

**A Computational Model Driven Design of a Novel Therapeutic Strategy for Targeting
*Klebsiella pneumoniae***

The Causations and Implications of the Overuse of Antibiotics in Healthcare

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Strains of drug resistant bacteria, specifically called carbapenem-resistant Enterobacteriaceae (CRE), have emerged as a growing and urgent issue in healthcare facilities, resulting in around 3 million infections in the United States each year (CDC, 2021). Carbapenem antibiotics, currently considered to be the most potent and highly effective class of antimicrobial agents, are often considered a last-resort, reserved specifically for the treatment of severe multidrug-resistant bacterial infections (Iovleva & Doi, 2017; Papp-Wallace, Endimiani, Taracila, & Bonomo, 2011). By far the most common cause of these infections is the bacterial pathogen *Klebsiella pneumoniae*, with over 50% of *K. pneumoniae* infections now being resistant to carbapenem antibiotics in parts of the Eastern Mediterranean and Europe (Iovleva & Doi, 2017; Zhu, Yuan, & Zhou, 2020).

This rise in drug-resistant bacteria is being accelerated by the vast over-prescription of antibiotics in the healthcare field. As of 2016, it was found that at least 30 percent of antibiotics prescribed in the United States are unnecessary, equating to over 47 million excess prescriptions every year in the United States alone (CDC, 2016). As antibiotics are more and more commonly prescribed, the bacteria that these drugs are meant to kill begin to have increasingly more exposure to these drugs, and thus, more chances to evolve resistance to them (CDC, 2020). Exemplifying the effect of this, data has been collected showing that countries with a higher consumption of antibiotics also show higher rates of bacterial resistance (Llor & Bjerrum, 2014). As these microbes become resistant to antibiotics, they are able to multiply and spread into new settings and even between countries. In addition, some resistant bacteria have even developed mechanisms to spread their antibiotic-resistant adaptations directly to other germs, further accelerating this problem (CDC, 2020).

As antimicrobial resistance is now recognized as one of the greatest threats to human health worldwide, a striking lack of development of new drugs to combat these superbugs has been noticed, threatening a future of a post-antibiotic world where simple infections may once again become unmanageable (Llor & Bjerrum, 2014; Papp-Wallace et al., 2011). *K. pneumoniae* has been rapidly acquiring antibiotic resistance and rendering almost all available treatments ineffective. Thus, the discovery of new treatment strategies for this bacterial pathogen are critical. To address this, I will first identify and validate novel therapeutic targets in *K. pneumoniae* through leveraging computational-modeling and genetic engineering approaches, and then research the social aspects influencing the overprescribing of antibiotics in the healthcare field.

Novel Therapeutic Targets in *K. pneumoniae*

The vast majority of *K. pneumoniae* clinical isolates now demonstrate a wide range of resistance to antibiotics, and thus there are currently almost no effective therapeutic options to combat these infections (Nirwati et al., 2019). The current standard of treatment for *K. pneumoniae* infections begins with a course of antibiotics tailored around the known antibiotic sensitivities of that particular geographic region. If the specific clinical isolate of *K. pneumoniae* causing infection is found to be antibiotic resistant, carbapenem therapy will be initiated. Further, if a CRE-related infection is diagnosed, common treatment options include more antibiotic regimens, high-dose prolonged-infusion of carbapenem, dual therapy carbapenems, or combination antibiotic therapy. Even with optimal therapy from the currently available treatments, *K. pneumoniae*-related infections carries a mortality rate of 30-50% in previously healthy patients, and an even worse prognosis in immunocompromised individuals (Ashurst &

Dawson, 2021). Thus, there is a clear need to develop novel treatment options for *K. pneumoniae* infections to improve patient outcomes around the world.

