Project Abstract: Cystic Fibrosis (CF) associated chronic respiratory infections are notoriously hard to treat with antibiotics and limit patients' life expectancy and quality. Traditional antibiotic sensitivity testing is often inadequate for such infections in part due to non-heritable environmental determinants of phenotype *in vivo* and adaptation within timescales of treatment administration. To address this shortcoming we seek to design an assay to robustly predict a pathogen's distribution of likely future drug resistance phenotypes from clinically attainable data.

We will analyze available RNA-seq, whole genome sequence, and MIC data from 21 clinical isolates of *P aeruginosea* from a patient with cystic fibrosis. We hypothesize that *P. aeruginosa* transcriptomic profiles can be used to predict future antibiotic resistance, facilitating more effective clinical antibiotic selection. We additionally hypothesize that mathematical models and simulations of *P. aeriegnosa* evolution in the context of CF can provide insight into the rate over time and space at which heterogeneity develops. To test this hypothesis we will (i) identify statistical mechanical properties of the RNA-seq dataset that are correlated with future resistance profile, (ii) elucidate the relationships between time elapsed, space traversed, dynamics of antibiotic administration, hypoxic conditions, and genetic diversity using a hybrid multi-scale cellular automata model of *P. aeruginosa* evolution in the respiratory, and (iii) validate the predictive capability of our identified transcriptomic features and modeled spatiotemporal dynamic relationships on antibiotic sensitivity with our *P. aeruginosa* clinical isolates, and other experimental and clinical data sets.

The proposed project will achieve a significant step toward a predictive, evolutionarily rational antibiotic sensitivity assay.

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By incorporating methodology that is agnostic to individual, pathogen-specific gene transcripts, we expect our methods and results to be transferable to sensitivity testing of other microbial pathogens and broader work on predicting complex eco-evolutionary dynamics in natural populations.

Personal Background: I am currently a fourth year medical student at Case Western Reserve University School of Medicine (CWRU SOM). During the past three years, my research time has been spent in the laboratory of Dr. Jacob Scott at the Cleveland Clinic Lerner Research Institute's Translational Hematology Oncology Research Department, as well as that of Dr. Robert Bonomo's at the CWRU-Cleveland VAMC Center for Antibiotic Resistance Epidemiology (CARE). Doing research within these groups as a medical student has provided me a supportive research environment where my unique skillset and creativity can thrive. In this time, I have been awarded a research stipend funded by an NIH T35 grant, co-authored two manuscripts in pre-print, and contributed to three others in preparation, including a clinical case report. In addition, I have presented a poster talk at an American Society for Microbiology Microbe national meeting, an international talk at the Max Planck Institute for Evolutionary Biology Conference on Evolutionary Dynamics, as well as numerous poster presentations. Besides the research thesis time granted by my curriculum, this work has been done outside of my medical school program, where I am currently in excellent standing, having received honors grades in four of seven of my core clinical rotations. Balancing my clinical and research interests, I have greatly developed my skillset as an aspiring physician-scientist. However, to achieve my goals of becoming an independent investigator, I must take dedicated time to further galvanize my knowledge and research skills.

I propose to take this time after graduating medical school this spring. I will be continuing my research with Dr. Scott in collaboration with Dr. Bonomo, as a Research Associate affiliated with the Cleveland Clinic Lerner Research Institute. In addition, I will pursue a PhD in Physics at University of Cambridge, where I have received conditional admission. I will spend a majority of my physical time at University of Cambridge, but my thesis will be co-advised by CWRU and Cambridge faculty. My work will be focused on building and validating mathematical models, computational pipelines, and simulations of the evolution of antibiotic resistance in spatially complex host environments, thus connecting and extending my previous work.

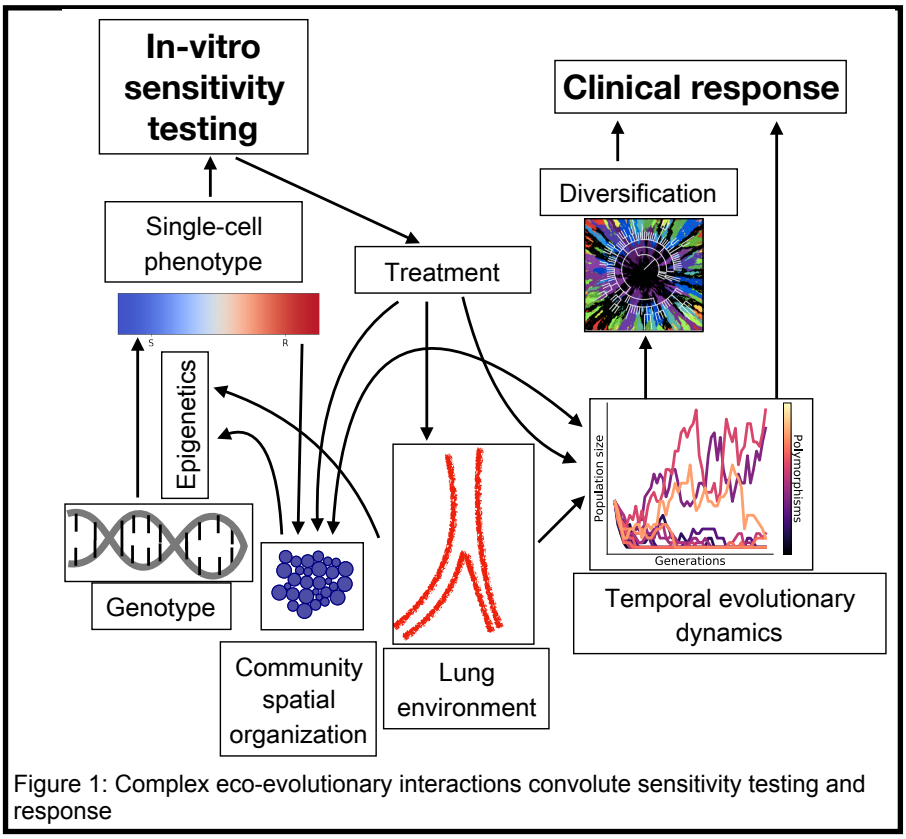


Figure 1: Complex eco-evolutionary interactions convolute sensitivity testing and response

