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Impact of Breathing Pattern and Nebulization on Expelled Viral Content During Mechanical Ventilation Using an Ex Vivo Porcine Lung System

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BACKGROUND: Little is known about the fate of expelled viral particulates during the aerosolization of inhaled medications during mechanical ventilation. We hypothesized that breathing patterns that generate a greater degree of shear stress and turbulent air flow will produce a greater concentration of exhaled viral RNA with the presence of a nebulizer during mechanical ventilation. METHODS: Eight ex vivo pig lungs were utilized as the physiological model. Each lung was dedicated to a specific breathing pattern that consisted of tidal breathing, respiratory distress, cough, and sneeze. Breath simulations were carried out through a commercial mechanical ventilator. Ninety mL of a bacteriophage stock at a concentration of 10^8 PFU/mL were introduced into the lungs during a 10-min sample collection session. The number of viral particles collected in exhalate was measured using quantitative polymerase chain reaction. The impact of breathing pattern on measured viruses was analyzed through two-way analysis of variance. RESULTS: The interaction effect between nebulization and breath pattern on exhaled viral quantity was not statistically significant $P = .80$, partial $\eta^2 = 0.167$. The analysis of the main effects indicated that the effects of the breathing pattern and nebulization phase were not statistically significant $P = .26$, partial $\eta^2 = 0.519$; $P = .98$, partial $\eta^2 = 0$, respectively. There were no statistically significant differences among the breathing patterns related to measurable viral RNA. Coughing produced the most measurable increase in measured viral quantity during the nebulization phase and non-nebulization phase with a mean exhaled viral quantity (3.5×10^5 ng/μL [95% CI 1.6×10^5–5.5×10^5] and 2.7×10^5 ng/μL [95% CI 7.1×10^3−5.5×10^5], respectively). Tidal breathing with the presence of a nebulizer and respiratory distress without a nebulizer produced the lowest measured viral quantities ($M = 1.1 \times 10^5$ ng/μL [95% CI -1.7×10^5 to 3.9×10^5]; $M = 1.2 \times 10^5$ ng/μL [95% CI -1.6×10^5 to 4.0×10^5]). CONCLUSIONS: In this ex vivo porcine model, the introduction of a nebulizer did not increase the mean viral RNA captured throughout all of the breathing patterns. *Key words: mechanical ventilation; aerosol; nebulization; breath patterns; inhaled medication; animal model.* [Respir Care 0;0(0):1–●. © 0 Daedalus Enterprises]

Introduction

Bioaerosols are an ever-present vector of disease transmission from a natural and human-made etiology. As a causative factor, the attribution of bioaerosols to the development of chronic illness and the transmission of infectious pathogens in aerosol droplets is not a novel concept.[1] Health care workers are at an increased risk of exposure to pathogenic bioaerosols from physiologic propulsion (eg, coughing, sneezing) as well as machine-generated particulates released from mechanical ventilators, aerosol medication administration, and other cardiopulmonary support mechanisms.[2,3] In general, aerosolized medications, and lifesaving measures that generate pathogenic aerosols, are provided to those with chronic lung and vascular disease as well as patients with microbial colonization and viral infections. These instances are not mutually exclusive in the majority of cases.

With the emergence of SARS-CoV-2 and subsequent pandemic, many procedures suspected of generating pathogenic aerosols are suggested to be limited or significantly altered for a more invasive means of cardiopulmonary support (ie, reduction of the instances of noninvasive ventilatory support for invasive mechanical ventilation). This sentiment also applies to the nebulization of medications in the home and health care environment.[4] Whereas there is limited evidence to support a complete omission of various aerosol-generating support mechanisms, it is not advised that the end user alter their treatment plan in cases associated with asthma and COPD.[5,6] The risks associated with discontinuing aerosolized medication do not outweigh the conflicting body of

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evidence associated with the increased transmission risk of SARS-CoV-2 during these procedures.[7]

Droplet nuclei formation through aerosolized medication administration is not restricted to the nebulization device itself, or its exhaust, but also can be attributed to potential induction of cough from the patient or persons in the immediate environment. The role of coughing in virus-laden aerosol dispersion is multifaceted and encompasses a heterogenous body of evidence that seems to support both direct and indirect transmission of virus to individuals and surfaces through a wide range of particle size dispersion.[8] Not all coughs are physiologically the same, and this applies to other forceful and passive exhalation maneuvers. As such, this phenomenon leads to the stratum of super-spreaders that generate higher viral loads per exhalation when compared to what is assumed to be an expected exhaled viral load.[9] It is difficult to apply an average to a variable that is highly dependent on many physiological, environmental, and equipment-related influences; but in a laboratory environment, the standardization and extrapolation of viral signals through different breathing patterns and equipment interactions are a feasible endeavor.

