Contribution ID: **38** Type: **Poster**

Quantifying surrogate respiratory pathogen inactivation in respiratory droplets

Objective:
Respiratory pathogens, including viruses, bacteria, and fungi, are transmitted through respiratory droplets and aerosol particles and exhibit significant variation in their airborne stability. Prominent examples such as influenza viruses, Mycobacterium tuberculosis, and SARS-CoV-2 highlight this diversity and their potential for widespread transmission and outbreaks. To better understand the viability range of airborne pathogens, this study investigates the inactivation rates of three surrogate organisms—Escherichia coli (representing gram-negative bacteria), Staphylococcus epidermidis (representing gram-positive bacteria), and MS2 coliphage (representing viruses)—chosen for their differing susceptibilities to pH and NaCl molality. These non-pathogenic surrogates are used instead of actual pathogens to enable downstream experimentation outside of biosafety confinements. Building on these inactivation kinetics, a secondary objective is to integrate the derived relationships into the Respiratory Aerosol Model (ResAM), a comprehensive shell-diffusion framework, to predict pathogen viability in respiratory droplets under different relative humidities. The model predictions are then validated via controlled droplet experiments conducted in an environmental chamber.
Methods:
To obtain the inactivation rate constant as a function of pH and salt, solutions of 0.1M Citric Acid and 0.2M Trisodium Phosphate at varying volumes to obtain a specific pHs between 2-10. Additionally, each solution had a parallel containing 5m NaCl to investigate the synergistic effects of pH and salt. Samples were taken at different time points and residual infectious concentrations were enumerated. For each experiment, an inactivation rate was calculated assuming pseudo first order kinetics using least-squares regression in R. Then each rate constant was plotted versus pH and salt molality and a surface regression was performed to obtain k=f\left(pH,\ \left[NaCl\right]\right), also performed using R.
Droplet experiments were performed in an environmental chamber where temperature and relative humidity are kept constant. Relative humidity levels of 30%, 50%, and 70% were tested. Artificial saliva was used as the droplet media to simulate realistic respiratory conditions. At specified time intervals, samples of the drying droplet were resuspended and enumerated by both infectivity assay, as well as quantitative PCR. Additionally, droplets were filmed under different conditions in order to determine the change in the particle radius and to detect the onset of efflorescence. The numerical relationship derived in from the bulk experiments was then encoded in ResAM where the droplets from the bulk experiments were modeled.
Results :
Among the surrogates tested, E. coli is the most sensitive to pH and is stable around pH 4-9. It is more sensitive to alkaline pH levels compared to acidic ones making it comparable to influenza. S. epidermidis is also stable around pH 4-9 and is less sensitive to extreme pH levels comparable to rhinovirus. MS2 is significantly less sensitive to extreme pH and is stable from pH 3-10. This is comparable to coronaviruses like SARS-CoV-2 and HCoV-229E. With the additional of NaCl, a synergistic effect was observed for E. coli at acidic pH ranges where the inactivation was enhanced. This synergistic effect was not observed for S. epidermidis and MS2.
For both E. coli and S. epidermidis, inactivation in droplets is increased as the relative humidity decreased, due to high supersaturation of salt prior to efflorescence. This rapid change in salt molality in the droplet is associated with inactivation. For MS2, higher inactivation is seen in the middle relative humidity range. Efflorescence was observed at all three relative humidities for both E. coli and S. epidermidis due to the bacteria acting as nucleation points –while only observed at 30% for MS2. The observed inactivation was compared to the predicted one using ReSAM and exhibited a good correspondence.

Conclusion:
These results highlight the complex nature of pathogen inactivation in respiratory droplets, where factors such as pH, salt concentration, and relative humidity play pivotal roles. The differing sensitivities of E. coli and S. epidermidis to high salt concentrations, contrasted with MS2's relative resilience, underscore the importance of using multiple surrogate organisms to reflect the diversity of pathogen responses. By integrating these experimental inactivation rates into the Respiratory Aerosol Model (ResAM) and validating them through droplet experiments, this study provides a strong framework for predicting pathogen viability under real-world conditions. Ultimately, these insights can inform more effective strategies for infection prevention and risk assessment, contributing to better control of respiratory pathogen spread.

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