In vitro synergistic activity of antibiotics and *Trachemys scripta* plasma on bacterial growth

Mackenzie N. Martin, Laura M. Zimmerman
Millikin University Biology Department, 1184 W. Main Street, Decatur, IL 62522

Introduction

- There is a growing concern for antibiotic resistance
  - From the use, overuse, and misuse of antibiotics, microorganisms in our environment are constantly altered, making bacterial illnesses tougher to overcome
  - Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* are notorious superbugs in human population[1]
- Prior research
  - Siroski et al. found that ciprofloxacin eliminated *E. coli* at higher rates when in the presence of caiman serum[2]
  - Taylor et al. used frozen *T. scripta* plasma and streptomycin against *E. coli* to show there was a trend towards plasma yielding higher killing[3]
- Turtle plasma contains immunogenic capacities, such as the complement cascade
  - Utilizing fresh versus frozen plasma
    - Beck et al. observed that 6-week-old frozen plasma samples at -80°C did not reduce bactericidal capacity[4]
  - Dynamic aspects of innate versus adaptive reptilian immune system
    - Immune function may vary with age, season[5]
- Objective of project is to study alternative method of attack against microorganisms
  - We hypothesize that plasma from *T. scripta* will act in a synergistic way with streptomycin to improve *E. coli* and *S. aureus* killing

Methods

- 14 *E. coli* and *S. aureus* trials were incubated in TSB at 37°C
- 1:5, 1:10, 1:15, and 1:20 bacteria to saline concentrations were added to 4 petri dishes
  - Aim was to grow <200 CFU per plate
- After an overnight incubation period, the amount of bacteria needed to create an assay was determined
  - 0.25 MIC prepared using 16 µg streptomycin/20 mL saline
  - Fresh and frozen *T. scripta* plasma were used
    - Plasma was added to yield a concentration of 1:40
- Data was collected from colonies after 0, 0.5, 1, 2, 3, and 4 hours to determine the percentage of colonies killed from bacteria, bacteria + plasma, bacteria + antibiotic, and bacteria, plasma, + antibiotic
- Repeated measures ANOVA and independent-sample t-tests were used to aid in data analysis

Results

- The effect of time was significant in all trials (Fig. 1 p=0.001, Fig. 2 p=0.002, Fig. 3 p=0.017)

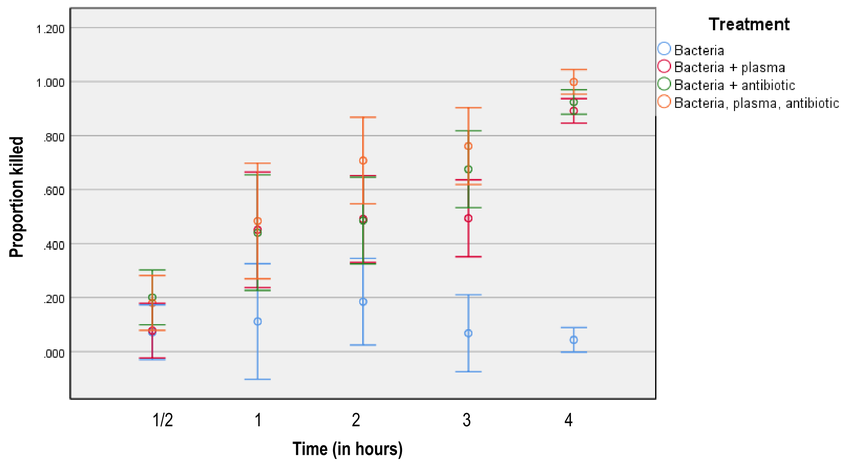

Figure 1. *E. coli,* fresh plasma, and antibiotic. The estimated marginal mean proportion killed (+/- 1 standard error) for each treatment group involving the effect of fresh *T. scripta* plasma and antibiotic on killing *E. coli.* 4 different treatments were employed and measured after ½, 1, 2, 3, and 4 hour intervals.

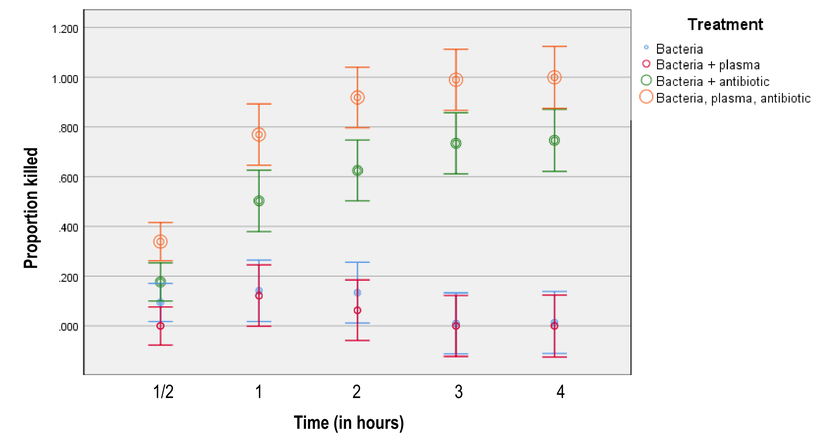

Figure 2. *E. coli,* frozen plasma, and antibiotic. The estimated marginal mean proportion killed (+/- 1 standard error) for each treatment group involving the effect of frozen *T. scripta* plasma and antibiotic on killing *E. coli.* 4 different treatments were employed and measured after ½, 1, 2, 3, and 4 hour intervals.