Through the development of this novel, ex vivo, porcine model and a invasive mechanical ventilator, this investigation was designed to illustrate the impact of 4 common breath patterns in patients receiving inhaled aerosolized medication and the subsequent impact on viral RNA measurement. We hypothesized that breathing patterns that generate a greater degree of shear stress and turbulent air flow produce a greater concentration of exhaled viral RNA with the presence of a nebulizer during mechanical ventilation. This investigation aimed to evaluate the effect of nebulized medication delivery on exhaled viral quantity for mechanically ventilated patients.

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QUICK LOOK

Current knowledge

Aerosol-generating procedures are highlighted as potential sources of exposure to viral pathogens for health care professionals during the COVID-19 pandemic. The lack of consensus related to inhaled medication delivery, as it relates to viral exposure, contributes to alterations of aerosolized medication delivery strategies and potential adverse effects for patients. The negative impact of potentially unnecessary alterations in inhaled medication delivery may cause secondary impacts on medication adherence, dosing, and costs.

What this paper contributes to our knowledge

We demonstrated that in this ex vivo porcine model, the addition of a nebulizer to provide aerosol therapy did not increase the amount of viral RNA found in the environment. In this model using mechanical ventilation, breathing pattern did not alter the environmental contamination.

Methods

Study Objectives

The primary objective of this study was to determine the impact of nebulization on the presence of viral RNA during a 10-min aerosolized medication administration session in the immediate environment.

This experiment consisted of two phases of lung inflation trials. The first phase of this investigation explored the feasibility of the developed porcine model to serve as a control for the second phase of the project. Phase 2 introduced a nebulizer into the breathing circuit to determine the impact of the procedure of bacteriophage expression in exhaled breath. A total of 8 porcine lungs were used. All lung material was purchased from a retail processing facility and was sourced from animals slaughtered for meat production. This investigation's protocol was reviewed and approved by the University of North Carolina at Charlotte Institutional Animal Care and Use Committee prior to conducting experiments.

Porcine Lung Model

The porcine lung has an anatomical structure that is sufficient to be utilized as a surrogate for the human lung; the porcine lung has a similar lobar structure (ie, multiple lobes), representation of the generations of the human tracheobronchial tree, and histological composition.[10,11] These

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anatomical similarities are the cornerstone of translational clinical research as well as a tool to reduce exposure to pathogens, radiation, and other harmful agents/procedures in human subjects. Mechanical ventilation of the ex vivo porcine lung is physically feasible when an airway is introduced into the trachea and sealed to create a closed circuit in series with a pump (mechanical ventilator).[12-14] Over the past decade, there have been multiple investigations regarding the ex vivo perfusion of lungs to provide insight on donor lung preservation and management to improve organ availability for those in need of lung transplantation; the ex vivo ventilation of the pig lungs in these lung perfusion studies has been conducted successfully for both volume and pressure control modes of mechanical ventilation.[15]

Propagation of MS2 Bacteriophage

Bacteriophage MS2 was chosen as a viable viral RNA tracer for this investigation due to the relative lack of immune stimulation, biological composition/structure, and its ability to be aerosolized and subsequently quantified upon exhalation.[16,17]

The bacteriophage host *Escherichia coli* strain C3000 (ATCC 15597) was cultured in flasks containing ATCC medium 271 under agitation at 37°C. Once the OD_{600nm} reached 0.1 in an actively growing *E. coli* culture, 100 µL MS2 bacteriophage (ATCC 15597-B1) was added and allowed to incubate overnight at 37°C. The phage culture was then centrifuged at 4,000 g for 10 min, and the cell lysate supernatant was filtered in a 0.2-µm polyethersulfone sterile filter and stored at 4°C. Plaque assays were performed to measure phage amplification, which was determined to be approximately 10^{11} PFU per mL. The phage buffer was composed of 69 mL Tris-HCl 10 mM, pH 7.4, NaCl 100 mM, MgSO410 mM, and 1 mL phage lysate with an estimated 10^{10} PFU per mL.[18]

