Antimicrobial resistance of Staphylococcus aureus in the dust microbiome

AYURG | Natural Sciences and Engineering (NSE) | *Tags: Lab-based*

This cover page is meant to focus your reading of the sample proposal, summarizing important aspects of proposal writing that the author did well or could have improved. ***Review the following sections before reading the sample****. The proposal is also annotated throughout to highlight key elements of the proposal's structure and content.*

Proposal Strengths	Areas for Improvement
The introduction narrows from a broad topic to the specific issue addressed in this project with a clear logic flow. Additionally, there is a clear focus at the end of the first paragraph.	The background/literature review reads too much like a summary. The section would be improved by adding sentences that explicitly connect the literature being reviewed to the broader importance/specific project being proposed. This act of interpreting the past literature, rather than summarizing, helps a reader follow your argument for why a project should be done.
The preparation section of the proposal addresses the specific methods needed to conduct the proposed study.	The analysis procedure should be made clearer. It is important to go beyond saying simply that the results "will be compared" or "will be analyzed". Think through what that analysis will entail step-by-step, and what your parameter for success will be: What would your output look like and what would that mean for your research question?
While much of what determines this project comes from work within this lab, the researcher cites more than their own lab and does the work of reviewing other relevant past work.	The inclusion of an explicit research question (ending with a question mark) or statement of objectives would strengthen the proposal.

Other Key Features to Take Note Of
A figure is included in this proposal within the 2 page limit. Figures are permitted, but unless the figure is *critical* for understanding your project, you could consider moving it to an appendix after your works cited and referring to the appendix much as you would an in-text citation (Appendix A).
All Academic Year URGs require a budget. There is no required format; however, we do provide a template on our website. The scope of the proposal should focus on what the funding covers.

Titles are unnecessary since a cover page is generated automatically

Antimicrobial resistance of *Staphylococcus aureus* in the dust microbiome

Broad importance made clear

Antibiotic resistance is a growing problem in modern medicine that has become consistently more worrisome. However, the issue of antibiotic resistance is not unique to the medical field; recent studies suggest that, with increasing occurrence of antimicrobial chemicals in consumer products, the evolution of antibiotic resistance is an emerging environmental problem, present everywhere from homes and schools to hospitals and athletic facilities (1). Research conducted on the indoor microbiome indicates that indoor microbial communities interact significantly with occupants; microbial communities originate primarily from local sources, such as the activities of human occupants (2). Therefore, the use of antimicrobial chemicals on indoor surfaces plays a crucial role in the development of indoor microbiomes. This realization has caused concern about the prevalence of antimicrobials in consumer products. The goal of my project is to expand on current research by determining the antimicrobial characteristics of *Staphylococcus aureus* in dust collected from antibacterial-treated surfaces. In doing so, I will learn how to conduct a research project from beginning to end, a skill that will greatly aid me in my academic success and beyond.

Microbiome not defined in simple terms

Clear research focus in the 1st paragraph

Triclosan is a common bacteriostatic drug that prevents bacterial growth, found in products ranging from domestic toothpastes and lotions to clinical antiseptics (3). While it effectively controls growth in bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) by blocking fatty acid synthesis – essential to reproducing cell membranes – species with high efflux capacities are able to circumvent this mechanism by dispelling the drug, and are therefore naturally resistant to triclosan. Several previously susceptible microbes seem to be adopting similar tolerances (4). The Food and Drug Administration has responded to concerns that overuse of triclosan and other antimicrobials is contributing to antibiotic resistance by issuing a ban against triclosan in consumer soaps. Similar regulation of another common antibacterial ingredient, benzalkonium chloride, is still up for debate, and will be revisited later this year (5). Benzalkonium chloride is a quaternary ammonium salt; it functions by altering the permeability of cell membranes and inhibiting normal cell activities (6). Therefore, benzalkonium chloride has bacteriostatic properties similar to triclosan, but works through a different mechanism.

Should include an explicit research question

Reads like a summary. Interpretive sentences should be added that connect literature to research aim

Identifies gap in knowledge

Situates project in lab's previous work

I will be working in Dr. Hartmann's laboratory, where we are currently testing dust samples collected from educational and athletic facilities treated with triclosan-containing products for antibiotic resistant forms of *Staphylococcus aureus* (Hartmann). Bacterial colonies from the dust samples are cultured on tryptone soy agar plates containing the fungicide itraconazole to prevent fungal growth from interfering with results. Dust is inoculated onto the plates from a buffer suspension. After 2-3 days of growth, the plates are replicated onto plates containing one of three antibiotics – ampicillin, clarithromycin, and tetracycline – and their growth is observed. I would like to add another element to this study by replicating these same colonies onto plates containing triclosan and plates containing benzalkonium chloride. Assuming the colonies exhibiting antibiotic resistant properties have developed such characteristics due to prolonged exposure to antimicrobials, I would expect the bacteria to grow relatively uninhibited by triclosan. After observing which colonies are resistant to triclosan, I would like to see how

Focuses on independent contribution, includes expected results

many of those same colonies are also resistant to benzalkonium chloride. This antimicrobial has a different mechanism of action than triclosan – rather than preventing reproduction of the cell membrane, benzalkonium chloride disrupts the cell membrane, altering its permeability and interrupting cell functions (Maris). Consequently, I would not expect to see significant growth in the presence of this compound; however, I would like to confirm that other antimicrobials are still effective on bacteria rendered resistant to triclosan.

