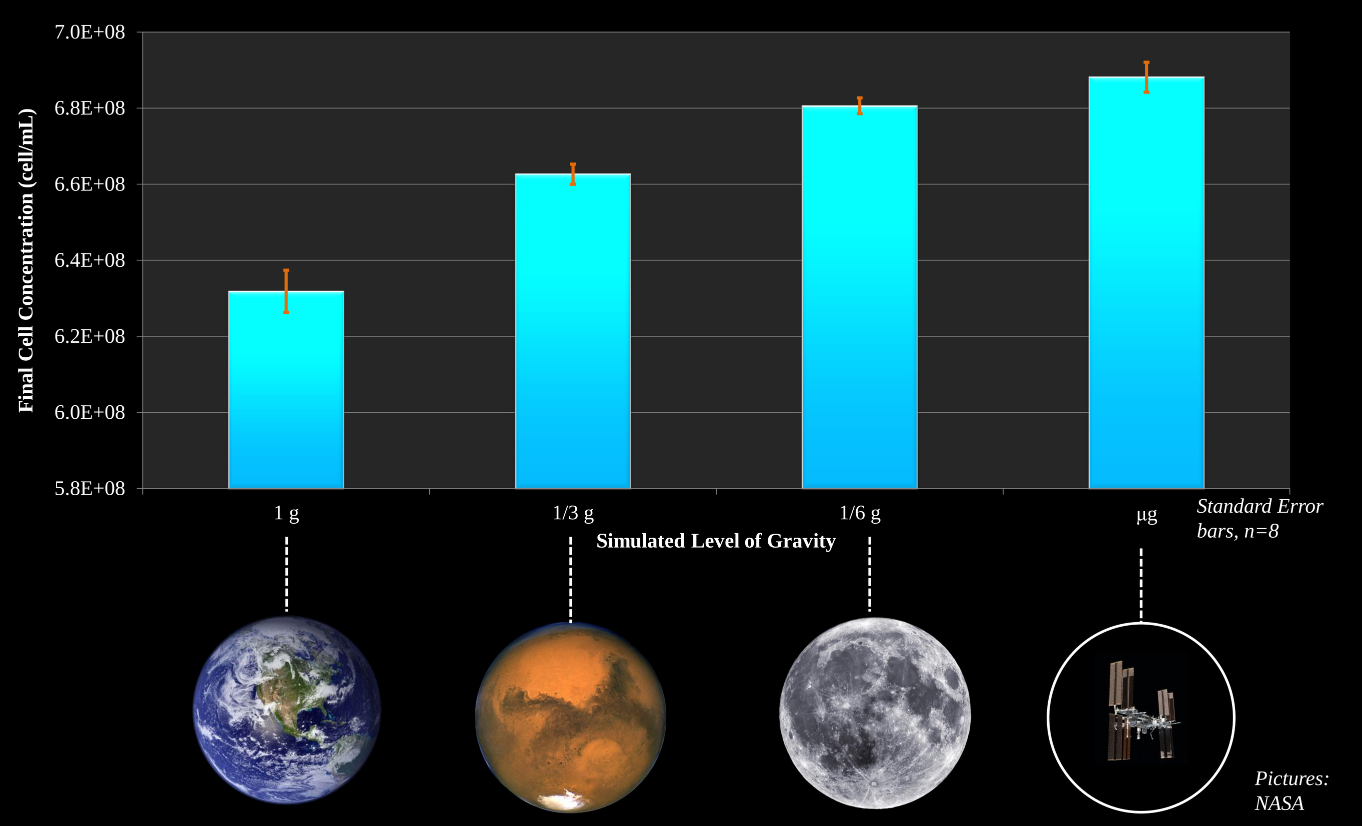
1. Klaus, & Howard. (2006). Antibiotic efficacy and microbial virulence during space flight. Trends in Biotechnology, 24(3), 131–136. doi:10.1016/j.tibtech.2006.01.008
2. Benoit, & Klaus, D. M. (2007). Microgravity, bacteria, and the influence of motility. Advances in Space Research, 39(7), 1225–1232. doi:10.1016/j.asr.2006.10.009
3. Mermel, L. A. (2013). Infection Prevention and Control During Prolonged Human Space Travel. Clinical Infectious Diseases, 56(1), 123–130. doi:10.1093/cid/cis861
4. Tixador, R., Richoilley, G., Gasset, G., Planel, H., Moatti, N., Lapchine, L., … Kirilova, F. M. (1985). Preliminary results of cytos 2 experiment. Acta Astronautica, 12(2), 131–134. doi:10.1016/0094-5765(85)90082-7
5. Benoit. (2005). Responses, applications, and analysis of microgravity effects on bacteria. ProQuest Dissertations And Theses; Thesis (Ph.D.)--University of Colorado at Boulder, 2005.; Publication Number: AAI3188462; ISBN: 9780542311154; Source: Dissertation Abstracts International, Volume: 66-09, Section: B, page: 4616.; 247 p
6. Bhaskaran, Dudhale, R., Dixit, J., Sahasrabuddhe, A., & Vidyasagar, P. B. (2011). Growth of bioluminescent bacteria under modelled gravity of different astronomical bodies in the Solar system. arXiv:1109.5786. Retrieved from http://arxiv.org/abs/1109.5786
7. He, M., Miyajima, F., Roberts, P., Ellison, L., Pickard, D. J., Martin, M. J., … Lawley, T. D. (2013). Emergence and global spread of epidemic healthcare-associated Clostridium difficile. Nature Genetics, 45(1), 109–113. doi:10.1038/ng.2478
8. Kacena, Merrell, G. A., Manfredi, B., Smith, E. E., Klaus, D. M., & Todd, P. (1999). Bacterial growth in space flight: logistic growth curve parameters for Escherichia coli and Bacillus subtilis. Applied microbiology and biotechnology, 51(2), 229–234.
9. Klaus, Luttges, & Stodieck. (1994). Investigation of Space Flight Effects on Escherichia coli Growth (SAE Technical Paper No. 941260). Warrendale, PA: SAE International. Retrieved from http://papers.sae.org/941260/
10. Klaus, D., Simske, S., Todd, P., & Stodieck, L. (1997a). Investigation of space flight effects on Escherichia coli and a proposed model of underlying physical mechanisms. Microbiology, 143(2), 449–455. doi:10.1099/00221287-143-2-449
11. Nickerson, C. A., Ott, C. M., Mister, S. J., Morrow, B. J., Burns-Keliher, L., & Pierson, D. L. (2000). Microgravity as a Novel Environmental Signal Affecting Salmonella enterica Serovar Typhimurium Virulence. Infection and Immunity, 68(6), 3147–3152. doi:10.1128/IAI.68.6.3147-3152.2000
