This is provided as an example proposal.
it is important that you follow the current guidelines.

The mentor letter has been removed.

The John J. Kopchick Molecular and Cellular Biology/Translational Biomedical Sciences Undergraduate Student Support Fund Cover Page Academic Year 2019-2020

NAME OF APPLICANT: Gillian Null
E-MAIL ADDRESS: gn454115@ohio.edu
DEPARTMENT: Biological Sciences
EXPECTED GRADUATION DATE (Month and Year): 05/2020

TITLE OF PROJECT: Investigating the importance of homeostatic regulation of global supercoiling levels to *in vivo* virulence of *Staphylococcus aureus*.

FACULTY MENTOR INFORMATION:

NAME: Ronan Carroll
E-MAIL ADDRESS: carrolr3@ohio.edu
DEPARTMENT: Biological Sciences
NUMBER OF YEARS FACULTY IN THE MCB OR TBS PROGRAM: 5

BUDGET: Total Request $1,500
(May not exceed $1,500)

STATEMENT OF HOW THE RESEARCH IS RELEVANT TO TRANSLATIONAL BIOMEDICAL SCIENCES* (500 character limit)

As *S. aureus* continues to evolve ways of resisting current treatments, ongoing work is needed to understand how virulence is regulated on a DNA level. Supercoiling is a dynamic property of DNA that acts as an intrinsic regulatory switch for gene expression, including for some virulence genes in *S. aureus*. Modulation of supercoiling is a vital feature of infection progression. Elucidation of the importance of this mechanism *in vivo* could inform progress of new treatments for *S. aureus* infection.

*For the purposes of this program, translational biomedical sciences is defined as the translation of basic research effectively into enhanced healthcare outcomes for the entire population in fields such as biomedical research, bioengineering, drug development, informatics, communications, health policy and planning.

IRB AND IACUC APPROVAL:

To ensure that the University is in compliance with all federal regulations, complete the checklist below. *Note: your proposal can be approved prior to IRB or IACUC approval (put "pending" or "to be submitted" instead of approval number), but funding will be withheld until notification of approval or exemption.*

Yes	***No***	***Office of Research Compliance***	***Policy #***
	x	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
x		Animal Species: Mus musculus Institutional Animal Care & Use Committee (IACUC) Approval #:17-H-019 Expiration Date:12/06/2021	19.049

SIGNATURES

Applicant's Signature		**Faculty Mentor's Signature**	
Signature	Gillian Null	Signature	Ronan Carroll
Name	Gillian Null	Name	Ronan Carroll

Optional: x Yes No

If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Gillian Null

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Research Apprentice in the Lab of Dr. Ronan Carroll

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Ohio University Honors Tutorial College Athens, Ohio	B. S	05/2020	Biological Sciences (4.0 GPA)

A. Personal Statement

I am currently in the process of applying to medical school and plan to obtain my MD degree. I am applying primarily to dual MD/MPH degree programs and plan to concentrate in infectious disease for my MPH studies. This project will help me to better understand the mechanisms regulating virulence in a pathogen that poses a formidable risk to public health. I can use the knowledge I gain from this project, which is very translational and clinically relevant, to better treat patients as a physician. This project will also enable me to further my research skills and experience. I plan to continue doing research in pathogenic bacteriology throughout medical school and residency as well as throughout my career as a physician.

B. Positions and Employment

Summer 2017 Data Collection Assistant under Dr. Gillian Ice, Social Sciences, OU-HCOM
2017- Undergraduate Research Assistant under Dr. Ronan Carroll, Dept. of Biological Sciences, Ohio University
Summer 2019 National Oceanic and Atmospheric Administration (NOAA) intern under the mentorship of Dr. Reagan Errera, Great Lakes Environmental Research Laboratory
2019- HTC Research Apprentice under Dr. Ronan Carroll, Dept. of Biological Sciences, Ohio University

C. Contributions to Science and Scholastic Honors

Publications Keogh, R.A.; Zapf, R.L.; Trzeciak, E.; Null, G.G.; Wiemels, R.E.; Carroll, R.K. Novel Regulation of Alpha-Toxin and the Phenol-Soluble Modulins by Peptidyl-Prolyl cis/trans Isomerase Enzymes in Staphylococcus aureus. Toxins 2019, 11, 343.
Funding John J. Kopchick Undergraduate Support Fund Awardee, 2018
2017 Gilman International Scholar
2018 Hollings Scholarship Program Awardee
2018, 2019 Poster Presentation at Ohio University Research Expo, First Place Awardee, Second Place Awardee for Beta Beta Beta Biological Honor Society Research Award

