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 ***Doctor of Philosophy*** *of*

ALLISON LEONARD

on June 10th, 2024 at 9:30 a.m. (SSC 1511)

Thesis Title: **Investigating and inhibiting *Staphylococcus aureus* host adhesion**

Examination Committee:

Dr. Krassimir Yankulov, Dept. of Molecular and Cellular Biology (Exam Chair)
Dr. Georgina Cox, Dept. of Molecular and Cellular Biology
Dr. Marc Coppolino. Dept. of Molecular and Cellular Biology
Dr. Priyanka Pundir Dept. of Molecular and Cellular Biology
Dr. John McCormick Dept. of Microbiology and Immunology, University of Western Ontario (External Examiner)

Advisory Committee:

Dr. Georgina Cox (Adv)
Dr. Marc Coppolino
Dr. Cezar Khursigara

Abstract: The emergence of antibiotic-resistant strains of *Staphylococcus aureus* has threatened our antibiotic arsenal. As such, it is crucial new therapeutic strategies are developed for treating *S. aureus* infections. One such example is anti-virulence therapeutics that target the mechanisms used by bacteria to infect the host. Adhesion to the host environment is crucial for colonization and infection. In *S. aureus*, this process is mediated by numerous cell wall-anchored (CWA) proteins that are covalently anchored to the peptidoglycan. This research describes the optimization and validation of a whole-cell ELISA-based adhesion assay for genetic and chemical screening of *S. aureus* adhesion to host ligands. This assay delineated the genetic determinants of *S. aureus* adhesion to the host ligands fibronectin, keratin, and fibrinogen. This screen highlighted the major murein hydrolase, autolysin (Atl), for its widespread role in adhesion. Here, the role of this hydrolytic protein in the surface display of CWA proteins was characterized, revealing that Atl-mediated daughter cell separation is critical for maintaining optimal surface levels of *S. aureus* CWA proteins. As such, disrupting autolysin function reduced the affinity of *S. aureus* for host ligands, negatively impacting the early stages of bacterial colonization in a systemic model of *S. aureus* infection. A chemical screen was also performed to identify anti-adhesive compounds that attenuate *S. aureus* adhesion to keratin, a major component of the anterior nares and the primary ligand associated with nasal colonization. This screen identified a polyunsaturated branched-chain fatty acid, Geranylgeranoic acid, exhibiting broad-spectrum anti-adhesive activity against fibronectin, keratin, fibrinogen, and immunoglobulins. This research highlights the multifaceted role uFAs play in the host-pathogen interaction while providing a better understanding of the regulation of CWA proteins. Overall,

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this research highlights two new classes of anti-adhesive agents with distinct mechanisms of action. These compounds are promising candidates to be advanced in future studies as new therapeutics to prevent and/or treat *S. aureus* infections.

Curriculum Vitae: Allison obtained her Bachelor of Science (Honours) at the University of Guelph in 2019. In the summer of 2019, she entered the MSc. program under the supervision of Dr. Georgina Cox. In the winter of 2021, she transferred into the Ph.D. program.

Awards: CIHR Canadian Graduate Scholarship – Doctoral (2022-2024), Donald R. Phillips Molecular and Cellular Biology Scholarship (2023), Queen Elizabeth II Graduate Scholarship in Science and Technology (2021-2022)

Publications:

1. **Leonard AC,** Petrie LE, Cox G. 2019. Bacterial anti-adhesives: inhibition of *Staphylococcus aureus* nasal colonization. *ACS Infect Dis*. **5**(10), 1668–1681

2. Petrie LE*, **Leonard AC***, Murphy J, Cox G. 2020. Development and validation of a high-throughput whole-cell assay to investigate *Staphylococcus aureus* adhesion to host ligands. *J Biol Chem*. **295**(49), 16700-16712.

3. **Leonard AC**, Stoica S, Cox G. 2022. "Chinks in the armour: novel pathogenesis-based strategies to combat bacterial infections". *In* John Prescott, John Boyce, Janet I. MacInnes, Andrew N. Rycroft, Filip Van Immerseel, and José A. Vázquez-Boland (eds.) Pathogenesis of Bacterial Infections in Animals, Wiley & Sons, Incorporated, Hoboken.

4. Berry KA, Verhoef MTA, **Leonard AC,** Cox G. 2022. *Staphylococcus aureus* adhesion to the host. Ann *NY Acad Sci*. **1515**, 75-96.

5. **Leonard AC,** Goncheva MI, Gilbert SE, Shareefdeen H, Petrie LE, Thompson LK, Khursigara CM, Heinrichs DE, Cox G. 2023. Autolysin-mediated peptidoglycan hydrolysis is required for the surface display of *Staphylococcus aureus* cell wall-anchored proteins. *Proc Nat Acad Sci*. **120**(12), e2301414120.

*Authors contributed equally