I am drawn to this problem and these approaches because of the immense difficulty in treating these infections clinically. While available antibiotics and clinical assays are very effective in a variety of contexts, they often fall short, especially in chronic infections in compromised hosts, such as cystic fibrosis. The crux of this challenge is both antibiotic resistance and resilience as well as immense pathogen diversity and heterogeneity, which conventional antibiotic selection and delivery methods fail to adequately capture (fig. 1)[1]. However, theoretical

and experimental work has begun to uncover the evolutionary mechanisms underlying these processes, revealing strategies to measure and perturb them clinically[2,3]. A greater understanding of how antibiotic resistance evolves in cystic fibrosis chronic infections, and what kind of clinical data can be leveraged to optimally select treatment would allow for improved therapy of this notoriously unrelenting process. I plan to study this process by carrying out two complimentary approaches, one focused on a computational method to predict resistance from clinical isolates, and the other focused on modeling of the evolutionary dynamics resulting in these resistance profiles. These approaches are inspired from evolutionary principles, but oriented toward clinical decision making.

Research Plan: The average life expectancy of a patient with cystic fibrosis is 37.5 years, 41 fewer than the average American. Among its various sequelae, perpetual decline in respiratory function owing to chronic respiratory tract colonization and subsequent inflammation, nearly universally causes death. By virtue of widespread mucus plugging and later bronchiectasis, the mucociliary elevator, which clears pathogens and toxins from healthy lungs, is severely dysfunctional in the lungs of cystic fibrosis patients[4]. The result is polymicrobial colonization of the respiratory tract and the formation of complex, robust spatial organizations, such as biofilms, within these microbial communities[5]. Due to the distinct ecological niches supported by the various portions of the diseased respiratory tract, these communities undergo divergent evolution[6].

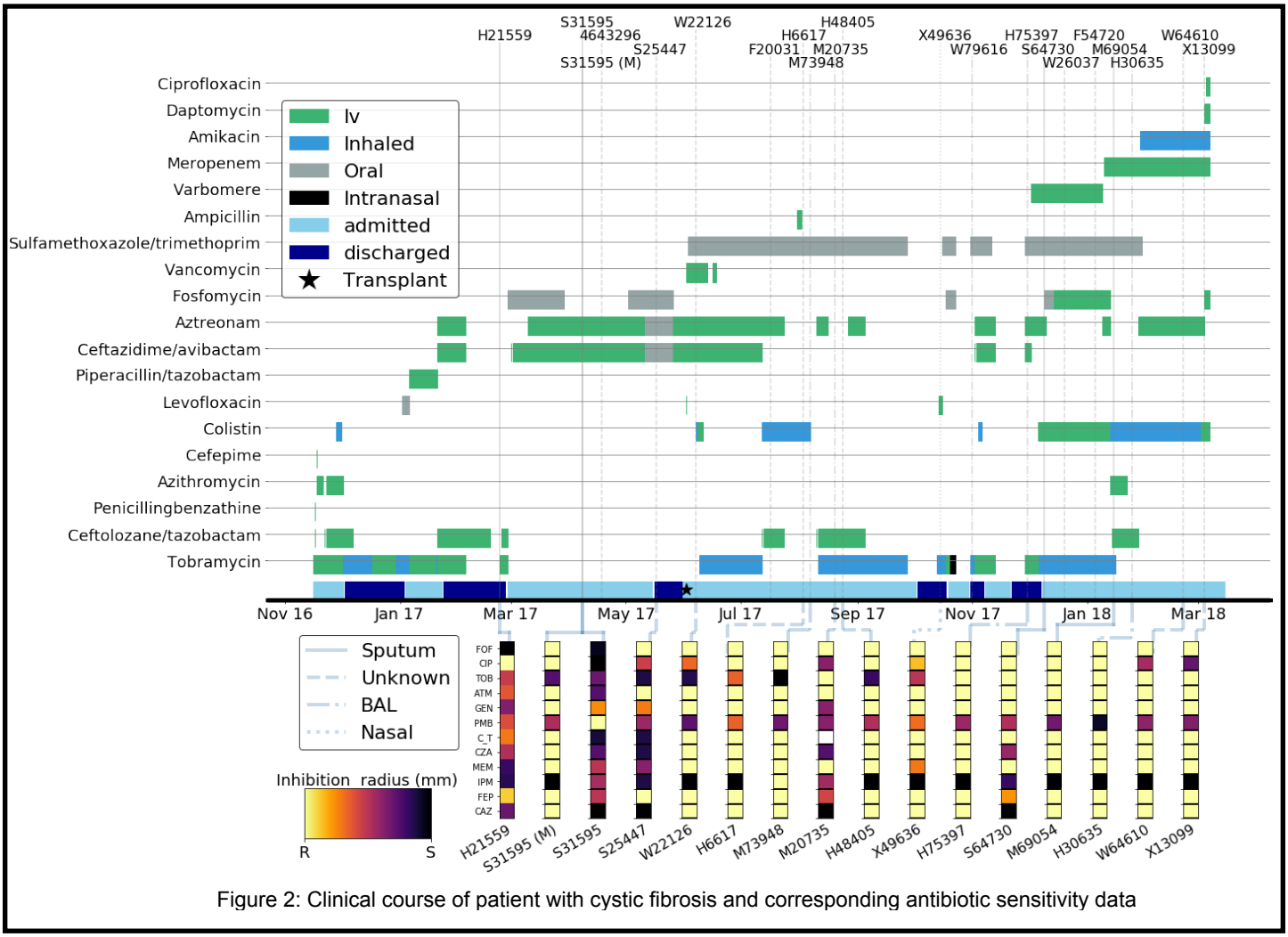
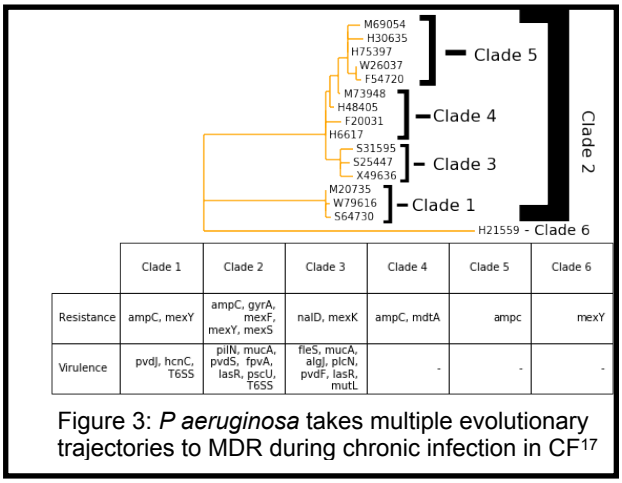