Breath Patterns

The 4 different breathing patterns consisted of tidal breathing, respiratory distress, cough, and sneeze. Tidal breathing and respiratory distress were characterized by a delivered tidal volume approximately 500 mL (8 mL/kg of average-sized adult male); breathing frequencies were 15 and 30 breaths/min, respectively. Coughing and sneezing were reproduced by a series of tidal breaths with an abrupt increase in extrapulmonary pressure to generate an expiratory flow 90–800 L/min at the end of inspiration. The expiratory phase of the cough was limited to 0.5 s; the sneeze was limited to 0.75 s for its expiratory phase.[19] This pattern was repeated 5 times during the 10-min experiment trial for both the cough and sneeze maneuvers.

Lung Inflation and Aerosol Collection

The porcine model was developed from lungs extracted from farmed Yorkshire hybrid pigs with an average weight of 118 kg and 6 months of age (Animal Biotech Industries, Doylestown, Pennsylvania). The lungs were provided with the pleural membrane, trachea, and larynx attached. An inflation protocol was developed and initiated prior to beginning the breathing trials, which involved inflating the lungs in pressure-controlled mechanical ventilation with an inspiratory pressure 20 cm H_2O, PEEP 20 cm H_2O for 10 min, and breathing frequency 15 breaths/min. A size 7.5 endotracheal tube was utilized as the conduit for lung inflation. During the inflation procedure, the lungs were assessed for air leaks and repaired if necessary (> 100 mL inhaled tidal volume to exhaled tidal volume difference). All tissues used in the experiments were not treated with fixation agents prior to inflation. Ventilation was provided through the mechanical ventilator (Hamilton-G5, Hamilton Medical, Bonaduz, Switzerland); volume control ventilation was the primary mode of ventilation during the experimental trials.

Prior to inflation, the lungs were placed in the supine position at 30° relative to the bench space with a support ramp. The lungs were subjected to 4 different breathing patterns to generate different aerosol profiles during a 10-min breathing session, with a new lung utilized for each breathing trial. Airway temperature and humidity were provided and measured by MR850 humidification system (Fisher & Paykel Healthcare, Auckland, New Zealand) between 35°C–40°C. After completing the lung inflation protocol, 90 mL MS2 stock (10^8 PFU/mL) was instilled into the airways via the endotracheal tube. A schematic and image of the model can be seen in Figures 1 and 2.

During the breathing trials, exhaled aerosol was collected on a polypropylene bacterial and viral filter (Hudson RCI, Teleflex, Wayne, Pennsylvania) in the expiratory limb of the ventilator circuit. Immediately after ventilation sessions the 1-inch diameter filter that was exposed to exhaled aerosol was cut and deposited in a conical tube with 5 mL 1X phosphate-buffered saline and placed on a shaker at maximum velocity for 20 minutes at room temperature to elute MS2 bacteriophage from the filters.

Phase Two

Upon the completion of Phase 1 of the experiment, a modified nebulizer (LC Sprint, PARI, Starnberg, Germany) was introduced into the breathing circuit to provide the model with aerosolized albuterol sulfate (2.5 mg/3 mL) (Fig. 3). Phase 2 followed the same inflation, ventilation, and sample collection protocols that were outlined in the previous section.

