UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Master of Science*** *of*

NOAH KUEHFUSS

On Tuesday, September 3rd, 2024 at 9:30 a.m. (SSC 1511)

Thesis Title: **An antibacterial combination drug against *Enterobacteriaceae***

Examination Committee:

Dr. Rebecca Shapiro, Dept. of Molecular and Cellular Biology
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Robert Harkness, Dept. of Molecular and Cellular Biology
Dr. Gerard Wright, Dept. of Chemistry and Biomedical Sciences, McMaster University

Advisory Committee:

Dr. Georgina Cox (Advisor)
Dr. Matthew Sorbara
Dr. Gerard Wright

Abstract: Intrinsic resistance mechanisms found in Gram-negative bacteria render many clinically used antibiotics ineffective and have challenged the discovery/development of novel antibiotics. Consequently, inhibitors of intrinsic resistance mechanisms, also known as antibiotic adjuvants, could rejuvenate antibiotic activity against resistant bacteria. Here, I report the identification of a novel antibiotic-adjuvant combination exhibiting efficacy against high-priority Enterobacteriaceae pathogens. A high-throughput screen of over 11,000 actinomycete fermentation extracts, a group of bacteria responsible for over 70% of the current clinically used antibiotics, identified a highly synergistic and specific interaction between the amino-sugar kanosamine, isolated from Streptomyces (WAC# 1529), and the RNA polymerase (RNAP) inhibitor rifampicin. I describe the activity-guided purification and identification of kanosamine from the fermentation broth of WAC# 1529, the mechanistic reasoning for synergy with rifampicin and other RNAP inhibitors, and the compound's antibacterial spectrum of activity. Synergy was restricted to members of the Enterobacteriaceae family, which was attributed to the conservation of the glucosamine-6-phosphate (GlcN6P) regulation mechanism. GlcN6P is a major precursor of the bacterial cell wall and is synthesized by GlcN6P synthase (GlmS) in Enterobacteriaceae. GlmS regulation in Enterobacteriaceae is controlled by the conserved regulatory microRNAs (miRNAs) GlmY and GlmZ, which increase GlmS abundance. The chemical inhibition of GlmS by the GlmS inhibitors bacilysin (tetaine) and L-norvalyl-N3-(4-methoxyfumaroyl)-(S)-2,3-diaminopropanionic acid (NVA-FMDP) decreases GlcN6P levels, and in response the conserved GlmY GlmZ regulatory network creates an overabundance of GlmS providing intrinsic resistance through target overexpression. I demonstrate that this regulatory network additionally provides intrinsic resistance to the GlmS inhibitor kanosamine. Indeed, mechanistic studies revealed that synergy between kanosamine and rifampicin is likely associated with kanosamine-mediated inhibition of GlmS. Rifampicin was shown to impact the relative expression of glmS, and I hypothesize that RNAP

inhibitors disrupt the GlmS feedback control mechanism governed by the GlmY and GlmZ regulatory system, ultimately reducing the abundance of GlmS, enhancing the activity of kanosamine against Enterobacteriaceae pathogens. Thus, in this instance, rifampicin acts as the adjuvant and kanosamine the antibiotic. In summary, this thesis presents a new antibiotic-adjuvant combination with an unprecedented mechanism of action that displays efficacy against high-priority Enterobacteriaceae pathogens. This work highlights a novel approach to address the clinical challenge of drug resistance in Gram-negative pathogens, and future work should further investigate the viability of kanosamine and RNAP inhibitor combination therapy as a potential therapeutic.

Curriculum Vitae: Noah completed his Bachelor of Science (Honours) in Molecular Biology and Genetics with a Minor in Biochemistry at the University of Guelph in 2021. In the Fall of 2022, he began his M.Sc. in Molecular and Cellular Biology under the supervision of Dr. Georgina Cox.

Awards: Ontario Graduate Scholarship (2023-2024)

Publications: Teelucksingh, T., Thompson, L.K., Zhu, S., **Kuehfuss, N.M**., Goetz, J.A., Gilbert, S.E., MacNair, C.R., Geddes-McAlister, J., Brown, E.D., and Cox, G. (2022). A genetic platform to investigate the functions of bacterial drug efflux pumps. Nat. Chem. Biol. *18*, 1399–1409.

Goetz, J.A., **Kuehfuss, N.M**., Botschner, A.J., Zhu, S., Thompson, L.K., and Cox, G. (2022). Exploring functional interplay amongst *Escherichia coli* efflux pumps. Microbiology *168*.