Figure 2: Clinical course of patient with cystic fibrosis and corresponding antibiotic sensitivity data

Preliminary Data: To examine how this eco-evolutionary context shapes clinical response to antibiotics over time we are engaged in a collaboration with a clinical team at the University of Miami, who collected 21 isolates of *P. aeruginosa* from a chronically infected patient with cystic fibrosis *during* her clinical course immediately before her lung transplant up until 'withdrawal' of care. To guide antibiotic selection, frequent genomic sequencing and wide antibiotic sensitivity testing was sought. However, the results of this testing and the subsequent clinical course highlight challenges to translating this data to treatment. Considering 8420 possible drug regimens of up to 3 drugs from a choice of 20, for example, systematic testing or empirical treatment of each is intractable practically, as well as inappropriate stewardship; any profiled subset of these antibiotic regimens is an incomplete survey of the complete pathogen phenotype[7]. In addition, even if a suitable antibiotic is found through sensitivity testing, theoretical and experimental evolution studies indicate that adaptation to antibiotics can occur on the order of days and even hours - within the time it takes for microbiological data to be gathered and analyzed and a corresponding treatment administered[8,9]. Indeed, our results show isolates resistant to antibiotics to which an isolate taken days prior was sensitive (fig. 2). Finally, as our phylogenetic reconstruction demonstrates that these isolates represent a genotypically and phenotypically heterogeneous population, profiling of a single isolate to inform antibiotic selection may yield a treatment that is ineffective against the rest of the population and hastens further resistance (fig. 3)[10].



	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6
Resistance	ampC, mexY	ampC, gyrA, mexF, mexY, mexS	nalD, mexK	ampC, mdtA	ampc	mexY
Virulence	pvdJ, hcnC, T6SS	pilN, mucA, pvdS, fpvA, lasR, pscU, T6SS	fleS, mucA, algJ, plcN, pvdF, lasR, mutL	-	-	-

Figure 3: *P aeruginosa* takes multiple evolutionary trajectories to MDR during chronic infection in CF[17]

These results highlight the need for a more comprehensive initial sampling of population heterogeneity when gathering microbiological data. While it's not clear how many samples and which mode of collection best captures this heterogeneity, a single sample is almost certainly too few[11]. We posit that a more robust assay would determine the probability distribution of *future* evolved phenotypes rather than current phenotype of a single isolate to facilitate selection of the most appropriate antibiotic given the set of evolutionary possibilities[12]. Indeed, previous theoretical work has demonstrated that with complete knowledge of possible drug resistance profiles conferred by each genotype, drug sequences can be designed to systematically steer evolution away from resistance[13].

Predicting antibiotic sensitivity: Aside from drug sensitivity and genome sequencing, gene transcription data may be informative to this end. As the rate of change of mRNA expression, or RNA velocity, has been used to characterize terminal cell fates in experimental models of murine embryo development, pathogen cDNA or RNA-seq expression profiles may provide clinically important data on *future* antibiotic sensitivity.[14] In addition to its role biologically as a link between genetic and cells state, RNA-seq data has the benefit of bearing computational features that are conveniently explored using frameworks not traditionally associated with microbiology, such as **network analyses, machine learning,** and other **statistical methods** variously associated with the emerging canon of 'gene set enrichment analyses', particularly in the human genomic literature[15,16].

In regard to bacterial transcriptomics, previous work has shown that human infection modulates the transcriptome of *P aeurginosa.* Among the specific genes with increased expression, those associated with antibiotic resistance, antibiotic tolerance quorum sensing, and cell-cell signaling have been noted to be involved in host-pathogen dynamics[17]. Furthermore, previous work has demonstrated that transcriptomics can

account for phenotypes crucial to pathogenesis in the absence of a mapping to a specific gene or other genetic elements[18]. In light of this, our central hypothesis is that ***P aeruginosa*** **transcriptome is predictably and measurably modulated during adaptation to antibiotics before standard antibiotics sensitivity assays detect resistance.** We aim to develop a computational pipeline to predict future antibiotic sensitivity from standard bacterial RNA-seq data. Our approach will both incorporate both known antibiotic resistance conferring gene, as well as computational features, unbiased by this *a priori* biological knowledge to maximize our ability to capture the complex interactions between genetic diversity, single cell state, and environmental feedback on the evolution of resistance[19]. We will achieve this through the following specific aim:

SA1: Design an 'evolvability metric' computed from standard bacterial RNA-seq data to predict future antibiotic resistance to in *P. aeruginosa*

SA1a: *Evaluate the correlation between expression of individual cDNA sequences and future resistance profile using statistical regression models.*

I will initially examine individual transcript expressions for correlation with subsequent resistance profiles. Harnessing previous work on characterizing gene expression associated with bacterial stress response, increased mutation rate, and other cell functions possibly related to rapid evolution, I will examine the expression levels of these known genes. Using a multivariate regression model we will note if the 'up' or 'down' regulation of any specific genes is associated with future drug resistance. Over the entire set of samples I can examine correlations with resistance to specific drugs, as well as the overall resistance profile. The results of the most predictive regression model can be reflected in a simple mathematical formula to predict resistance from expression profile.

SA1b: *Identify statistical mechanical properties of the entire transcriptomic profile that are correlated with future resistance profile.*

Noting that genes of unknown function as well as complex interactions of genes confer antibiotic resistance, we will simultaneously pursue an approach that is 'naive' to known gene function. I will explore various properties of the entire transcriptomic profile using principles from statistical mechanics and information theory, such as entropy, Gibbs free energy, Kullbach-Liebler divergence, as well as more conventional statistical measures of variability to examine a correlation between these metrics and resistance profile.

SA1c: *Quantify the likelihood of subsequent antibiotic resistance based off of the statistical properties identified in* ***SA1a*** *and* ***SA1b*** *for other RNA-seq datasets of temporally sequential P. aeruginosa samples*.

Using the various measures explored between **SA1a** and **SA1b,** I will evaluate their generalizability to various other datasets of temporal *P. aerugionsa* and generate a final 'evolvability metric' that maximally accounts for variation in future resistance profile. These validation datasets will be derived from publicly available databases, generated from experimental evolutionary biologist in the lab Dr. Bonomo at the Cleveland VA Medical Center (VAMC), and collected from patients admitted at VAMC in collaboration with the clinical infectious disease department with which my co-mentor Dr. Robert Bonomo is deeply integrated in a clinical and research capacity.

Role in investigation: My role in this proposed investigation is to perform all the statistical and computational analysis of the raw RNA-seq data to the final results. The clinical isolates collected by collaborators at the University of Miami are currently physically located in the Bonomo lab, where the initial whole genome sequencing and MIC disc assays were performed by the experimental evolution team. The Bonomo lab experimental personnel will subsequently culture and extract RNA-seq data from the available clinical isolates to allow for the proposed analysis. This work will comprise 50% of my research activities, during my training program.

Modeling heterogeneity: Even if the time course of evolution can be predicted from a given clinical isolate, the represented proportion of the total heterogeneity of the evolved phenotype is unclear. While phenotypic diversity in CF respiratory infections is well established, predicting the degree of heterogeneity in patients remains an open area of investigation and is not explicitly factored in current clinical paradigms[20]. Given the considerable obstacles in making these predictions clinically, mathematical models and simulation of evolution in the complex spatial environment of the respiratory may allow us to begin to uncover the fundamental drivers of this heterogeneity. To this end we can harness a powerful framework of hybrid multi-scale spatial cellular automata (CA) to stochastically model the evolution of cells and their dynamic interaction with environmental factors in space under a simple set of rules[21]. This framework has been used to extrapolate powerful characteristics of the tumor-host environment and its influence on evolution in cancer and may provide similar insights in host-pathogen modeling of CF respiratory infections.

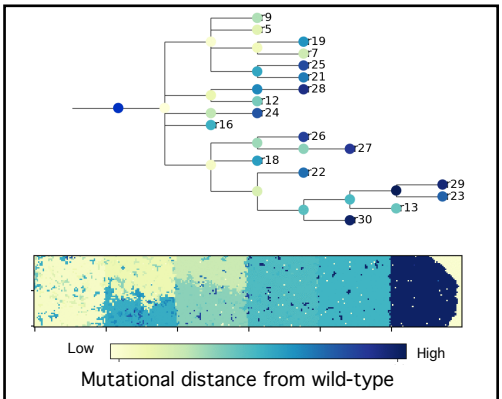


Figure 4: Example visualization of cellular automata model of bacterial growth

The strength of this framework is its incorporation of space, antibiotics, oxygen, and genetic diversity and their collective effective on antibiotic resistance evolution (fig. 4). **Our main hypothesis is that individual-based modeling of chronic *P. aeruginosa* infection in the respiratory tract will provide fundamental insights into how diversity develops over time and antibiotic administration, which can be sufficiently applied to experimental and clinical sequence data to make quantitative predictions.** This hypothesis will be tested by first building a simulation of bacterial evolution in the context of chronic respiratory infections[22]. We will subsequently use our model to extrapolate relationships between final genetic diversity across space given varied time, spatial organization, and parameters of antibiotic administration[23,24]. The relationships uncovered will be validated through data from experimental collaborators and used to infer underlying evolutionary dynamics in our set of clinical isolates. We will achieve this through the following specific aim:

SA2: Use an individual-based model of *P. aeruginosa* chronic respiratory infection to infer the scales of time and space on which diversification occurs and consequently make predictions about clinical/ experimental datasets.

SA2a: Design a multi-scale hybrid discrete-continuous cellular automaton (CA) of P. aeruginosa spread under dynamic constraints of antibiotics and oxygen in two dimensions.

I will begin by modeling discrete subpopulations of *P aeruginosa* as spaces on a two-dimensional rectangular grid with growth rate determined by antibiotic and oxygen concentration at that location according to a simple pharmacokinetic relation. Antibiotic and oxygen concentration across the grid will be modeled using a partial differential equation reflecting drug pharmacodynamics and known bacterial oxygen consumption dynamics respectively.