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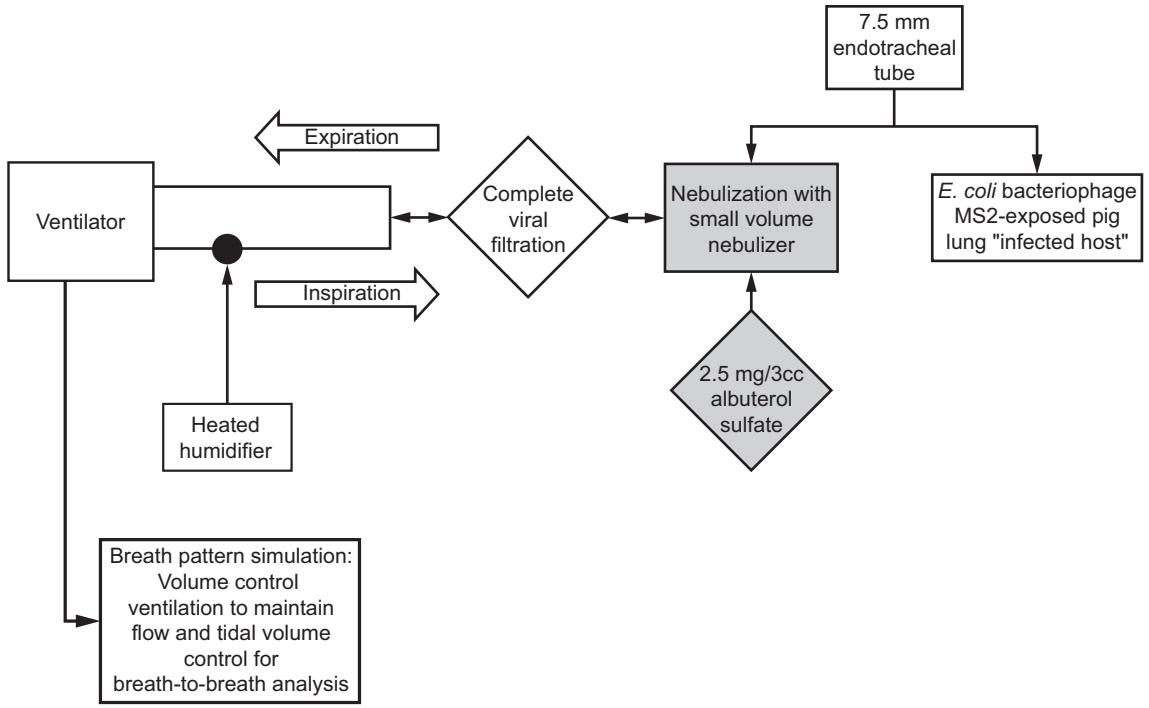

Fig. 1. Schematic of the elements of the model developed for this investigation for both phases of the experiment. Filtration of air, as shown in the diagram, occurs proximal to the endotracheal tube and nebulizer for phases 1 and 2, respectively. Events shaded gray were initiated in Phase 2 of the study.

RNA Extraction and cDNA Synthesis

RNA extraction was completed using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Modifications to the manufacturer's protocol were done as suggested by a previous study, which noted a decrease in MS2 viral recovery.[18] Complementary DNA (cDNA) synthesis was completed with Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific, Waltham, Massachusetts) per manufacturer's protocols in a 20-μL solution composed of 4 μL of 5× Reaction Mix, 2 μL of Maxima Enzyme Mix, and 5 μg of extracted template RNA.

qPCR Detection of MS2

For the detection and quantification of the MS2 bacteriophage, previously established protocols were utilized.[18] All quantitative polymerase chain reaction (qPCR) reactions were performed in triplicate with the Thermo Fisher QuantStudio 3 Real-Time PCR System. The 13-μL qPCR reaction mix was composed of 7 μL of 2× TaqMan Fast Advanced Master Mix (Applied Biosystems, Waltham, Massachusetts), 1 μM of forward primer (5′-GTCCATACCTTAGATGCG TTAGC-3′), 1 μM of reverse primer (5′-CCGTTAGCGAAGTTGCTTGG-3′), 150 μM of a dual-labeled probe (5′-/56-FAM/ACGTCGCCAGTTCCGCCATTGTCG/3BH), and 2 μL of the cDNA template. The amplification cycle was as follows: 94°C for 3 min followed by 35 cycles of 94°C for 15 s and 60°C for 1 min with a plate read after an elongation step.

Positive MS2 Controls

In order to isolate the MS2 amplicon, PCR was performed utilizing the same primers used in the qPCR protocol with similar conditions as previously described. Gel electrophoresis was used to separate the amplicons. The amplicon was then extracted from the gel using the QIAquick Gel Extraction Kit (QIAGEN). After this process, the amplicon was then quantified, and serial dilutions were performed and used to generate a standard curve.

