Investigating the Effects of Fluid Composition on Bacterial Aerosol Production

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Summary

Airway lining mucus (ALM) is a complex hydrogel composed of 98% (w/v) water and 2% (w/v) solids content.[1] Biological aerosols, including infectious aerosols, are believed to originate from shear-stress induced breakup of the ALM. Understanding how the composition and viscoelastic properties of the ALM affects infectious aerosol production can provide valuable insight into airborne transmission mechanisms. In this study we examined the effect of fluid composition on bacterial aerosol production. Solutions containing different concentrations of peptone water, a microbial growth medium rich in protein content, were used to suspend the bacterial pathogen, *Staphylococcus aureus (S. aureus).* Peptone water solutions were aerosolized using the single jet Blaustein Atomizer module. Polydisperse bacterial aerosols were size fractioned using a viable six-stage cascade impactor. Size and counts of bacterial aerosols generated from the three peptone water solutions were compared. Mean particle size of bacterial aerosols increased as the concentration of the peptone solution was increased from 1.5% (w/v) to 5.0% (w/v) and 10% (w/v) peptone content. Additionally, the number of bacterial aerosols generated from a 1.5% (w/v) peptone water solution was significantly greater as compared to the number of aerosols produced from a 10% (w/v) peptone water solution.

Key Message

Bacterial aerosols were generated from solutions formulated to mimic the solids concentration of the ALM. Significant changes in size and quantity of bacterial aerosols were observed as the concentration of the peptone water solution was altered, demonstrating the influence of fluid composition on the production of pathogen containing aerosols.

Introduction

A superspreader transmission event is a phenomenon where a small fraction of infected individuals account for a disproportionately high number of transmission events within a population.[2] Studies of superspreader events have produced the 20/80 rule, where 20% of infected individuals cause 80% of all transmission events.[2,3] Individuals with active respiratory infections capable of producing high quantities of infectious aerosols may exhibit greater transmission probability.[3] Exhaled bioaerosol quantification studies indicate airborne transmission may follow a superspreader distribution pattern.[3–5] Within a group of 194 healthy humans participants, approximately 18% of individuals were shown to produce an extremely high number of bioaerosols, accounting for 80% of total aerosol production by the group.[3,4] Furthermore, changes to surface tension properties of the ALM, induced through saline or surfactant delivery, transiently attenuated or exacerbated bioaerosol production from human participants.[4,5] Relating changes in ALM with changes in infectious aerosol production can provide valuable insights into the mechanism of airborne transmission.

Evidence exists that physiological factors that alter the ALM leading to changes in aerosol production, can impact transmissibility of respiratory diseases.[1,3] For instance, the solids concentration of the ALM is altered with the onset and severity of muco-obstructive diseases.[6] Solids concentration of the mucus layer, described as the dry weight percentage per given volume, is made up of a mixture of salts, lipids, globular proteins, mucin biopolymers and cellular debris.[1] While sputum samples from healthy individuals contain between 1.5 – 2.5% (w/v) solids concentration, samples from individuals with cystic fibrosis contain between 5 – 9% (w/v) solids concentration.[6] Thus, physiological differences in surface tension and viscoelastic properties of the ALM resulting from muco-obstructive lung diseases can contribute to variability in bioaerosol production.[2,4]

In this study we investigated the effect of peptone water concentration on bacterial aerosol production by characterizing size and quantity of bacterial aerosols. Peptone water solutions were formulated to reflect physiologically relevant airway mucus solids concentrations. A 1.5% (w/v) peptone water solution was used to simulate the solids concentration in healthy ALM, whereas 5% (w/v) and 10% (w/v) peptone water concentrations were used to mirror mucus solids concentrations in moderate and severe cases of muco-obstructive lung disease, respectively.[1]

Experimental Methods and Materials

Media Preparation: Tryptic soy broth was prepared by dissolving 30 g/L tryptic soy broth (BD Bacto™) in deionized water. Tryptic soy agar (TSA) media was prepared by dissolving 30 g/L tryptic soy broth (BD Bacto™) and 15 g/L agar (Fisher BioReagents™) in deionized water. Peptone water solutions were prepared by dissolving 15 g/L, 50 g/L, and 100 g/L of peptone water (Thermo Scientific™ CM0009) in Milli-Q® water. All solutions were sterilized by autoclaving at 121°C for 20 minutes.

