An Innovative Approach to Improving Antibiotics: Investigating the Efficacy of Inhibiting Pathogen Glycosylation to Synergistically Improve Existing Antibiotics

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The rise of antibiotic-resistant strains of bacteria and limitations of broad-spectrum antibiotics have motivated urgent research efforts towards the development of novel antibiotics. In 2017, the World Health Organization released a publication deeming particular pathogens "high priority" for the research and development of new antibiotics (1). This list includes pathogens of interest such as *Enterococcus faecium, Staphylococcus aureus, Helicobacter pylori, Campylobacter, Salmonella,* and *Neisseria gonorrhoeae* (1). These bacteria share important similarities in the glycoprotein molecules present on the exterior of their cells that can be targeted in therapeutic interventions.

Bacterial glycoproteins contribute to a pathogen's pathogenicity, participating in the interactions that allow bacterial motility and biofilm formation (2). Furthermore, these cell surface glycoproteins have a direct role in a bacterium's ability to evade host immune detection (3). These molecules are an intriguing target for inhibition because of their distinctive expression across species; their structures differ from those of eukaryotes and contain rare monosaccharides (4).

Interfering with glycan elongation and consequently glycoprotein structure is hypothesized to negatively affect the biological fitness of a particular bacteria. Inhibitors based upon sugar scaffolds theorized to selectively interfere with glycosylation in some species of bacteria and not others were synthesized by a collaborating laboratory at the Indian Institute of Technology Bombay to test this hypothesis. My research focused on evaluating the selectivity of these inhibitors across different species of bacteria including the pathogenic *Campylobacter jejuni* and the symbiotic *Bacteroides fragilis*.

Metabolic oligosaccharide engineering (MOE) is a technique utilized in the Dube laboratory to label, detect, and identify glycoproteins within bacteria of interest (5). I first screened a panel of inhibitors in *C. jejuni* using a Western Blot. In the presence of an effective inhibitor, a reduction in signal should be observed. Changes in biological fitness were also assessed using motility and biofilm assays. The inhibitors tested did not elicit any defects in the fitness of *C. jejuni*.

Next, I screened the panel of inhibitors in *Bacteroides fragilis*, a symbiotic bacterium known to colonize the healthy gut. In this species, some inhibitors caused a reduction in Western Blot signal reflective of a decrease in glycosylation. This result was further supported by Flow Cytometry experiments that quantify this change in glycosylation on the cellular level.

Overall, in the context of the work of previous Dube Laboratory members, these results indicate some of the inhibitors demonstrate species-specificity. In other words, some inhibitors work selectively only against the pathogenic *H. pylori* and not *C. jejuni* nor *B. fragilis*. This information is very encouraging, as a main objective of our work is to selectively target specific pathogens. Ultimately, we aim to improve existing antibiotics by reducing the biological fitness of specific disease-causing bacteria using these inhibitors.

I will continue my investigation as an Honors Project during the upcoming academic year. My future work will focus on further exploring the effective inhibitors. After determining the biological changes caused by these inhibitors in *B. fragilis,* I will likely explore the effects of these inhibitors on mammalian cells, a critical step towards understanding how this therapeutic approach could work one day in patients in need.

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