Aiming to combat one of the most detrimental antibiotic-resistant bacterial pathogens, I am working to develop and experimentally validate a computational-model-driven design of a novel therapeutic strategy for targeting *K. pneumoniae* infections. Metabolism has been previously shown to be an effective target for antibacterial therapy (Panayidou et al., 2020). Genome-scale metabolic network reconstructions (GENREs), which are mathematical representations of the metabolism in a given organism, have provided a powerful means to accelerate identification of potential targets via computational experiments (Gu et al., 2019; Ruppin, Papin, de Figueiredo, & Schuster, 2010). Therefore, computational models of the bacteria's metabolism are being utilized as a framework to identify the areas of metabolism with the highest potential efficacy for infection inhibition if used as a therapeutic target. Leveraging a GENRE in conjunction with high-throughput biological data from both laboratory and clinical *K. pneumoniae* isolates (Bruchmann et al., 2015; Guilhen et al., 2016; Lee et al., 2019; Liao et al., 2011; Long et al., 2019), context-specific models of the bacteria's metabolism were generated, representing the metabolism of the bacteria either while actively infecting or while growing in a laboratory environment (Figure 1). Analyses of these context-specific models revealed that both model composition (what genes and reactions were contained in each model) and metabolic activity (what genes and reactions were simulated to be active) of clinical isolate-associated models significantly differs from laboratory isolate-associated models of the bacteria. Further, through this analysis, increased consumption of the amino acid L-valine in clinical isolate-specific growth simulations was identified. This observation is suggested to be biologically important because, in previous studies, valine has been shown to stimulate the immune system

by augmenting macrophage phagocytosis, resulting in the death of foreign cells such as *K. pneumoniae* (Chen et al., 2017). Therefore, the observed result of increased valine consumption in clinical isolates could be indicative of an immunosuppressive strategy evolved by *K. pneumoniae* for survival during infection.

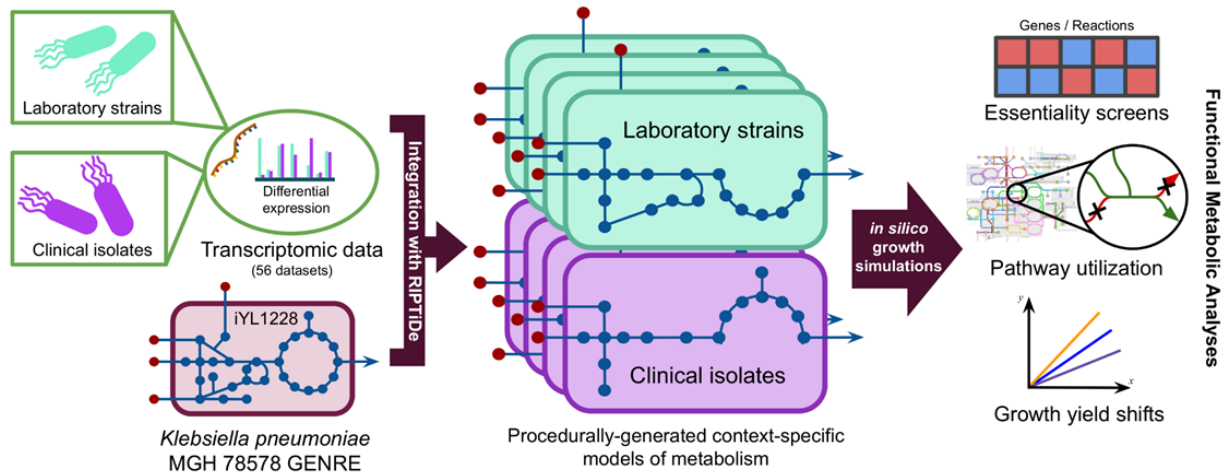


Figure 1. General procedure for generating context-specific models of metabolism from transcriptomic data. All 56 datasets from the transcriptome meta-analysis were used to generate distinct context-specific models of *K. pneumoniae* metabolism (Dickenson, 2021).

Due to these promising model-based results, these identified potential targets (all *K. pneumoniae* valine transaminases) are being validated through laboratory experiments using genetic engineering approaches. I aim to create *K. pneumoniae* mutants for each gene of interest, where, within that mutant, the particular gene of interest is inactive. To compare each of these mutants to the wild-type strain, I will design and utilize growth assays, such as a valine utilization and a macrophage phagocytosis assay, to evaluate the effects of the mutations. Using these observations, I will then iteratively improve the current metabolic model of *K. pneumoniae* to better represent physiological conditions and to aid in future computational model-based studies. Cumulatively, this will allow me to evaluate the potential effects each of these genes

have on the bacteria's ability to colonize a host organism, thus determining their potential efficacy as a therapeutic target.