Bacterial Culture Preparation: A 3 mL volume of tryptic soy broth was inoculated from a glycerol stock of *Staphylococcus aureus* subsp. *aureus* Rosenbach 6538™ stored at -80°C. Bacteria was cultured for 20 – 24h at 37°C in a shaking incubator set to perform 180 orbital rotations per minute. Appropriate volumes of bacterial culture were added to peptone water solutions to achieve a concentration of $5x10^4$ CFU/mL. Solution concentrations were verified by plating 100 µL of twice diluted sample on TSA plates in triplicates. Plate colony counts were performed after 20 – 24 h of incubation at 37°C.

Nebulization and Size Fractioning of Bacterial Aerosols: Figure 1A depicts the experimental test setup used to generate and size fraction bacterial aerosols. The test setup was constructed based on specifications outlined in the ASTM International standard F2101-19.[7] Polydisperse bacterial aerosols were generated with the Blaustein Atomizer Single-Jet Model (CH Technologies ARGBLM2) operated in atomizer mode using the 10-40 single jet expansion plate (CH Technologies ARGBLM0031). Bacterial solution was dispensed from a 5 mL syringe (BD Luer-Lok™ Tip) at a rate of 200 µl/min controlled using a syringe pump (Fisherbrand™14831200). Air flow through the Blaustein nebulizer was maintained at 1.5 LPM, controlled using an IMI NORGREN pressure gauge and monitored using a TSI flow meter (5300 Series). A downstream vacuum pump was used to draw air through the impactor at a rate of 28.3 LPM and was monitored using an OMEGA flow meter (FMA-A2317). The 6 Stage Viable Impactor (Tisch Environmental TE-10-800) was used to fraction bacterial aerosols into six aerodynamic size ranges: 0.65 – 1.1, 1.1 – 2.1, 2.1 – 3.3, 3.3 – 4.7, 4.7 – 7.0, and > 7.0 µm (Fig. 1A). Glass petri dishes (Corning® 3160-100 Pyrex®) containing 27 mL of TSA were inserted beneath each of the six stages of the impactor to collect aerosols through impaction (Fig. 1B). TSA collection plates were incubated in a static incubator at 37°C for 20 – 24 hours. Bacterial colonies were counted, and the positive-hole correction factor was applied to account for co-incidence error.[8]

Total Particle Counts $= C1 + C2 + C3 + C4 + C5 + C6$

Mean Particle Size (µm) $= \frac{P1*C1+P2*C2+P3*C3+P4*C4+P5*C5+P6*C6}{C1+C2+C3+C4+C5+C6}$

$1 - 6 =$ Impactor Stages; $P =$ Stage cut − off diameter (µm); $C =$ Corrected colony counts as described by Macher et al. 8

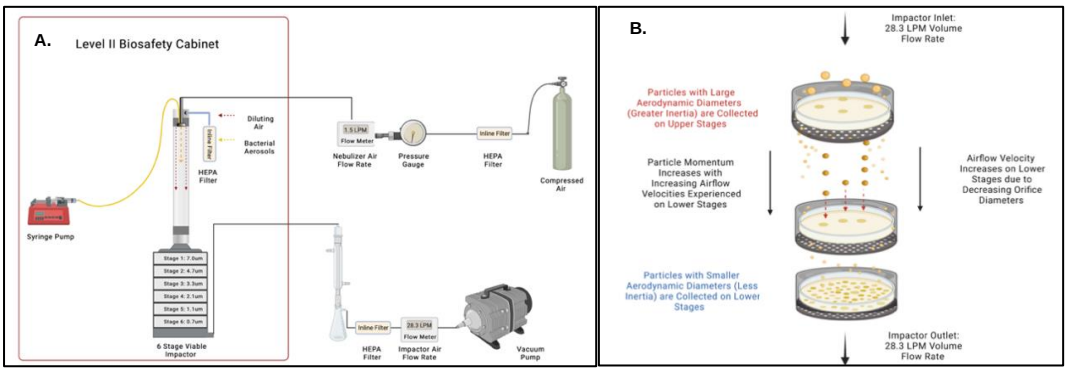

Figure 1 – (A) Aerosol sampling apparatus; (B) Fractioning of bacterial aerosols by aerodynamic size.