SA2b: Expand the CA model to accommodate beneficial mutations and model spatial constraints of the respiratory tract on the macroscopic scale.

I will expand the CA model to accommodate beneficial mutations that increase fitness to antibiotics in a linear manner. Furthermore I will employ a 2-dimensional lattice space with complex branching patterns to more closely model the geometry of the respiratory tract. This model will record mutational events allowing for simulation of bacterial evolution to antibiotics in the respiratory tract with a fully resolved phylogenetic description of this evolution.

SA2c: Extrapolate the time and space scales of evolution based on the final measured genetic heterogeneity

with respect to space using the CA model

I will simulate evolution using my CA across a range of parameters and varying spatial scales. This will allow for the extrapolation of the relationship between genetic diversity, time elapsed, and the spatial scales traversed, during the evolution of resistance to antibiotics. I will quantify this relationship using statistical regression models.

SA2d: Apply these validated statistical models to predict the time and sales of evolution from experimental and clinical data

Harnessing the experimental evolution expertise of collaborators in Bonomo and Scott labs, simple *in vitro* experiments of *P. aeruginosa* evolution over spatiotemporal antibiotic landscapes will be performed, with subsequent sequencing and sensitivity testing across the growth space. Using the data, I will validate predictions of temporal and spatial scales of evolution from final genetic distributions generated from modeling work in aim **SA2c**. The results of these validated predictions will then be applied to our current set of clinical isolates of *P. aeruginosa* and subsequent data that can be collected from the Cleveland VA Medical Center patients as in **SA1d**.

Role in investigation: My role in this proposed investigation is to design and perform the simulations, as well as the corresponding analysis. Again, the Bonomo lab experimental personnel will subsequently culture and extract RNA-seq data from the available clinical isolates to allow for the proposed analysis. This work will comprise 50% of my research activities during my graduate program.

Scientific impact: Completion of the proposed project will achieve a significant step toward a predictive, evolutionarily rational antibiotic sensitivity assay. By incorporating methodology that is adaptable to a range of host-pathogen contexts, we expect our methods and results to be transferable to sensitivity testing of other microbial pathogens and broader work on predicting complex eco-evolutionary dynamics in natural populations.

Impact of proposed research on personal career development:

My career goal is to become a physician specializing in infectious disease and a scientist researching antibiotic resistance (fig. 5). I hope to maximize continuity between theoretical, experimental, and clinical phases of research through dual-training in medicine and systems biology. As a scientist, I will elucidate the complex evolutionary dynamics underlying antibiotic resistance through research grounded in computational models. As a physician, I will frame my scientific inquiry through the lens of clinical practicality and experience. With a deeper understanding of microbial evolution and mechanisms of resistance in both the population and the individual, my research will enrich my clinical acumen, enabling me to better serve and advocate for my patients. I strive to connect collaborators from seemingly dissimilar fields to encourage future scientists to challenge the boundaries of traditional scientific silos and enhance innovation through diverse, interdisciplinary perspectives.

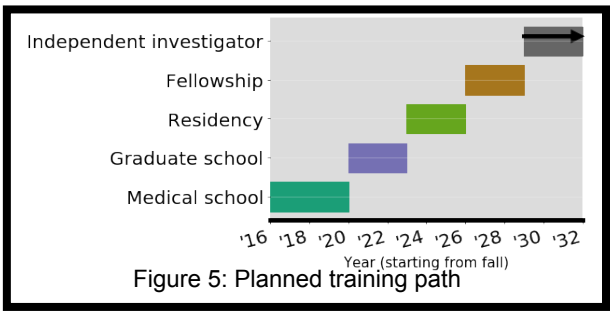


Figure 5: Planned training path

In carrying out the above research plan, I will use mathematical models to simulate evolution in chronic respiratory infections and computational tools to predict antibiotic resistance from clinically relevant data. I will gain a strong foundation in statistics, statistical mechanics and information theory and how these tools can be applied to biological data. In addition, I will gain experience in translating these data and the resulting analysis in a directly clinical manner. In addition to developing a deep technical knowledge through the methods utilized

in my research project, they will provide me with experience working and communicating in a cross-disciplinary scientific team of clinicians, microbiologists, and geneticists. As such, this experience will give me a strong foundation to be an independent physician-scientist investigator performing interdisciplinary research through the quantitative study of antibiotic resistance evolution, closely reflecting my career aims.

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I will be pursuing research training as a **PhD student in the Physics department at the University of Cambridge**, where I have gained conditional admission to work with Dr. Diana Fusco in the Biology and Soft Systems group. As part of this program I will be conducting research co-mentored by faculty at Cambridge as well as Dr. Scott and Dr. Bonomo who are affiliated at CWRU. **At least 20 hours a week of my research and salary will be supported by Dr. Scott at the Cleveland Clinic, where I will officially be a research associate.**

Impact of plan on career: This training program will grant me time from my clinical training to **deeply invest** in knowledge and experience in mathematical and computational models of evolution. My chosen project and mentors will foster the development of skills apropos to my goal of becoming a physician-scientist and approaching the challenge of antibiotic resistance evolution from an interdisciplinary perspective *focused* on clinical applications but *grounded* in fundamental physical models. The unique arrangement of collaborations in this project will greatly extend my ability to communicate and organize resources across disciplines and institutions. This experience and mode of collaboration is appropriately geared toward the increasingly interdisciplinary and global landscape of biomedical research.

