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Biofilm As A Predictor Of Attenuation In Francisella Tularensis

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Biofilms have been established as the predominant lifestyle of many bacteria found in the environment, yet the role of biofilm in the biothreat agent Francisella tularensis is poorly understood. Non-substantial biofilms are often observed in vitro with clinically relevant subspecies F. tularensis tularensis (Type A) and holarctica (Type B). However, we have identified conditions that induce biofilm in a stochastic, but highly reproducible manner using clinically relevant isolates from both subspecies. We found that the frequency of biofilm formation increased temporally and appeared switch-like as progeny from the initial biofilm quickly formed a biofilm in a predictable manner. This suggested a heritable mechanism of biofilm induction. Upon plating biofilm-forming cultures, multiple colony morphologies were observed leading to the identification of a particular subset of variants that constitutively produce biofilm. All biofilm-forming variants displayed alterations of the O-antigen that is incorporated into LPS and capsule. While biofilm is often considered a virulence factor in many pathogens, previous reports have shown variation of the O-antigen leads to attenuation in F. tularensis. Given these antithetical findings, we utilized a murine tularemia model to study the effects of biofilm formation on virulence in clinically relevant subspecies. For humans, the glandular and ulceroglandular forms of tularemia contracted from an arthropod bite are most common while aerosol exposure represents the most serious defense threat. With this in mind, animals were challenged via subcutaneous and intradermal routes to mimic tick and mosquito bites, respectively, as well as intranasal to mimic aerosol exposure. By all routes of infection, biofilm-forming variants of both subspecies were found to be highly attenuated. This work suggests that biofilm formation occurs at the cost of virulence in F. tularensis and that biofilm may potentially be used as a predictor of attenuation in this bacterium.