Results

The size of bacterial aerosols is based on the cut-off diameter (d_{50}) of the stage on which it was collected. Impactor stages have the following cut-off diameters: 0.65, 1.1, 2., 3.3, 4.7, and 7.0 µm.[8] Stage cut-off diameter (d_{50}) describes a 50% collection efficiency for particles with the specified aerodynamic

diameter.[8] Aerodynamic diameter of a particle is based on its inertial properties, specifically the settling velocity in a fluid medium, such that airborne particles with different sizes, shapes, and densities can be compared.[8] All references to particle size is based on the aerodynamic diameter determined using the six-stage cascade impactor. The mean size of bacterial aerosols is calculated from the fraction of aerosols collected on each of the six stages of the impactor. Mean particle size of bacterial aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) peptone water solutions was found to be 3.23 ± 0.03 µm, 3.72 ± 0.05 µm, and 3.99 ± 0.04 µm, respectively (Fig. 2A). Total bacterial aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) peptone water solutions was found to be 4053 ± 834, 2933 ± 284, and 2467 ± 478, respectively (Fig. 2B).

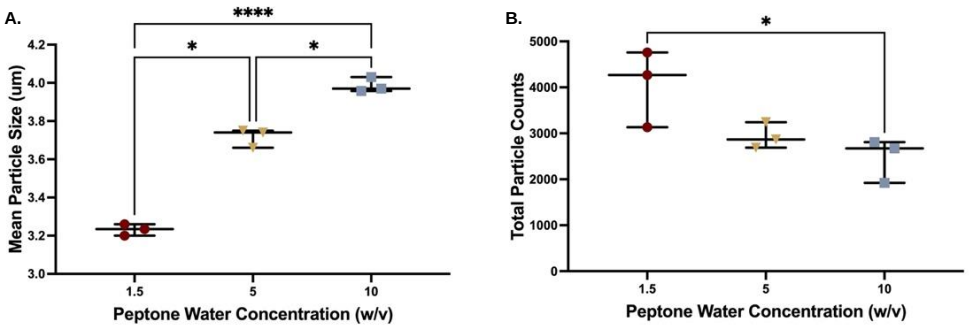

Figure 2 - Effect of peptone water concentration on (A) mean aerodynamic size and (B) total particle counts of bacterial aerosols generated. Results from three replicate trials are presented. Results were analysed by one-way ANOVA followed by Tukey's post-hoc test, *p<0.05, **p< 0.01, ***p<0.001, ****p<0.0001.

Bacterial aerosol fractions collected on each of the six stages of the impactor are shown in Figure 3A. The 7.0 µm cutoff diameter stage collected 8.03%, 13.42% and 17.53% of aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) solutions, respectively (Fig. 3A). Similarly, the 4.7 µm cutoff diameter stage collected 19.03%, 25.06%, and 28.24% of aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) peptone solutions, respectively (Fig. 3A). The 3.3 µm cutoff diameter stage collected 32.98%, 33% and 29.7% of aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) solutions, respectively (Fig. 3A). The fraction of aerosols collected on 4.7 µm and 7.0 µm cutoff diameter stages increased with peptone concentration (Fig. 3A). The opposite trend was observed for stages with 0.65, 1.1, and 2.1 µm cutoff diameters, where the collected fraction decreased as peptone water concentration increased (Fig. 3A). The 2.1 µm cutoff diameter stage collected 24.57%, 19.87%, and 18.09% of aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) solutions, respectively (Fig. 3A). The 1.1 µm cutoff diameter collected 15.12%, 8.61% and 6.43% of aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) solutions, respectively (Fig. 3A). Lastly, the 0.65 µm cutoff diameter stage collected 0.28%, 0.04%, and 0.01% of aerosols generated from 1.5% (w/v), 5% (w/v) and 10% (w/v) solutions, respectively (Fig. 3B).