Statistical Analysis

Statistical analysis was conducted through IBM SPSS Statistics for Mac, Version 27.0 (IBM, Armonk, New York). The main effects of this investigation and the comparison of group means were analyzed through factorial analysis of variance (ANOVA). In addition to the measurement of the main effects through factorial ANOVA, pairwise analysis of the simple effects was conducted to determine interactions between the individual variables. A priori power analysis (G*Power) concerning statistical outputs yielded a total of 25 sample measurements for a

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Fig. 2. Physical setup of the porcine model. The lungs are placed on a ramp to situate the lungs 30° relative to the bench surface. The connection between the ventilator circuit and the endotracheal tube is shown with the viral filter proximal to the endotracheal tube and distal to the Y-piece of the circuit connection. The background demonstrates the location of the mechanical ventilator used for the series of experiments.

desired alpha 0.05 and power 0.80 (3 degrees of freedom). All *P* values are given for 2-tailed tests.

Results

A two-way ANOVA was conducted to examine the effects of the presence of a nebulizer and breathing pattern on measured viral quantities. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot; normality was assessed using Shapiro-Wilk normality test for each cell of the design, and homogeneity of variances was assessed by Levene test. There were no outliers; residuals were normally distributed, and there was homogeneity of variances ($P = .05$).

The interaction effect between nebulization and breath pattern on measured viral quantity was not statistically significant $P = .80$, partial $\eta^2 = 0.167$. Therefore, an analysis of the main effects for breath pattern and nebulization phase was performed. The analysis of the main effects indicated that the effects of the breath pattern and nebulization phase were not statistically significant $P = .26$, partial $\eta^2 = 0.519$; and $P = .98$, partial $\eta^2 = 0$, respectively. All pairwise comparisons were run, reported 95% CI and *P* values are Bonferroni adjusted. The unweighted marginal means of measured viral concentrations (ng/μL) for the breath patterns of tidal breathing, respiratory distress, coughing, and sneezing were 1.21×10^5 ng/μL (SE = 7.7×10^4), 1.3×10^5 ng/μL (SE = 6.3×10^4), 3.1×10^5 ng/μL (*SE* = 6.6×10^4), and 1.6×10^5 ng/μL (*SE* = 6.3×10^4), respectively.

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Fig. 3. The bottom black arrow indicates the location of the nebulizer in the ventilator circuit during Phase 2 of the experiment. The top red arrow in this image is the location of the viral filter to collect the simulated breath from the model.

The unweighted marginal means of exhaled viral quantity for the nebulization phases of the presence of a nebulizer and the absence of a nebulizer were 1.8×10^5 ng/μL ($SE = 4.6 \times 10^4$) and 1.8×10^5 ng/μL ($SE = 5.0 \times 10^4$), respectively.

All pairwise comparisons among the breath patterns, as well as between nebulizer phases, did not reveal statistically significant mean differences in measured viral quantity (Fig. 4). Of note, the presence of a nebulizer was associated with the reduction of mean measured viral quantity (1.8×10^3 ng/μL [95% CI -1.7×10^5 to 1.7×10^5], $P = .98$) but with minimal clinical relevance. Whereas there were no statistically significant differences among the breathing patterns related to measurable viral RNA, coughing produced the most measurable increase in captured viral quantity during the nebulization phase and non-nebulization phase with a mean measured viral quantity (3.5×10^5 ng/μL [95% CI 1.6×10^5–5.5×10^5] and 2.7×10^5 ng/μL [95% CI 7.1×10^3–5.5×10^5], respectively). Tidal breathing with the presence of a nebulizer and respiratory distress without a nebulizer produced the lowest exhaled viral quantities ($M = 1.1 \times 10^5$ ng/μL [95% CI -1.7×10^5 to 3.9×10^5]; and $M = 1.2 \times 10^5$ ng/μL [95% CI -1.6×10^5 to 4.0×10^5]).

Descriptive statistics associated with captured viral quantity (ng/μL) in each breathing pattern and nebulizer phase are expressed by the means and SD in Table 1.