Specifically, growth will be assessed by counting colonies on all plates to determine the number of colony forming units (CFUs). The number of CFUs on the original, triclosan, and benzalkonium chloride plates will be compared to determine the percentage of resistant colonies. Figure 1 shows a series of antibiotic plate replicas – the result of my experiment will resemble this. I may pick some colonies to be suspended in tryptone soy broth so that their DNA may later be analyzed using the polymerase chain reaction (PCR) method. This would allow me to definitively determine if a colony is *Staphylococcus aureus* by isolating the 16S region, and if genes related to resistance and virility are present. By analyzing the amount of antibacterial-resistant colonies in the dust samples, my project aims to further our understanding of how widespread use of antimicrobials might be contributing to antibiotic resistance.

Clear output

There should be more detail on the analysis. How will the results be compared? What amount will be considered significant?

After working in Dr. Hartmann's lab this quarter, I have significant experience with the culturing, inoculating, and replicating techniques described above. In the next few weeks, I will be learning about PCR analysis. I will use predetermined minimum inhibitory concentrations to calculate the amount of antimicrobial needed in the plates. Using this information and my prior experience, I will adapt the procedures I am using in the laboratory to fit my experiment. Conducting this research study will provide me with the opportunity to apply my academic interests toward investigating a relevant issue; this will help me to explore practical applications of biological sciences and prepare myself for future internship and career opportunities. This grant is an opportunity to gain experience in research, and may even contribute to a senior thesis for my major.

Preparation section mentions specific methods needed for the project. Student was not in lab long but shows a specific training plan to reach independence

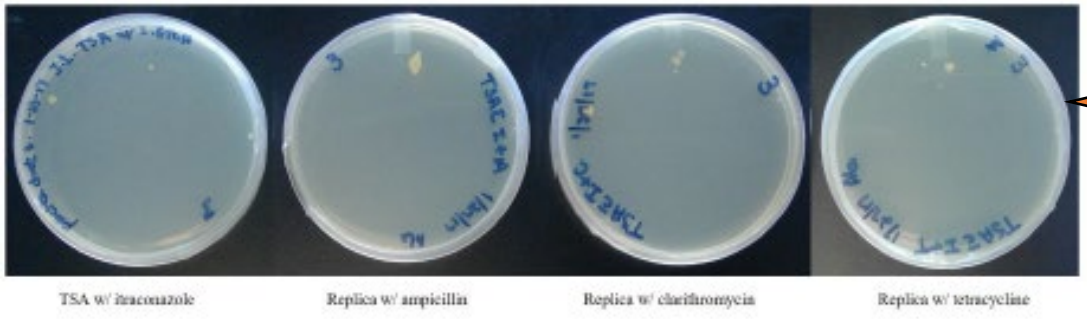

Figure could be added to an appendix after the works cited

Figure 1: TSA plates with ampicillin, clarithromycin, and tetracycline replicated from the original (far left). Colony growth at the top of the plates indicates multi-resistance.

References

1. Hartmann, E. M.; Hickey, R.; Hsu, T.; Betancourt Román, C. M; Chen, J.; Schwager, R.; Kline, J.; Brown, G. Z.; Halden, R. U.; Huttenhower, C.; Green, J.L. Antimicrobial Chemicals Are Associated with Elevated Antibiotic Resistance Genes in the Indoor Dust Microbiome. *Environ. Sci. & Technol.* **2016**, *50*(18), 9807-9815.

2. Stephens, B. What Have We Learned about the Microbiomes of Indoor Environments? *mSystems* **2016**, *1*(4).

3. Møretrø, T.; Sonerud, T.; Mangelrød, E.; Langsrud, S. Evaluation of the Antibacterial Effect of a Triclosan-Containing Floor Used in the Food Industry. *Journal of Food Protection* **2006**, *69*(3), 627-633.

4. Pawlowski, A. C.; Wang, W.; Koteva, K.; Barton, H. A.; McArthur, A. G.; Wright, G. D. A diverse intrinsic antibiotic resistome from a cave bacterium. *Nature Communications* **2016**, 7.

5. FDA issues final rule on safety and effectiveness of antibacterial soaps. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm517478.htm (accessed February 9, 2017).

6. Maris, P. Modes of action of disinfectants. *Rev. sci. tech.* **1995**, *14*(1), 47-55).

Itemized Budget

A. Research-Related Expenses (Data Collection; Analysis)

TYPE	COST	NOTES
1. Consumable Materials	$850.00	triclosan, benzalkonium chloride, tryptone, soytone, agar, culture plates, sterile cell spreaders, itraconazole
2. Non-Consumable Materials		
3. Equipment/Durable Goods	$40.00	Lab notebook
4. Research Subject Compensation		
5. Fees		
6. Transcription Services		
7. Tuition/Mandatory Fees		
8. Instructional Materials		
9. Living Expenses		
10. Other		

B. Travel-Related Expenses

TYPE	COST	NOTES
1. Airfare (round trip)		
2. Housing		
3. Food		
4. Local Travel Expenses		
5. Other		

C. International-Related Expenses

TYPE	COST	NOTES
1. Entry Visa or Visas		
2. Required Vaccines		
3. Recommended Vaccines		
4. Travel Health Insurance (HTH)		

5. Passport		
6. Other		

TOTAL EXPENSES

TYPE	COST	NOTES
Total Research Expenses (A)	$890.00	
Total Travel Expenses (B)	$0.00	
Total International Expenses (C)	$0.00	
TOTAL EXPENSES	**$890.00**	

D. POTENTIAL FUNDING

SOURCE	AMOUNT	NOTES
TOTAL FUNDING	**$0.00**	