12. Todd, P., Klaus, D. M., Stodieck, L. S., Smith, J. D., Staehelin, L. A., Kacena, M., … Bukhari, A. (1998). Cellular responses to gravity: Extracellular, intracellular and in-between. Advances in Space Research, 21(8–9), 1263–1268. doi:10.1016/S0273-1177(97)00397-9
13. Moatti, N., Lapchine, L., Gasset, G., Richoilley, G., Templier, J., & Tixador, R. (1986). Preliminary results of the “Antibio” experiment. Naturwissenschaften, 73(7), 413–414. doi:10.1007/BF00367282
14. Ilyin, V. K. (2005). Microbiological status of cosmonauts during orbital spaceflights on Salyut and Mir orbital stations. Acta Astronautica, 56(9–12), 839–850. doi:10.1016/j.actaastro.2005.01.009
15. Kitts, C., Ronzano, K., Rasay, R., Mas, I., Acain, J., Neumann, M., … Beck, E. (2009). Initial Flight Results from the PharmaSat Biological Microsatellite Mission. In Proc. 23nd Annual AIAA/USU Conf on Small Satellites, Logan UT. Retrieved from http://webpages.scu.edu/ftp/iamas/papers/SSC09-IV-10.pdf
16. Mermel, L. A. (2013). Infection Prevention and Control During Prolonged Human Space Travel. Clinical Infectious Diseases, 56(1), 123–130. doi:10.1093/cid/cis861
17. Parra, M., Ricco, A. J., Yost, B., McGinnis, M. R., & Hines, J. W. (2008). Studying space effects on microorganisms autonomously: genesat, pharmasat and the future of bio-nanosatellites. Gravitational and Space Biology, 21(2), 9–17.
18. Ricco, A. J., Parra, M., Piccini, M., Ly, D., Niesel, D., McGinnis, M., … Beasley, C. (2010). PharmaSat: drug dose dependence results from an autonomous microsystem-based small satellite in low Earth orbit. In Technical Digest, Solid-State Sensor, Actuator and Microsystems Workshop (pp. 110–113).
19. Taylor, & Sommer, A. P. (2005). Towards rational treatment of bacterial infections during extended space travel. International Journal of Antimicrobial Agents, 26(3), 183–187. doi:10.1016/j.ijantimicag.2005.06.002
20. CDC. (2011, April 7). CDC Online Newsroom - Press Release: April 7, 2011. Retrieved March 20, 2013, from http://www.cdc.gov/media/releases/2011/p0407_antimicrobialresistance.html
21. Vogel, H. J., & Bonner, D. M. (1956). Acetylornithinase of Escherichia Coli: Partial Purification and Some Properties. Journal of Biological Chemistry, 218(1), 97–106.
22. Arunasri, K., Adil, M., Venu Charan, K., Suvro, C., Himabindu Reddy, S., & Shivaji, S. (2013). Effect of Simulated Microgravity on E. coli K12 MG1655 Growth and Gene Expression. PLoS ONE, 8(3), e57860. doi:10.1371/journal.pone.0057860
