Chapter 1

Introduction

1.1 Background

Streptococcus pyogenes, also known as Group A *Streptococcus* (GAS), refers to a Gram-positive bacterium infamously responsible for strep throat, skin infections such as pharyngitis and impetigo, and life-threatening conditions including septicaemia, streptococcal toxic shock syndrome, myositis, cellulitis, and necrotizing fasciitis. Other serious sequelae of GAS infection include rheumatic fever and poststreptococcal glomerulonephritis (Kanwal & Vaitla, 2024). Pathogenic bacteria, including GAS, commonly utilize two-component signal transduction systems to regulate the expression of genes encoding toxins, adhesins, and other virulence factors that interact with the host (Hirakawa et al., 2020).

A two-component signal transduction system commonly follows a mechanism of a ligand binding to an extracellular sensory domain of a sensor histidine kinase (HK), that induces a conformational change. This conformational change allows HK autophosphorylation where HK transfers a phosphoryl group from ATP to a conserved histidine residue within its own structure. HK contains histidine phosphotransferase activity that transfers the phosphoryl group to a response regulator (RR), inducing yet another conformational change that activates the output domain. Activated output domains commonly bind to DNA, causing a regulation of gene expression. A reduction of the sensor kinase activity happens when phosphatase activity removes the phosphate from the RR, returning the RR to a non-phosphorylated state to reset the system (Buschiazzo & Trajtenberg, 2019).

The two-component transduction signal system that is responsible for the regulation of virulent factors in GAS is called the CovRS system. The mechanism of the CovRS system follows where CovS acts as the transmembrane protein containing the sensory domain, while CovR acts as the response regulator that would be phosphorylated by CovS for further downstream signaling process of genes that repress various lethal virulence factors of GAS such as production of streptolysin S (SLS) and

streptokinase. Meanwhile, inactivation of this CovRS system follows the removal of phosphate group from the RR that leads to a hypervirulent state of GAS that includes an overexpression of the hyaluronic acid capsule and promote virulent factors such as streptokinase, streptolysin S, and streptodornase for infection in humans (Sarkar & Sumby, 2016).

Another crucial regulatory two-component signal transduction system in GAS refers to the LiaFSR system that has a responsibility in maintaining the survival of the bacteria. LiaSFR works in a way where LiaS acts as the transmembrane protein with the sensory domain, in which in the presence of signal binding, LiaF component dissociates from it, allowing the autophosphorylation of LiaS. Following this, LiaS transfers its phosphoryl group to LiaR that acts as the response regulator (Shankar et al., 2015). Phosphorylated LiaR acts as an activator that binds to specific DNA sequences that lead to several gene expressions such as *spx*A2 a transcriptional regulator that regulates bacterial response to stress and proteins such PspC, LafB, and YidC2 that collectively modify and alter the cell envelope for the survival of the bacterium (Vega et al., 2024). Thus, a disruption of the cell membrane or ExPortal of GAS from human neutrophil peptide 1 (hNP-1) treatments or polymyxin B treatments, antimicrobials which targeted the cell membrane, resulted in the activation of LiaFSR to restore membrane composition and remodel the cell envelope (Lin et al., 2020).

Recent findings discover that this CovRS system has an interconnected regulatory network with the LiaFSR system where the expression of *spx*A2 gene happens to regulate the expression of the CovRS system. An abundance of *spx*A2 would allow more binding to RNAP enzymes, causing upregulation of CovR-regulated gene expression and mediating CovR release. This allows more expression of virulence factor, causing a hypervirulent state of the bacterium. Meanwhile, low levels of SpxA2 would activate the RNA polymerase (RNAP) enzyme that downregulates CovR-regulated gene, causing its repression (Sanson et al., 2021). Thus, a GAS with a non-phosphorylated CovR mutant prevents the inhibition of CovR-regulated genes, allowing more expression of virulence factors even in non-threatening or stressful conditions. However, the outcome of these unregulated expression of

virulence factors, especially towards the LiaFSR system, remain a mystery when the bacterium encounters stressful conditions such as when exposed to hNP-1 treatments and polymyxin B treatments. Understanding more in depth on the effects of non-phosphorylated CovR towards the sensitivity response of LiaFSR could improve more understanding towards GAS regulatory system interaction with antimicrobial treatments, developing understanding on its resistance mechanism that could assist in the invention of a more precise medication that eliminates or prevents the rise of virulent GAS.

1.2 Objective

The aim of this research focuses on investigating the role of non-phosphorylated CovR on ExPortal integrity and inducing insensitivity on the LiaFSR system with regards to polymyxin B treatments through:

- Protein secretion analysis of SLO protein using Western Blot method
- Gene expression analysis of *spx*A2 gene using RT-qPCR method
- Protein expression analysis of LiaR~P using PhosTag Western blot method

1.3 Hypothesis

The study would apply an alternative hypothesis and null hypothesis as the following:

- Null Hypothesis (H_o): Non-phosphorylated CovR do not cause insensitivity towards the LiaFSR transduction system and disrupt ExPortal integrity, allowing it to have more susceptibility towards polymyxin B
- Alternative Hypothesis (H_1): Non-phosphorylated CovR cause insensitivity towards the LiaFSR transduction system and have more intact ExPortal integrity, allow it to have more resistance towards polymyxin B