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Determining the Effects of Human Serum on Pseudomonas Aeruginosa Physiology

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Extended Abstract

The opportunistic bacterium *Pseudomonas aeruginosa (P. aeruginosa)* causes severe infections that are difficult to treat due to its intrinsic high antimicrobial resistance[1,2]. Research and development into new treatments often start with *in vitro* antimicrobial assays traditionally performed in environments[3] that are not an accurate reflection of the *in vivo* wound environment. For example, unpublished data from our lab suggests that the effectiveness of antimicrobial silver in clinical trials is surprisingly low despite its routine use for wounds in Canada[4] and high antimicrobial success *in vitro*[5]. Recent studies have started to use more biologically relevant environments by adding serum. Serum is the part of blood without red blood cells or clotting factors, and can be divided into complement (part of the body's innate immune defenses) and non-complement. While the effects of complement on *P. aeruginosa* have been investigated extensively[6–9], little to no research exists on the effects of complement-inactivated serum, which contains over 3000 proteins. My work aims to address this knowledge gap. Our research shows that complement-inactivated serum elicits antimicrobial resistance and tolerance from *P. aeruginosa. P. aeruginosa* displayed tolerance to imipenem and colistin, and resistance to silver, when incubated with 10% complement-inactivated human serum. Through bioactivity-guided fractionation, we found a >100 kDa component in serum that elicits strong silver resistance and a component between 50-100 kDa that elicits weak tolerance and resistance. The addition of proteinase K, an enzyme that digests proteins, to the >100 kDa component resulted in a loss of silver resistance which suggests that this component is a protein. *P. aeruginosa* is also known to exhibit nutrition-dependent phenotypes, like virulence. We added proteinase K-digested human serum that was <50 kDa to provide the bacteria with an alternate nutrition source which elicited intermediate *P. aeruginosa* silver resistance. My research shows that bioactive moieties, chemistry, and physiology likely all play a part in serum-dependent antimicrobial resistance and tolerance. Further research will examine how complement-inactivated serum affects *P. aeruginosa* physiology through RNA-sequencing, which allows me to assess what parts of the DNA have been translated into RNA. Outputs will inform further research into creating better wound environment models that will provide the basis for *in vitro* antimicrobial R&D and wound-related research.

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