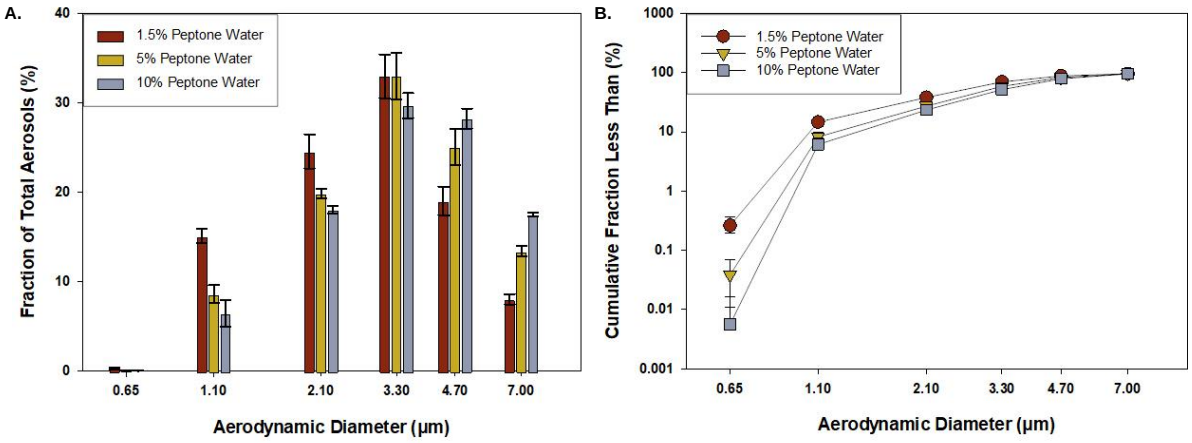

Figure 3 - Effect of peptone water concentration on (A) aerodynamic size distribution and (B) cumulative distribution of bacterial aerosols. Data represented as mean ± SD (n = 3).

Discussion

In this study we intended to answer whether changes in protein and salt concentrations influence bacterial aerosol production and if so, whether these changes alter airborne transmission potential. *S.*

aureus containing aerosols were produced from peptone water solutions formulated to mimic physiologically relevant solids concentrations of the ALM. Significantly fewer bacterial aerosols were produced from 10% (w/v) peptone solutions as compared to 1.5% (w/v) peptone solution (Fig. 2B). Furthermore, the mean size of bacterial aerosols increased significantly with increase in peptone water concentration (Fig. 2A). Thus, changing the concentration of the peptone water solution produced significant changes in the quantity and size of bacterial aerosols. The sizes of bacterial aerosols produced through nebulization of peptone water solutions correlates well with sizes of pathogen containing aerosols identified in clinical studies. Lindsley et al. in a 2009 study used personal cascade samplers to collect airborne particles containing Influenza A RNA exhaled by patients at an urgent care clinic.[9] Approximately 48% of Influenza A RNA was isolated from particles < 4.9 µm in size, while 32% of Influenza A RNA was isolated from particles < 1.7 µm in size.[9] Additionally, in the study by Tsay et al., particle sampling was performed in surgical and medical ICU areas to detect airborne bacterial pathogens. Bacterial pathogens were predominantly isolated from three size fractions of airborne particles: 1.1 – 2.1, 3.3 – 4.7, and > 7 µm.[10] This demonstrates our system can generate pathogen containing aerosols of clinically relevant size ranges.[9–11]

Airborne transmission depends on the quantity as well as the size of infectious aerosols produced.[11,12] Particle size is the most important determinant of particle behaviour in the air and dictates the duration of time a particle remains airborne.[11,12] Larger infectious aerosols will have a shorter lifespan in the air due to faster settling velocities which in turn decreases the risk of airborne transmission.[11] Settling velocities of 7.5, 30 and 119 µm/sec were calculated for 0.5, 1 and 2 µm sized particles, respectively.[12] Additionally, settling velocities of 746 and 2985 µm/sec were calculated for 5 and 10 µm sized particles, respectively.[12] The different rates of settling means that while a 5 µm particle can remain airborne for 22 minutes, a 1 µm particle can remain airborne for over 9 hours before settling 1 meter in still air.[12] A greater percentage of bacterial aerosols generated from 1.5% (w/v) peptone water solution was collected on 0.65, 1.1, and 2.1 µm cutoff diameter stages in comparison to bacterial aerosols generated from 5% (w/v) and 10% (w/v) peptone water solutions (Fig. 3B). The opposite trend was observed for stages with 4.7 and 7.0 µm cutoff diameters which collected larger fractions of bacterial aerosols produced from 5% (w/v) and 10% (w/v) peptone water solutions (Fig. 3A). These results suggest that more of the finer aerosols generated from 1.5% (w/v) peptone water solution can remain airborne for longer which in turn increases the risk of transmission.

Conclusion

In this study, we have demonstrated that changing the concentration of the fluid produces significant changes in both the quantity and size of bacterial aerosols. Specifically, increasing the concentration produced fewer, larger bacterial aerosols, whereas decreasing the concentration produced smaller, more numerous bacterial aerosols. Thus, the transmission potential of bacterial aerosols was effectively altered by changes in solution concentration.

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