Discussion

In line with current reports of the contribution of aerosol-generating procedures and the distribution of viral particulates into the immediate environment, this investigation demonstrated that the introduction of a nebulizer in the breathing circuit of this porcine lung model did not increase the viral deposition on the filter during the expiratory phase of the breath during simulated coughing.[20,21] In conjunction with this overarching finding, it was found that different breathing patterns produced varying levels of measured viral particulates that can be stratified into coughing—generating the most measured viral particulates—and tidal breathing, with a nebulizer in line producing the least (Table 1). This finding is not necessarily surprising from the perspective that the physiological mechanism of a cough is to expel pathogens from the tracheobronchial tree and generate a high degree of shear stress in the airways.

There are many considerations when assessing the etiology of viral transmission during the exhalation phase of the

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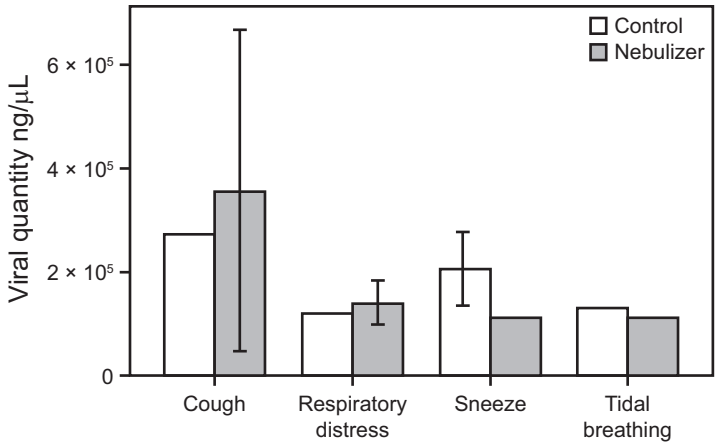

Fig. 4. Viral quantity as measured by quantitative polymerase chain reaction during each of the 4 different breathing patterns. The samples collected from the model were run in triplicate to calculate desired parameters and standard error bars shown in the graph (of note, samples that only had one valid measurement do not have an associated standard error [SE]). Measured viral quantity (ng/μL) was not significantly different among the 4 breathing patterns with and without the nebulizer present ($P > .05$). Error bars denote ± 2 SE.

Table 1. Exhaled Viral Quantity by Breath Pattern and Phase

Dependent Variable: Quantity, ng/uL				
Breath Pattern	Phase	Mean	SD	No.
Cough	Nebulizer	356,475.49	92,233.72	2
	No nebulizer	271,887.25	*	1
	Total	328,279.41	92,233.72	3
Respiratory Distress	Nebulizer	140,411.21	37,268.15	3
	No nebulizer	120,551.18	*	1
	Total	135,446.20	32,008.57	4
Sneeze	Nebulizer	112,330.86	*	1
	No nebulizer	205,076.55	62,466.70	3
	Total	181,640.13	69,270.89	4
Tidal Breathing	Nebulizer	111,099.83	*	1
	No nebulizer	129,955.27	*	1
	Total	121,027.55	12,625.69	2
Total	Nebulizer	193,945.04	92,233.72	7
	No nebulizer	189,603.89	68,730.15	6
	Total	191,941.43	92,233.72	13

* SD could not be calculated for quantitative polymerase chain reaction measurements that produced one value.

breath. In the context of this experiment, a hypothesized source of viral contamination, and thus spread, is the nebulizer itself. This assumption is an extrapolation of information related to nebulization and microbial contaminants found in nebulizers used by patients with cystic fibrosis and the stability of SARS-CoV-2 on plastics.[22,23] In theory, this is a proposed avenue of SARS-CoV-2 transmission that has yet to be fully elucidated through scientific exploration. For each breath pattern, a new set of lungs and equipment was utilized to reduce the compounding effects, and mismeasurement, secondary to viral adhesion to various parts of the ventilator and associated equipment; therefore, we cannot safely assume that the nebulizer has functioned as a viral reservoir for the bacteriophage in this model. This investigation did demonstrate mixed, although not statistically significant, results when a nebulizer was introduced to the breathing circuit during different breathing patterns. In the context of mechanical ventilation and medication nebulization, this investigation provides the perspective of potential viral RNA exposure during accidental, or purposeful, ventilator circuit disconnections that are a common occurrence in critical-care environments.