This project will determine the potential of valine catabolism inhibition as a viable therapeutic target against *K. pneumoniae* infections. Furthermore, it will provide a better understanding of the role of L-valine in *K. pneumoniae* colonization and virulence, which may warrant further research around this in future studies. This study could help lead to novel clinical treatment regimens for *K. pneumoniae* infections. Finally, this research might be applied to other recalcitrant bacterial pathogens in the future as a platform for accelerated drug target discovery and validation.

The Sociotechnical Aspects of Antibiotic Overuse

Although I am working to identify novel therapeutic targets in the drug resistant bacteria *K. pneumoniae*, it is also necessary to understand the social aspects affecting antibiotic overprescribing in the healthcare field. The unnecessary prescribing of antibiotics is commonplace, even though antibiotic overuse is costly and leads to antibiotic resistant pathogens (Ackerman & Gonzales, 2012). Most often, these antibiotic prescriptions are being written unnecessarily in primary care settings where viruses, which do not respond to antibiotics, cause the vast majority of infections seen (CDC, 2016; Llor & Bjerrum, 2014). The technical and social aspects of this project go hand in hand. These two aspects of this problem are trapped in a viscous cycle; clinicians over-prescribe antibiotics, bacteria grow more resistant, clinicians prescribe continually more courses of antibiotics to be able to treat these resistant bacteria, new therapeutics become in dire need, and the cycle begins all over again (CDC, 2020; Llor & Bjerrum, 2014; Thakolkaran, Shetty, D'Souza, & Shetty, 2017). Furthermore, the common

expectations of patients to receive medication, and of healthcare professionals to administer medications, results in a demand for new treatment options that vastly outweighs the ability of the pharmaceutical industry to research, develop, and get approved for new therapeutics. This viscous cycle of bacterial resistance and doctor-patient interactions is putting the health of the world at risk, and therefore urgently needs to be addressed.

Central to the issue of antibiotic overuse are the clinicians prescribing these drugs and the patients who are receiving them. Studies have shown that oftentimes the doctor will feel pressured by the patient's expectations to prescribe antibiotics and will then succumb to those pressures, even if the doctors themselves do not feel as if they should be prescribing these drugs (Ramachandran et al., 2019). This has now seemingly become the standard of care. If a patient has an appointment with a healthcare professional regarding an illness, both parties involved expect the result to be the writing of a prescription. Further, while it may be expected that the interest in, and the level of knowledge about a given subject would align well, this is oftentimes not the case regarding the general public and science. Surveys have been collected about self-reported public interest in antibiotics and the correlating public knowledge about antibiotics. These studies have shown that while the public can be considered highly interested in antibiotics, they are very ill-informed on the topic overall (Durant, Evans, & Thomas, 1989). Further, another study demonstrated that, while approximately 90 percent of Americans claimed to support scientific and medical advances, less than half of Americans (~45 percent at the start of the 2000s) understood that antibiotics do not kill viruses (Miller, 2004). This exemplifies that there are huge discrepancies between what people support and what they actually understand, highlighting why the public may unnecessarily desire, and pressure clinicians towards, antibiotic prescriptions.

To analyze the myriad of factors thought to influence the unnecessary prescribing of antibiotics (Thakolkaran et al., 2017), I will use Star's article on infrastructure. Star's article, "The Ethnography of Infrastructure," describes infrastructure as hidden, yet incredibly important, mechanisms which underly the abundance of processes necessary for systems to function properly. Specifically, infrastructure is framed as a fundamentally relational concept, investigated in the context of those actors using the system put in place. Star additionally presents a series of characteristics that all good infrastructures should have. Of these, four of the characteristics most applicable to the problem of antibiotic overuse are embeddedness (being sunk into and inside of other existing structures), learned as part of membership (defined as communities of practice taking their infrastructures for granted), becomes visible upon breakdown (the invisibility of an infrastructure fades away when it breaks), and finally is fixed in modular increments (infrastructures are not installed or fixed in one single pass) (Star, 1999). Using this framework to analyze infrastructure allows for the easier identification of deficiencies within a given system, so that these shortfalls may be addressed.