Developmental activities: As a University of Cambridge graduate student I will be engaged in Physics departmental seminars and conferences, which will expose me to applications of mathematics, physics, and data science to solving problems in healthcare and biology at-large. I will further be engaged in teaching of undergraduates, which will cement critical teaching, outreach, and organizational skills. In addition, across the University I will have access to to relevant journal clubs and seminars in the Evolutionary Genetics Club to keep me abreast of relevant methodologies in molecular evolution and genomic research. In addition, I will have access to the periodic cross-disciplinary seminars in the Infectious Disease department which will expose me to emerging public health and clinical challenges in infectious disease and antibiotic resistance. Finally, I will endeavor to support myself and work with annual grant-writing. As part of my development as an independent investigator, I am keen on developing the crucial skills of grantsmanship to support my research now and beyond.

Research methods to be learned: My PhD program will grant me academic resources for careful study of *canonical physical models of evolution*, as well as *computational and statistical mechanical* frameworks to analyze large-scale next generation sequencing data. As part of the program I will be granted the time and support for independent study and access to coursework and experts in the Physics, Medicine, and Biology departments, and beyond. These fundamental tools will inform my thesis project on modeling *P. aeruginosa* evolution in the respiratory tract, and using these insights, as well as computational frameworks, to *interpret* sequencing data from *P. aeruginosa* clinical isolates.

Mentorship Plan: As the primary motivation of my work is to design a rational, evolutionary minded approach to clinical antibiotic sensitivity assays, to this end, I will be jointly mentored by project co-investigators Dr. Jacob Scott and Dr. Robert Bonomo. Their overlapping interests and distinct yet complementary scientific backgrounds will **comprehensively** support my research project.

Dr. Jacob Scott is a Radiation Oncologist at the Cleveland Clinic and principal investigator of a laboratory researching evolutionary dynamics of cancer and microbial pathogens. He has a DPhil in mathematics from Oxford and specializes in theoretical models of evolution, especially in the context of drug resistance. In four years as a principal investigator, Dr. Scott is currently an advisor to a PhD student and two MD-PhD students. Additionally **he has successfully co-mentored** a post-doctoral researcher as well as a physician pursuing a graduate degree at Oxford University after receiving an MD, but before resuming further clinical training - **a training path similar to my own**. His laboratory is supported by an NIH R01 grant from the National Cancer Institute, as well as several private and internal grants. As a physician-scientist and NIH LRP recipient, Dr Scott will provide career and scientific guidance from the perspective of a successful physician-scientist with a math and physics background and unconventional career trajectory, **bridging theoretical evolution to target clinical challenges.**

Dr Robert Bonomo is a physician-scientist and principal investigator of a laboratory researching antibiotic resistance. He has years of experience as an infectious disease clinician and is currently the Chair of the Medicine department at The Louis Stokes Cleveland VA Medical center. As the principal investigator on the overarching work my project is a part of he supports the collaborations which generated the robust data I'll be analyzing, and his deep clinical knowledge will guide the practical motivations behind my investigation. Within the wide purview of his lab, supported by 13 NIH R01 grants and spanning experimental evolution, structural enzymology, and molecular epidemiology, he has mentored several PhD, MD-PhD, and post-doctoral students as well as clinical ID fellows. In addition, he has overseen several large multi-institutional collaborations, such as that which yielded the data I am analyzing. In addition he has a productive and long-standing history co-investigating projects on the evolution of antibiotic resistance with Dr. Scott. Given his clinical background in infectious disease, and **renown as a world-leading expert in anti-biotic resistance**, Dr. Bonomo is well-suited to mentor me as I pursue a career as an Infectious disease clinician and independent investigator in the area of infectious disease.

Mentor supervision: Dr. Scott will guide me in my statistical and mathematical analytic methods, as well as, the evolutionary motivations of the project. His background in mathematical oncology will be a great aid to me as I employ hybrid multi-scale cellular automata models to model bacterial infection, as **he has significant experience in their construction and analysis.** During my research so far as a medical student I regularly communicated my progress and results to him and received detailed feedback and guidance. In addition, I attend his laboratory's meetings and collaborate with other members on projects, gaining valuable skills and insight across the diverse projects in the lab. In this way, Dr. Scott's lab has serves as my **scientific 'home'**. Our rapport will facilitate a continued close mentoring relationship in collaboration with my University of Cambridge research advisor as I pursue my graduate training. The proximity of Dr. Scott's lab in the Cleveland Clinic Lerner Research Institute to the VAMC, will facilitate periodic meetings with Dr. Bonomo who through his clinical background will guide me as I navigate the clinical constraints of my scientific inquiry. In addition, his role in the data collection will crucially **facilitate and supervise** close collaborations with the clinical team, collection of additional data, and management of the physical samples.

I will regularly correspond and receive guidance from these two mentors, as well as my research mentor at Cambridge, and they will **maintain communication with each other** as collaborating investigators on this project. Between them, they have a wide network of collaborators across multiple clinical departments and the departments of physics, genetics, biochemistry, mathematics, and systems biology at Case Western Reserve University from which further mentorship and resources can be accessed as my project progresses. As **physician-scientists**, they will serve as **models of strong interdisciplinary researchers** of antibiotic resistance evolution and passionate physicians, as I too aim to be.

While I will be co-mentored by faculty at the University of Cambridge and Case Western Reserve University School of Medicine, I will physically be in the UK during the majority of that time. As such, my research project will be supported **by a vast and connected network** of collaborators and physical resources across University of Cambridge, as well as Case Western Reserve University School of Medicine and its **four** affiliated academic hospitals.

Physical Resources: The research environment at the University of Cambridge is world-class, with unbridled access to powerful computing services, including the Cambridge Service for Data Drive Discovery (CSD3). This service is supported by two GPU enabled supercomputers offering high-performance Intel scalable processor and clusters of Nvidia P100 GPUs. The newest of these supercomputers was ranked 75 out of the 500 fastest in the UK at 1.6 PFlops. Use of these resources is facilitated by training courses and technology support services, which will fully enable the computational aspect of my work.

