Abstract

Streptococcus pygones (GAS) virulence is governed by an intertwined regulatory network of CovR/CovS (CovRS) and LiaF/LiaS/LiaR (LiaFSR) two-component systems. While CovRS represses various virulence factors, LiaFSR senses membrane-perturbing antimicrobials (hNP-1, polymyxin B) and disrupts ExPortal integrity to reduce secretion of the virulence determinants such as streptolysin O (SLO). Recent work revealed that LiaFSR mediated SpxA2 upregulation would reduce CovR phosphorylation level, yet whether this state compromises LiaFSR-mediated ExPortal protection remains unknown. The consequence of hyperactivation of virulence towards the interaction between LiaFSR and CovRS systems during stressful conditions such as exposure to membrane-perturbing antimicrobials also remains unknown. This study then aims to quantify ExPortal-dependent SLO secretion and ExPortal integrity through *spx*A2 expression as well as LiaR~P expression in wild-type A20, Δ*lia*F, CovR-defective mutants with or without their $Lias_{D142A}$ counterparts, and CovS-defective mutants with or without their $Lias_{D142A}$ counterparts, before and after treatment with polymyxin B through protein and gene expression analysis. Interesting findings showed that non-phosphrylated CovR in CovR-defective mutants have more intact ExPortal integrity and insensitive LiaFSR system that leads to polymyxin B resistance shown through their higher SLO secretion, lesser *spx*A2 expression compared to wild-type, and lesser LiaR~P expression. By directly linking CovR phosphorylation status to LiaFSR sensitivity and ExPortal functionality under antimicrobial stress, this study will clarify how GAS balances virulence induction for their survival under stressful conditions that may lead to the identification of exploitable vulnerabilities for precision anti-GAS therapies.

Keywords: *Streptococcus pyogenes*, CovRS, LiaFSR, ExPortal integrity, non-phosphorylated CovR