Nebulization of medication is considered an aerosol-generating procedure and, as such, has inspired the use of alternative aerosolized medication delivery regardless of the lack of a strong evidence base.[24] Nebulization of inhaled medications is of concern because of aerosol dispersion versus generation; in essence, it is assumed that the nebulizer is acting as a distribution amplifier during the breathing patterns addressed in this investigation.[25,26]

Assumptions regarding nebulization and the enhancement of viral transmission, especially in the context of SARS-CoV-2, have created a set of theories that has translated into the change in practice in many health care facilities without consideration for the risks that these changes may have on patient care. For example, an alternative for the nebulization of aerosolized medications is the use of a pressurized metered-dose inhaler (pMDI). The pMDI requires coordination between the actuation of the device in coordination with the patient's breath. This is unachievable for patients with disorders such as neuromuscular disease, developmental issues, or simply lack the ability to use the pMDI effectively. During mechanical ventilation, the reliance on the practitioner to provide pMDI actuation, and timing with the breath, may exhibit the same issues as stated with self-administration. Finally, the precipitation of cough from inhaled medications is considered a primary source of viral transmission from the user to the

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environment; changing medication delivery devices does not mitigate this risk.[27]

Airway anatomy and physiology are major determinants in exhaled aerosol characteristics and viral transmission. This investigation focused primarily on the impact of the lower airway of the porcine lung in the generation of viral RNA through simulated tidal breathing, respiratory distress, coughing, and sneezing. A primary consideration is the standardization of these breathing patterns in the context of the human patient. For example, not every cough is the same, nor does it generate the same shear stress and expiratory force for every maneuver. There is also distinct variation in the structures of the upper airway from subject to subject. Furthermore, the structures of the upper airway (more specifically the oral cavity) generate large particle sizes that settle out of suspension more quickly than particles that are formed in the lower airways.[28] In this context, the isolation of the lower airway from the upper airway (ie, oropharynx, nasopharynx) was an appropriate method to standardize the particle sizes of interest that are commonly generated by the structures of the lower airway. Although bypassing the structures of the upper airways presents as one of the limitations of this investigation, the modeling of the human tracheobronchial tree through the development of a porcine model provides an approximation of the characteristics of exhaled gasses and, as such, viral transmission with a bacteriophage signal.[29]

Whereas the findings of this investigation are potentially useful in the decision-making processes related to the nebulization of aerosolized medications, there are significant limitations of this investigation. The translation from an ex vivo porcine lung model to the human is not well defined in this specific capacity. Furthermore, the incorporation of a mechanical ventilator, ventilator circuit, and associated potential alteration on viral deposition in aerosol needs to be considered, although the study design employed strategies to attempt to mitigate these effects.

The validation of the model developed for this investigation was based on the initial measurements provided during Phase 1 of the experiment; a robust description of the methods was made available to promote the reproduction of this experiment for further validation of this novel porcine model. The ability to quantify viral particles in exhalate via qPCR analysis provided the confidence to move forward with the second phase of this work and dissemination of findings. This investigation incorporated a proof-of-concept approach to determine clinically relevant outcomes related to aerosol-generating procedures, pulmonary physiology, and viral infection in the context of mechanical ventilation and the administration of aerosolized medications; further translational investigations using similar techniques need to be conducted to improve the validity of our specific approach. Finally, the infectivity of the exhaled viral RNA was not analyzed. Despite these limitations, we can conclude that the use of a nebulizer to deliver aerosolized medication does not necessarily increase the amount of exhaled viral RNA in this investigation.

Conclusions

This investigation was developed to compare the impact of nebulization during four common breathing patterns found in a clinical environment on exhaled viral RNA expression from the proximal airway during mechanical ventilation. Our findings suggest that there is not a statistically significant difference, or increase, in the amount of exhaled viral RNA during simulated tidal breathing, respiratory distress, coughing, and sneezing in this model with or without a nebulizer present in the circuit. Further study is needed to determine if the use of a nebulizer demonstrates an increase in infectious viral concentrations in exhaled breath during aerosolized medication administration.

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