23. Baker, P. w., & Leff, L. g. (2005). Intraspecific differences in bacterial responses to modelled reduced gravity. Journal of Applied Microbiology, 98(5), 1239–1246. doi:10.1111/j.1365-2672.2005.02593.x
24. Brown, R. B., Klaus, D., & Todd, P. (2002). Effects of space flight, clinorotation, and centrifugation on the substrate utilization efficiency ofE. coli. Microgravity Science and Technology, 13(4), 24–29. doi:10.1007/BF02881678
25. Galvez, R. (2013). Human Research Roadmap. NASA Human Research Roadmap. Retrieved March 20, 2013, from http://humanresearchroadmap.nasa.gov/
26. Kacena, M. A., Manfredi, B., & Todd, P. (1999). Effects of space flight and mixing on bacterial growth in low volume cultures. Microgravity science and technology, 12(2), 74.
27. Mattoni, R. H. T. (1968). Space-Flight Effects and Gamma Radiation Interaction on Growth and Induction of Lysogenic Bacteria: A Preliminary Report. BioScience, 18: 602-8(June 1968). Retrieved from http://www.osti.gov/energycitations/product.biblio.jsp?osti_id=4488819
28. Menningmann, H. D., & Heise, M. (1994). Response of growing bacteria to reduction in gravity. In Life Sciences Research in Space (Vol. 366, p. 83). Retrieved from http://adsabs.harvard.edu/full/1994ESASP.366...83M
29. Wilson, J. W., Ott, C. M., Bentrup, K. H. zu, Ramamurthy, R., Quick, L., Porwollik, S., … Nickerson, C. A. (2007). Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proceedings of the National Academy of Sciences, 104(41), 16299–16304. doi:10.1073/pnas.0707155104
30. Klaus, David M, Benoit, M. R., Nelson, E. S., & Hammond, T. G. (2004). Extracellular mass transport considerations for space flight research concerning suspended and adherent in vitro cell cultures. Journal of gravitational physiology: a journal of the International Society for Gravitational Physiology, 11(1), 17–27.
31. Klaus, David M. (2007b). Clinostats and bioreactors. Gravitational and Space Biology, 14(2). Retrieved from http://gravitationalandspacebiology.org/index.php/journal/article/view/267
32. Klaus, D.M., Todd, P., & Schatz, A. (1998). Functional weightlessness during clinorotation of cell suspensions. Advances in Space Research, 21(8–9), 1315–1318. doi:10.1016/S0273-1177(97)00404-3
33. Leidich, J., Thomas, E., Klaus, D., A Novel Testing Protocol for Evaluating Particle behavior inFluid Flow Under Simulated Reduced Gravity Conditions, ICES, 2009-01-2359