The infrastructure around antibiotic prescriptions in healthcare is learned as a member (be it doctor or patient), resulting in the constant pressures felt from both parties to continually rely on antibiotics (Ramachandran et al., 2019). Yet, the parties within this system are incredibly ill informed, and thus continue to make decisions blindly based on previous trends (Durant et al., 1989; Miller, 2004). The result is seen in our everyday lives; the infrastructure that has been so carefully built around keeping the world protected from infectious diseases is now very visibly broken and desperately needs to be repaired.

Research Question and Methods

Due to the countless problems caused by the over-prescription of antibiotics (Llor & Bjerrum, 2014), I aim to research: From the perspectives of clinicians and patients, what factors influence the unnecessary prescribing of antibiotics, and how does each factor influence this? This research is necessary because the vast overprescribing of antibiotics has led to a recent rise in antibiotic resistant pathogens, rendering even the most potent antibiotics useless (Llor & Bjerrum, 2014). Star's article on infrastructure will further be used frame the issue of antibiotic resistance prior to detailing the findings from my research question of interest (Star, 1999). If the details around why antibiotics are being overused can be better understood, proper actions will be able to be taken to ameliorate the situation.

To collect data on this particular research question, I will conduct surveys with both clinicians and the general public about knowledge on and tendencies towards antibiotic usage. In order to receive the most amount of survey responses from both groups, I will use the snowball sampling method, where each group of people who take the survey are asked to recommend new people to participate in this study (Kirchherr & Charles, 2018). I plan to ask clinicians a series of questions about general knowledge on antibiotics, pressures felt from a variety of sources towards or against antibiotic prescriptions, and frequency training updates on antibiotics. Sample questions include:

- Do antibiotics affect viruses or bacteria? Answers: Viruses/Bacteria/Both
- What percentage of antibiotics are prescribed unnecessarily per year? Answers: Less than 10% / 10% - 20% /20% -30% / 30% - 40% / More than 40%
- Do you ever feel pressure from patients to prescribe them antibiotics? Answers: Yes/No

- How often do you receive training updates on antibiotics? Answers: Almost never/Once to twice a year/Three or more times a year

I additionally plan to ask people from the general public to answer a similar set of questions about general knowledge on antibiotics, overall desire/tendencies to receive antibiotics when seeing a doctor, and general knowledge on antibiotic resistance. Sample questions for this survey include:

- Do antibiotics affect viruses or bacteria? Answers: Viruses/Bacteria/Both
- When you see a doctor regarding some form of illness, do you tend to want/expect to receive a prescription for drugs/antibiotics before you leave? Answers: Always/Sometimes/Never
- On average, how many times do you receive an antibiotic prescription in a year? Answers: 0/1-2/3-5/More than 5
- Are antibiotic resistant bacteria a major problem in the United States? Answers: Yes/No

Using the results from this survey, I will identify trends in the results (parsing out majority answers, minority answers, and any other unique trends) indicating the common knowledge around, and actions towards, antibiotics from both the clinical and the patient points of view. This will allow for the better understanding of the sociotechnical aspects causing antibiotic over-prescription, which may lead to plausible ways to work towards resolving this issue.

Conclusion

Through this project, I will first create and validate a model-driven design of novel therapeutic targets in *K. pneumoniae*, and then analyze the sociotechnical aspects surrounding antibiotic overuse in the healthcare field by performing a series of surveys with clinicians and the

general public. Positive results from the technical portion may result in the downstream development of a novel therapeutic approach to treat clinical *K. pneumoniae* infections. Results gleaned from the STS portion of this study may indicate the primary reasons antibiotics are prescribed and taken in the healthcare setting from the clinician and the patient points of view, allowing for actions to be taken to lower the rates of overuse. It is vital that the problem of antibiotic overuse be further studied and that new solutions to treating these antibiotic resistant drugs be found, so that the world may begin working to resolve this detriment to human health.

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