D. Professional Memberships

2018-2019 Service Chair, Beta Beta Beta Biological Honor Society
2019- President, Beta Beta Beta Biological Honor Society

Objectives and Scope: *Staphylococcus aureus* is a Gram-positive opportunistic human pathogen that comprises part of the normal skin and nose microbiota in 30% of people. However, it is capable of causing invasive disease in almost every niche of the human host, including systemic sepsis (1). Staphylococcal DNA exists in a supercoiled state. Supercoiling refers to the formation of superhelices in which the DNA helix is wound into a secondary helical shape. Among its practical purposes, supercoiling is responsible for regulation of larger-order processes within the cell such as gene expression. Supercoiling levels are dynamic, varying between a highly supercoiled and relaxed state, which enables this mechanism to act as a regulatory switch for genes. Levels are modulated by two topoisomerases: Topoisomerase I and DNA gyrase (2). Supercoiling has been shown to regulate some virulence genes in *S. aureus* (3-5). However, the importance of this mechanism during pathogenesis of infection *in vitro* has not been investigated.

The preservation of wild type global supercoiling levels has been shown to be necessary for full virulence of *Escherichia coli* in a murine UTI model of infection (6). In this study, mutations were induced in *gyrA,* which encodes one of the DNA gyrase subunits, ultimately disrupting the ability to carry out its normal function, which is to increase supercoiling levels (6-7). This resulted in partially attenuated virulence. The goal of this study is to recapitulate this in *S. aureus*, by first creating a strain that has a disruption in normal supercoiling modulation, and then comparing its *in vivo* virulence to wild type *S. aureus*.

Aim 1 of this study will be to produce the supercoiling-disrupted strain using ciprofloxacin, which is an antibiotic that targets GyrA. Sequential exposure of a bacterial population to increasing concentrations of the antibiotic over time will select for *gyrA* mutants (5). After ciprofloxacin resistance becomes apparent, potential *gyrA* mutants will be screened by sequencing the gene. Because there is the possibility that other parts of the chromosome were altered during antibiotic

exposure, Aim 2 of this study will be to create an isogenic mutant strain by exchanging normal *gyrA* for the mutant copy in wild type *S. aureus*. After the isogenic mutant has been successfully constructed, in Aim 3 it will be assayed for supercoiling levels using a chloroquine agarose gel to ensure the mutation in *gyrA* has resulted in global relaxation of superhelicity. Differences in virulence will be determined by examining survival rates over time in the animals and determining bacterial burden in organs at the end of the experiment. If maintenance of supercoiling levels is important for virulence, mice infected with the *gyrA* mutant will have better survival rates and decreased bacterial burden relative to wild type.

Materials and Methods

Selection for Ciprofloxacin Resistance: Induction of ciprofloxacin resistance will be carried out according to Sánchez-Céspedes *et al*. Briefly, the minimum inhibitory concentration (MIC) of ciprofloxacin on wild-type *S. aureus* will be determined, which provides an estimate for the appropriate starting concentration. Next, cells will be grown in increasing concentrations of antibiotic until the MIC is 100-fold higher than the starting MIC. Cells will be plated on ciprofloxacin-supplemented agar and single colonies will be selected for sequencing of *gyrA* to screen for mutants.

Generation of Isogenic Mutant: Once an appropriate *gyrA* mutant has been screened and selected, the mutant copy of the *gyrA* gene will be introduced onto the chromosome of wild-type *S. aureus* using a standard allelic exchange method to produce an isogenic mutant (8).

Chloroquine Gels: The degree to which supercoiling is altered by mutations induced in *gyrA* will be measured by analyzing a reporter plasmid on a chloroquine agarose gel. Chloroquine is a DNA intercalator that causes molecules to migrate by superhelicity rather than size.

Murine Model of Systemic Infection: This model is well established in the Carroll lab. Animals will be weighed daily, and survival rates monitored over the course of the 7-day infection. After euthanization, brain, heart, lungs, liver, spleen, and kidneys will be harvested and homogenized. Colony forming units per gram of tissue will be calculated by diluting and plating.

