Quantification of Hemolysis for Five Strains of GBS

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Introduction:

Streptococcus agalactiae (GBS) remains the leading cause of infectious neonatal morbidity and mortality in the United States. Although GBS is an important cause of chorioamnionitis (inflammation of the gestational membranes), preterm birth, and neonatal disease, little is known about the mechanism by which GBS causes increased inflammatory signaling in the gestational tissues. Professor Boldenow previously collected data showing that GBS strain deferentially alters inflammatory cytokine levels in human gestational explants in vitro. However, it is not understood why each GBS strain has a unique inflammatory profile. Recent data show that different GBS strains deferentially express a virulence factor known as beta hemolysin, which is responsible for the hemolytic activity of the bacteria. Five GBS strains (A909, GB37, GB112, GB411 & GB590) were tested for hemolytic activity using both blood agar plates and blood in suspension to quantify GBS hemolytic activity.

Method Description:

Blood Agar Plates: After enrichment in Todd-Hewitt broth overnight, GBS culture was streaked on a blood agar plates and incubated overnight at 37C 5%CO2.

Blood Suspension Assay: GBS supernatant was prepared by centrifuging overnight GBS cultures at 1,000 x g for 10 minutes. GBS cells were prepared by growing GBS cultures to OD_{600} 0.3, centrifuging and resuspending to $1x10^8$ CFU in 1 mL of PBS-1%glucose-2% starch. Either 250 µL of GBS supernatant or resuspended cells were incubated with 250 µL of sheep erythrocytes (1:10 dilution in PBS) at 37°C for 30 minutes with gentle shaking (100 rpm). Samples were then centrifuged and 100 µL of the supernatant was aliquoted in a 96-well plate. Hemoglobin release was detected by measuring the OD_{450} on a plate reader. All treatments were performed in duplicate and the assay was repeated six times. ANOVA was used to determine statistical significance.

Results:

On a blood agar plate, 4 of 5 strains showed hemolytic activity. GB37 was not hemolytic, whereas A909, GB112, GB411, and GB590 were hemolytic. In the blood suspension assay, hemolytic activity in all 5 GBS strains was statistically significant from control (-), but no significant difference were seen between GBS strains for both the supernatant and the cells. Hemolytic activity for GB37 was different depending on the assay (agar plate vs blood suspension). Future work will determine why the bacteria responds differently in different assays.

Personal Reflection:

This project is my first formal experience working in a lab, and I have loved it. Unlike in the classroom, I am able to practice problem solving in the lab and experience the satisfaction that only comes after troubleshooting. Examining this bacteria through the lens of microbiology and global health has allowed me a glimpse into what my own future could look like as I am interested in pursuing both medicine and public health.