Beyond the immense resources at Cambridge, as I will be a research associate affiliated with Cleveland Clinic Lerner Research Institute, my research and collaborations would benefit from access to versatile and powerful computational and biological resources through both the Cleveland Clinic and its affiliated CWRU School of Medicine's core facilities. The Research Core Services available at the Cleveland Clinic Lerner Research Institute include: Genomics, Flow Cytometry, Histopathology, Confocal Microscopy, Biostatistics, Digital Imaging, high performance computing, digital imaging microscopy and many others. In addition, through collaboration with the Bonomo lab, I will have access to patients within the Cleveland VA Medical Center for further clinical sampling. Additionally, by virtue of the network of affiliated hospitals, there are clinical collaborators within each hospital from which patient samples can feasibly be collected, as the Bonomo lab has utilized in previous investigations.

Intellectual rapport and experts: The environment of my training program will be supported by a very large and diverse network of collaborations, as well as access to experts across two world-class research university environments. The diverse and varied nature of my quantitative and medical academic training and research goals is *reflected* in the cross-disciplinary collaborative research environment in which my graduate training will take place. My research project itself is born out of a collaboration between four institutions in three states, and includes infectious disease clinics, geneticists, as well as my mentors, who will continue to support the generation of clinical and experimental data for my project. Additionally, between my mentors Dr. Scott and Dr. Bonomo, there are roughly **eight scientific and clinical affiliations** which will provide me with a broad network of support and resources during my training. Furthermore, at University of Cambridge I will be in a research environment with a nearly 800 year history of of academic excellence and 160 affiliated Nobel laureates. My home department of Physics, in the Biology and Soft Systems group, will provide me to access to biological, experimental and theoretical expertise all within the Cavendish laboratory. In this way, the clinical, experimental, mathematical, and computational/data science aspects of my project are all supported by mentorship and nearby experts who will allow me to make **unhindered** forward progress on my work.

Institutional Investment: As a student pursuing graduate studies at University of Cambridge, upon enrollment I will have healthcare and housing provided for me through my membership at one of the university's colleges. Though there is no mandated coursework structure, as an enrolled student I will have the full breadth of courses available for me to aid my training. At the onset of my enrollment, I will have a research lab and mentor guaranteed from the onset (tentatively Dr. Diana Fusco in Biology and Soft Systems group). Through the physics PhD program at Cambridge, **100% of my time will be dedicated to my research project.** Travel awards are available to me from three available funds of up to 1000 British Pounds.

As I aim to conduct research grounded in computational and mathematical models geared toward clinical problems informed by my perspective as a physician, CWRU, Cleveland Clinic, and University of Cambridge will provide me with an ideal environment for me to hone the skills to be a successful independent, inter-disciplinary researcher.

The past four years have profoundly impacted my personal and professional development. Caring for patients is truly inspiring. It has deepened my passion for medicine and strengthened my identification with the mission of my profession. While I cherish the connections that I have made with my patients, I have been disheartened by the limitations of medicine and the care I am able to provide as a clinician. As I developed an interest in infectious disease, I was greatly frustrated caring for patients who succumbed to antibiotic-refractory infection despite our most effective therapies. These experiences cultivated my commitment to future patients and dedication to the study of antibiotic resistance.

Drawing from my physics training, I wondered if one could learn and even perturb the fundamental dynamics that gave rise to this resistance. With my mentor, Dr. Jacob Scott, I applied stochastic reaction-diffusion, as well as experimental evolution to study the spatio-temporal dynamics of antibiotic resistance in both a theoretical and experimental setting (resulting in two bioRxiv pre-prints, contributions to two manuscripts in preparation). Under the additional mentorship of Dr. Robert Bonomo, I have begun to develop computational methods to predict antibiotic resistance from next generation sequencing of clinical isolates (case report in preparation). I have additionally earned support for my research as part of the Medical Student Summer Research Program at Case Western Reserve University, supported by an NIH T35 grant. I have been able to take part in this research while maintaining excellent standing in my medical curriculum. I feel deeply compelled to continue this work, harnessing my medical training to facilitate its clinical application.

As I approach the end of medical school, I am not ready to move forward in clinical training without developing the fundamental tools to carry out this research. The support of the NIH loan repayment program will allow me to pursue graduate studies in Biophysics, to immerse myself in the formal study of stochastic mathematical models and computational methods applied to antibiotic resistance evolution. My previous training has given me a strong clinical background and experience in producing creative theoretical and experimental research with a diverse set of collaborators.

This in-depth scientific training, in addition to my subsequent clinical training afterward, will uniquely poise me to facilitate interdisciplinary collaborations and support future scientists to challenge traditional scientific boundaries and enhance innovation through diverse perspectives as an independent physician-scientist. As I pursue this training, my unique background and career goals will be reflected and supported by the mentorship of two physician-scientists invested in cross-disciplinary, evolutionary-minded research of antibiotic resistance evolution. It is in such an environment with the support of the NIH loan repayment program that I can best evolve as a physician-scientist and gain an essential foundation for the career I envision, unraveling the puzzles of antibiotic resistance.