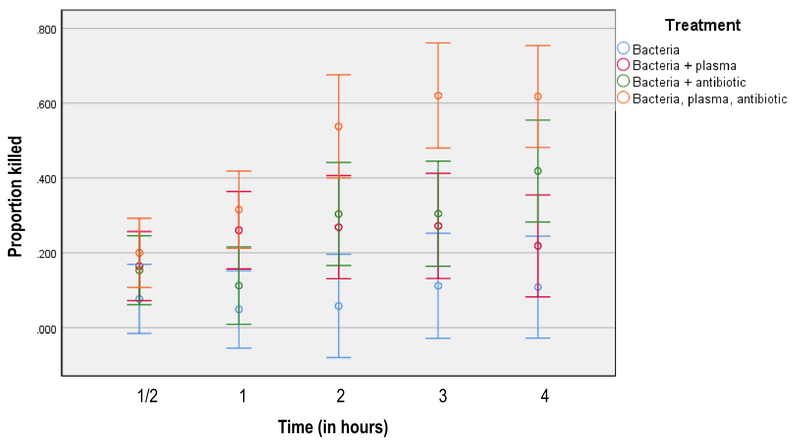

Figure 3. *S. aureus,* frozen plasma, and antibiotic. The estimated marginal mean proportion killed (+/- 1 standard error) for each treatment group involving the effect of frozen *T. scripta* plasma and antibiotic on killing *S. aureus.* 4 different treatments were employed and measured after ½, 1, 2, 3, and 4 hour intervals.

Results (continued)

- The effect of treatment was significant in fresh and frozen plasma in killing *E. coli* (Fig. 1 p=0.003, Fig. 2 p=0.000, Fig. 3 p=0.163)
- There was not a significant time by treatment interaction in any trials (Fig. 1 p=0.438, Fig. 2 p=0.358, Fig. 3 p=0.112)
- Though not statistically significant, there was a trend for plasma and antibiotic treatments to have greater capacities for killing, particularly with *E. coli* bacteria (Fig. 1 & 2)
  - For bacteria, plasma, and antibiotic, there was an average of 86.29% colonies killed after 4 hours
  - For bacteria and antibiotic, there was an average of 70.31% of colonies killed after 4 hours
- The effect of using fresh plasma alone was significant in increasing killing capacity (p=0.000)
  - 0.00% killed with *E. coli* and frozen plasma after 4 hours
  - 89.17% killed with *E. coli* and fresh plasma after 4 hours
- The effect of fresh plasma and antibiotic synergy was not significant in increasing killing capacity (p=0.840)

Discussion

- Hypothesis partially supported
  - Time by treatment interaction not significant
  - Trend towards greater killing capacity with plasma and antibiotic synergy
  - Significantly higher killing seen with fresh plasma alone
- Potential capacity for error
  - *In vitro* models, while useful in controllability, cost effectiveness, and ease of use, are not necessarily representative of clinical outcomes[6]
  - Small sample size could possibly lead to non-statistically significant interaction
- Future research and data in support of employing plasma for decreasing antibiotic use to treat bacterial infections
  - Research bactericidal synergy with other types of antibiotics
  - Continually lower the MIC amount of antibiotic used in treatment
  - Use of antibiotic-resistant bacterial strains[7]

Acknowledgements

I would like to thank Dr. Zimmerman for her guidance throughout the duration of this project. I would also like to thank the Millikin University Biology Department for providing me with the opportunity, funding, and facilities to participate in undergraduate research through the Leighty Science Scholars Program. Additional thanks to Katie Stromsland, Hannah Bond, and Selena Smail for collecting the plasma samples.

Contact

Mackenzie Martin
Millikin University
mnmartin@milikin.edu

References

1. Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. American Society for Microbiology. 74(3), 417-433.
2. Siroski, P.A., Russi, N.B., Ortega, H.H., Formentini, E.A., 2014. In vitro evaluation of synergistic activity between ciprofloxacin and broad snouted caiman serum against *Escherichia coli.* Research in Veterinary Science. 98, 98-105.
3. Taylor, S.J., Gray, W.A., Zimmerman, L.M, 2017. In vitro synergistic bactericidal activity between antibiotics and *Trachemys scripta elegans* plasma. Presented at Society for Integrative and Comparative Biology, New Orleans, LA.
4. Beck, M.L., Thompson, M., Hopkins, W.A., 2017. Repeatability and sources of variation of the bacteria-killing assay in the common snapping turtle. Department of Fish and Wildlife Conservation. 327(5), 293-301.
5. Zimmerman, L.M., Paitz, R.T., Vogel, L.A., Bowden, R.M., 2010. Variation in the seasonal patterns of innate and adaptive immunity in the red-eared slider (*Trachemys scripta).* Journal of Experimental Biology. 213:1477-1483.
6. Bergman, M., Nyberg, S.T., Huovinen, P., Paakkari, P., Hakanen, A.J., 2009. Association between antimicrobial consumption and resistance in *Escherichia coli.* Antimicrobial Agents and Chemotherapy. 53(3), 912-917.
7 Hoeksema, M., Jonker, M.J, Brul, S., Kuile, B.H., 2019. Effects of a previously selected antibiotic resistance on mutations acquired during development of a second resistance in *Escherichia coli.* BioMed Central Genomics. 20, 1-14.