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Title: Manipulation of immune cell function by hydrogen peroxide from *Streptococcus pneumoniae*.

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Sponsor: Canterbury Health Laboratories

Introduction:

Streptococcus pneumoniae is a bacterium that was first isolated by Pasteur over 130 years ago. Since then *S. pneumoniae* has been extensively studied, and has led to many important break throughs in science. Despite the availability of vaccines, *S. pneumoniae* remains a major cause of diseases such as ear infections, pneumoniae, meningitis and sepsis, with the greatest impact on young children, the elderly and immunocompromised patients. With the rise in anti-biotic resistant *S. pneumoniae* it is likely we will see an increase in impact of this pathogen, giving a sense of urgency in the development of new therapeutic treatments.

S. pneumoniae is reported to produce large amounts of hydrogen peroxide (H_2O_2), a chemical that is often harmful to bacteria, as it grows in the presence of oxygen. Interestingly this bacterium lacks catalase, the most common protein in biological systems that breaks down H_2O_2 to water. H_2O_2 can cause damage to neighboring cells and it can act as a signaling molecule to alter the function of immune cells trying to remove the bacterium. However, it does not appear to harm the bacterium itself. In this project I have examined exactly how much H_2O_2 is produced by bacteria isolated from patients at Christchurch Hospital, and how the bacterium can cope with additional H_2O_2 added while they grow.

Aim: To measure the production of H_2O_2 by various clinical isolates of *S. pneumoniae* obtained from the lungs of patients with pneumonia or blood of patients with sepsis.

Impact:

If we can identify how *S. pneumoniae* protects itself H_2O_2, it could be possible to target their defenses, making them easier to kill.

Method:

Clinical isolates, 9 from blood and 13 from lungs, of *S. pneumoniae* were obtained from Canterbury Health Laboratories.

Isolates were grown in a nutrient rich media until the start of the log phase (where the bacteria are growing the fastest). At this point, the bacteria were transferred into a nutrient poor media and incubated for a set amount of time. Samples were taken at time 0, 20, 40 and 60 minutes and analyzed to determine the amount of H_2O_2 that had accumulated in these samples.

The H_2O_2 was also captured as it was produced to determine the rate of production of H_2O_2, by each isolate.

Results:

Results from this assay demonstrated the clinical isolates could increase H_2O_2 in their surroundings by 8-24 µM after a one-hour culture (10 million bacteria per ml), see figure 1. While there was significant variation in H_2O_2 production between the isolates, it didn't matter if they were blood or lung isolates.

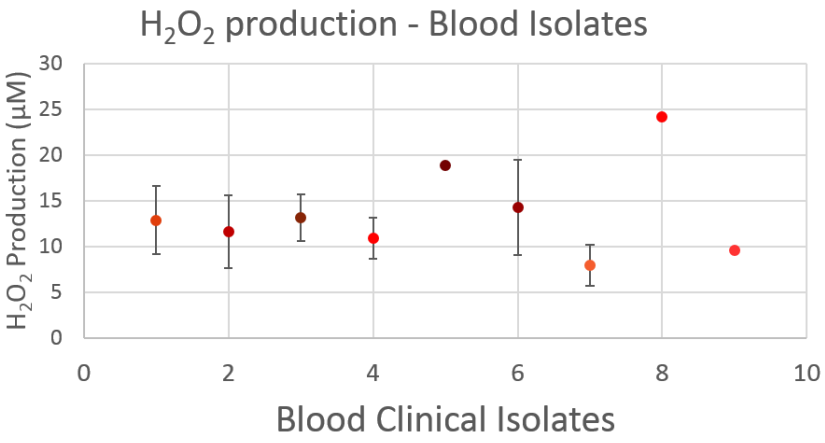

Figure 1 – Shows the H_2O_2 produced in a variety of blood clinical isolates after a one-hour culture (10 million bacteria per ml).

Conclusion:

S. pneumoniae can generate large amounts of H_2O_2 when they are grown in culture, and they don't seem to be able to effectively remove this H_2O_2. These concentrations of H_2O_2 are known to be sufficient to kill some types of white blood cells.

Further investigation will look at how the low and high producers, identified earlier, are able to cope after the addition of extra H_2O_2 in their environment. It would be interesting to explore how they are able to protect themselves when this amount of H_2O_2 would kill other cells.