BIOGRAPHICAL SKETCH

NAME: Krishnan, Nikhil

eRA COMMONS USER NAME (credential, e.g., agency login): nkrishnan1

POSITION TITLE: Medical student researcher

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Case Western Reserve University	B.S.	5/2016	Physics
Case Western Reserve Univ. School of Medicine	M.D.	5/2020 (projected)	Medicine

A. Personal Statement

I am a medical student with a background in physics, clinical interest in infectious diseases, and deep passion for addressing health disparities through policy changes and advances in medical knowledge. By virtue of my unique perspective and diverse training background, I am uniquely poised to bridge the gap between these fields and interests through cross-disciplinary collaboration as a graduate student and in the future as an independent physician-scientist. At the intersection of my training and interests, lies my desire to research antibiotic resistance through the lens of underlying evolutionary mechanisms and principles.
My curricular training as a Physics undergraduate and training in medical school as well as research experiences in structural biology, protein biophysics, biomedical engineering, and theoretical population genetics have given me a strong foundation from which to pursue my goals. As I pursue further training in applying quantitative and computational tools to my clinical interests, I hope to leverage both my clinical and basic science training to ultimately improve treatments for my patients.

B. Positions and Honors

Positions

2017 - Medical Student Summer Research Program (Supported by NIH/NIDDK 1T35DK111373−01), Case Western Reserve University, Digestive Disease Research InstituteI, Cleveland OH

2016 - Doctor of Medicine Candidate, Case Western Reserve University School of Medicine, Cleveland, OH

2014 - 2016 Research Student, van den Akker Lab, Department of Biochemistry Case Western Reserve University Federal Work-study program, Cleveland, OH

Honors

University Scholarship for Academic merit, Case Western Reserve University, Cleveland, OH, 2012
Huntington Fund for Cuyahoga co. STEM students, John F. Hunington Fund, Cleveland, OH, 2014
Alumni Scholarship Merit award, Case Western Reserve University, Cleveland, OH, 2015

Professional memberships and other experience:

Student member, Infectious Disease Society of America
Student member, American Society for Microbiology
Chapter leader, Students for a National Healthcare Program (SNAHP), CWRU SOM, Cleveland, OH, 2017

C. Contribution to Science
Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/nikhil.krishnan.1/bibliography/public/

1. **Inhibitors of β-lactamase provide potent enzyme inhibition but are limited by genetic diversity and natural selection:** I investigated the biophysical interactions that take place during inhibition of SHV1 β-lactamase via x-ray protein crystallography. I employed this study design to study inhibition by the then newly approved inhibitor avibactam and a novel boronic acid inhibitor. These studies revealed that these two inhibitors potently inhibit the two β-lactamases in our study including the *Klebsiella* derived carbapenamase responsible for marked resistance clinically to crucial last resort antibiotics. However, we uncovered that KPC2 gradually neutralizes avibactam; additionally our study was motivated by the identification of avibactam-resistance conferring mutations in each of the enzymes. This work was the subject of two publications, one in, PloS ONE and the other in Antimicrobial Agents and Chemotherapy. In addition, I contributed to the elucidation of the biophysical mechanisms of an *C. jejuni* exolytic lytic trans-glycosolase, published in PLoS ONE.

a. **Krishnan NP**, Nguyen NQ, Papp-Wallace KM, Bonomo RA, van den Akker F. Inhibition of Klebsiella β-Lactamases (SHV-1 and KPC-2) by Avibactam: A Structural Study. PLoS ONE (2015).
b. Nguyen NQ, **Krishnan NP**, Rojas LJ, Prati F, Caselli E, Romagnoli C, Bonomo RA, van den Akker F. Crystal structures of KPC-2 and SHV-1 β-lactamases in complex with the boronic acid transition state analog S02030. Antimicrobial Agents and Chemotherapy (2016).
c. Vijayaraghavan J, Kumar V, **Krishnan NP,** Kaufhold RT, Zeng X, Lin J, van den Akker F. Structural studies and molecular dynamics simulations suggest a processive mechanism of exolytic lytic transglycosylase from *Campylobacter jejuni.* PLoS ONE (2018).

2. **The dynamics of evolution through time and space beget phenotypic heterogeneity:**
I was a member of the design and implementation team for a bioreactor capable of autonomously and dynamically maintaining antibiotic selection pressure for cell culture in replicates and with sequences of drugs. This work was described in a pre-print on bioRxiv. In addition to experimentally investigating the stochasticity of evolution, I have theoretically explored this matter in simulations of well-mixed evolving populations, contributing to work on how to administer drugs to control this process. I have presented this in multiple posters and talks, which is presented in a manuscript in preparation. Finally, using a theoretical model I have explored how a population's spread to a new environment modulates the pattern of mutant fixation, published in a pre-print on bioRxiv. Take together the above work represents both theoretical and experimental approaches to investigating the eco-evolutionary determinants of antibiotic resistance evolution and strategies to disrupt this evolution.

a. **Krishnan NP,** Pelesko J, Wadhwa RR, Yoon N, Kaznatcheev A, Nichol D, Marusyk A, Hinczewski M, Scott JG. Evolutionary game theory and fitness landscapes as frameworks for predicting and preventing drug resistance in cancer. Review. The 2019 Mathematical Oncology Roadmap. Physical Biology. (2019).
b. **Krishnan NP,** Yoon N, Nichol D, Bonomo RA, Scott JG. Inference of fitness landscapes for antibiotics based on dynamics data. 'Poster talk'. American Society for Microbiology Microbe. New Orleans, LA. 2017.
c. Gopalakrishnan V, **Krishnan NP**, McClure E, Pelesko J, Guo D, Williamson DFK, Webster N, Ecker D, Nichol D Scott JG. A low cost, open-source EVolutionary biorEactor (EVE). bioRxiv. (2019)
d. **Krishnan NP,** Yoon N, Williams DFK, Bonomo RA, Scott JG. Exploring evolutionary trajectories of populations subjected to sequences of drugs *in silico* and *in vitro*. Oral presentation. Workshop on Modeling Diversity in Cancer and Virus Evolution. Max Planck Institute of Evolutionary B i o l o g y . Plön, Germany. 2018.
e. **Krishnan NP,** Scott JG. Range expansion shifts clonal interference patterns. bioRxiv. (2019)