Results

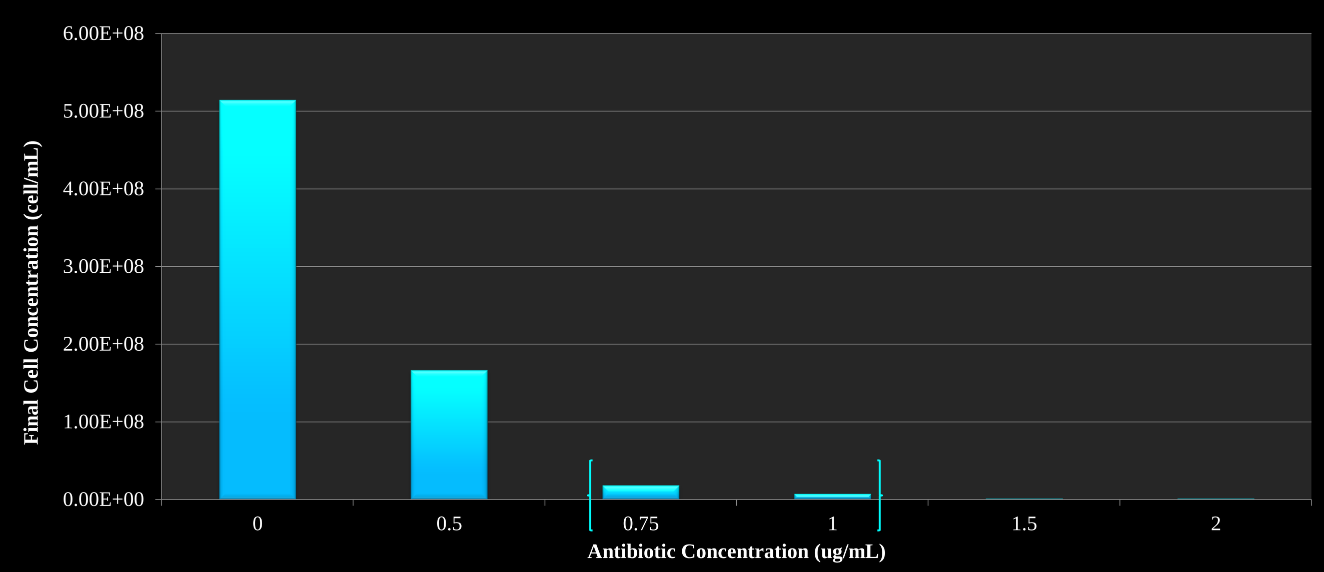
Example of *E.coli* Growth Curves at Different Simulated Levels of Gravity



Example of Final Cell Concentrations at Different Simulated Levels of Gravity



Colistin Sulfate Minimum Inhibitory Concentration at 1 g



Future Work

- More growth curves are currently being produced to achieve statistically significant values.
- Colistin Sulfate MICs at different simulated reduced levels of gravity will be identified.
- This investigation will be expanded to analyze Gentamicin Sulfate, an antibiotic with a different mechanism of action.
- This research will be expanded to include Bacillus Subitlis to have a gram positive model.

Conclusions

- Although more experiments are currently being conducted to achieve statistical significance, so far data indicates that there is a direct relationship between gravitational regime and *E.coli* proliferation: the lower the gravity, the higher final cell concentration.
- First tests show final cell concentration of *E.coli* to be 9% higher than the 1 *g* horizontal controls.
- This data suggest that, although bacteria could proliferate better in Lunar and Martian settlements compared to Earth, the worst case scenario could be the microgravity environment.

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