Significance: Studies addressing the expression of virulence genes that enable *S. aureus* to be highly pathogenic have largely been dominated by inquiries about regulation at the protein level. However, there is a plethora of information that can be gleaned about how pathogens operate in the human host from examining virulence regulation at the DNA level. These types of investigations are largely underrepresented in *S. aureus* research. The fact that *S. aureus* is such a diverse pathogen makes this type of work relevant as well. In order to be pathogenic in different environments, it must have ways of sensing environmental conditions and transmitting these to DNA so that virulence genes can be regulated appropriately. Evidence supports that supercoiling is part of the mechanism that does this (3-4). It would be a significant finding if disruption of the bacterium's supercoiling levels leads to an attenuation of virulence *in vivo*. This would suggest that supercoiling levels of a cell play a fundamental role in its pathogenicity, and that proper supercoiling modulation is necessary for a bacterium to sense it is in an environment where it can potentially cause disease. Supercoiling-mediated gene regulation has been largely studied *in vitro,* but it is important to correlate these results with infection *in vivo* to determine if these types of regulatory effects observed in the lab are biologically relevant during infection.

Intellectual Property: Infections caused by *S. aureus* are commonly associated with high levels of antibiotic resistance and consequently are becoming increasingly hard to treat. Bacterial resistance to antibiotics will continue to advance until the use of these drugs is totally obsolete (9). Though research has generally moved away from discovery of new antibiotics to reflect this,

progress on novel methods to treat resistant infections is limited. So, it is paramount that we use our remaining effective antibiotics responsibly. Part of this requires understanding the connection between resistance to clinically relevant antibiotics like ciprofloxacin (which is used in this study) and their effect on supercoiling levels, including how this alters virulence on the molecular level during infection. This kind of knowledge may inform treatment guidelines for better antibiotic use, helping us to make smarter clinical decisions when treating infections.

Elucidating the effect of disrupted supercoiling levels on virulence has the potential to inform new treatments for *S. aureus* infections. The proposed study is especially relevant in this context because it addresses this question using an *in vivo* model. If supercoiling disruption leads to an attenuation of virulence, this is evidence that a subtle alteration of superhelicity may be a viable alternative treatment to bactericidal antibiotics. If a therapy is able to finely target bacterial processes required for pathogenesis without being deadly, it may serve as an alternative treatment that does not produce antibacterial resistance so readily. In other words, if a drug can alter supercoiling levels so that bacterial cells are still able to live on a host as a commensal but can no longer cause disease, this eliminates selective pressure that favors drug resistance.

Bibliography

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3. Fournier, B. & Klier, A. Protein A gene expression is regulated by DNA supercoiling which is modified by the ArlS-ArlR two-component system of Staphylococcus aureus. *Microbiology (Reading, Engl.)* **150**, 3807–3819 (2004).
4. Sheehan, B. J., Foster, T. J., Dorman, C. J., Park, S. & Stewart, G. S. Osmotic and growth-phase dependent regulation of the eta gene of Staphylococcus aureus: a role for DNA supercoiling. *Mol. Gen. Genet.* **232**, 49–57 (1992).
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6. Sánchez-Céspedes, J., Sáez-López, E., Frimodt-Møller, N., Vila, J. & Soto, S. M. Effects of a Mutation in the gyrA Gene on the Virulence of Uropathogenic Escherichia coli. *Antimicrob. Agents Chemother.* **59**, 4662–4668 (2015).
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8. Lehman, M. K., Bose, J. L. & Bayles, K. W. Allelic Exchange. *Methods Mol. Biol.* **1373**, 89–96 (2016).
9. Spellberg, B., Bartlett, J. G. & Gilbert, D. N. The future of antibiotics and resistance. *N. Engl. J. Med.* **368**, 299–302 (2013).

Budget

Chemical reagent	Cost
Mice and per diem costs	$1000
Ciprofloxacin	$130
Chloroquine diphosphate salt	$110
Sequencing costs	$100
Primer sets for sequencing	$50
QIAprep Spin Miniprep Kit	$410
Total	*$1,800

*The remaining $300 of this budget will be funded by the Carroll lab.

Justification

Mice will be ordered through Envigo. For the infection assay, each group will contain 12 mice. From power analysis calculations, an n of 12 is the least number of animals that will produce statistically significant values. The experiment will require 24 animals total. This cost also includes per diem charges for care of mice, spanning over the entire course of time they will be in the facility. Ciprofloxacin will be purchased through Fisher Scientific and will be used to select for *gyrA* mutants. Chloroquine diphosphate salt will be purchased through Fisher Scientific as well and will be used for supercoiling analysis in chloroquine agarose gels. Screening of potential *gyrA* mutants by sequencing this gene will be done at the Ohio University Genomics Facility. Sequencing of multiple isolates will likely have to be performed before a suitable mutant is chosen. Sequencing will also require specific primer sets that span the length of the gene to ensure the entire locus is sequenced. These will be ordered through ThermoFisher. The QIAprep Spin Miniprep Kit will be purchased from Qiagen. This kit provides a reliable method for isolating plasmid DNA used for supercoiling measurement on chloroquine agarose gels.