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RESEARCH ARTICLE

Contamination of Aerosol with Pseudomonas Aeruginosa Introduced via Mouthpiece in Different Nebulizer Designs

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ABSTRACT

Background: Nebulizers have been associated with bacterial and viral contamination likely from drooling or expulson of oral secretions into the nebulizer mouthpiece. We hypothesized that simulated "drooling" could result in contamination of the nebulizer medication resulting in aerosolization of potential pathogens.

Method: We evaluated four nebulizers: Continuous jet nebulizer (CJN: MistyMax, Allegiance, USA), breath enhanced (BEN:LC Sprint; Pari, Germany), breath actuated (BAN; AeroEclipse Monaghan/Trudell, Canada) and vibrating mesh nebulizer (VMN; Aerogen with Ultra, Aerogen Ltd, Galway, Ireland) operated per manufacturer recommendations with 3 mL of NSS. Pseudomonas aeruginosa broth (2 mL) was pipetted into the mouthpiece of each nebulizer in an upright postion simulating a patient drooling into the device. Aerosol was produced for 30-60 seconds and collected on Triptic Soy Agar (TSA) plate, prior, immeadiately, and 4-5 hours post instillation. Colony counts were done post incubation (3-5 days).

Results: P. aeruginosa colony counts prior, immediately, and four hours after instillation; BAN (0, 110, and 122 CFU/m); and BEN (0, Too Numerous To Count (TNC), and TNC), VMN: (0, 0, and 0 CFU/mL) and CJN (0, 0, and 0 CFU/mL), respectively.

Conclusions: Nebulizer type and design influence impact of pathogen containing fluids passing through the mouthpiece contaminating the aerosol generated.

Keywords: nebulizer; contamination; infection prevention; aerosol; jet nebulizer; vibrating mesh nebulizer

Introduction

Nebulizers have historically been implicated as a potential risk for contamination for both patients and healthcare practitioners (HCP). Epidemics such as severe acute respiratory syndrome (SARS) coronavirus and the more recent Covid-19 infection caused by SARS CoV-2 raise concerns around potential contamination of patients, caregivers and personnel in the immediate vicinity of nebulized medication treatments. These concerns become more exaggerated during active epidemics and pandemics. Tran et al. suggested that aerosol generating procedures (AGP) were associated with increased risk of transmission of SARS to HCPs or were a risk factor for transmission.[1] They identified intubation, tracheotomy, non-invasive ventilation (NIV), and bagging before intubation as procedures that resulted in a high risk of transmission of infection.[1] In order to reduce potential risk of cross-contamination organizations such as the Centers for Disease Control (CDC) and the World Health Organization (WHO) have developed evidence based guidelines for care of patients with virulent respiratory infections which includes personal protective equipment (PPE) and isolation of patients with contagious diseases in single patient rooms whenever possible.[2] Isolation should include airborne, droplet and contact transmission.[3]

Medical nebulizers were identified in initial lists of AGPs. This would suggest that this therapy would increase the production of bioaerosols which contain pathogens such as viruses, or bacteria and which originate from a living organism.[4] In contrast to procedures which may stimulate coughing and generation of bioaerosols, medical aerosols contain no pathogens unless devices or medications are contaminated by the the health care provider or by retrograde contamination from the patient. Airborne transmission of bioaerosols including droplets and finer aerosols are generated with a sneeze, cough, laugh, talking and quiet breathing from an infected patient. The dispersion of those aerosols may occur with oxygen therapy, noninvasive ventilation, bag mask ventilation and mechanical ventilators.

Concerns have been raised that patient generated bioaerosols, droplets and patient secretions have potential for contamination of nebulizers. For example, drooling is not uncommon during aerosol administration via mouthpiece, and if patient saliva containing pathogens enter into a nebulizer reservoir the resulting aerosol would be contaminated. Jet nebulizers (JN) have historically been linked to the potential for contamination and generation of bioaerosols due to the nature of their design (Craven et al 1984).[5] The medication reservoir of a JN is where aerosol is produced and positioned below the patient interface; mouthpiece, mask and/or in-line in circuits. This creates the potential for patient secretions, and/or condensate and rainout, to flow into the medication reservoir and be aerosolized. In contrast, the mouthpiece and mask application with vibrating mesh nebulizers (VMN) are separated by a valved-holding chamber that is not in communication with the reservoir cup that contains the medication and aperture plate that produces aerosol. During mechanical ventilation, VMNs used with in line applications are positioned above patient circuits (typically on the dry side of the humidifier) with no potential for rainout and our secretions to flow from the circuit into the nebulizer reservoir.

In this study, we focused on aerosol delivery with a mouthpiece handheld application and attempted to simulate the effect of contaminated saliva introduced through the mouthpiece into the device in an in vitro model with adult simulated breathing patterns. We theorized that aerosol device design would have an impact on the potential for contaminated aerosol production in this scenario. The aim of the study was to determine whether simulated "drooling" during aerosol delivery could result in contamination of nebulizer medication and reservoirs resulting in aerosolization of pathogens.

Materials and Methods

An in vitro study was designed to compare the effect of instilled bacterial broth introduced through the mouthpiece on aerosol emitted from a nebulizer. A broth of solution with a known amount of pathogens (10^9 CFU Pseudomonas aeruginosa), was introduced through the mouthpiece of four nebulizers of different designs to determine whether the medical aerosol subsequently produced would be contaminated.

Nebulizers

An independent lab, Pacific Bio Labs (Hercules. CA) was contracted to evaluate four nebulizers: A continuous jet nebulizer (CJN: MistyMax, Allegiance, USA, Yorba Linda, CA, USA) breath enhanced jet nebulizer (BEN:LC Sprint; Pari,

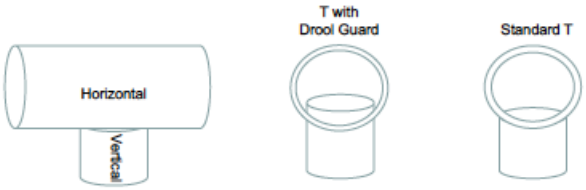
Germany); breath actuated jet nebulizer (BAN; Aeroeclipse, Monaghan/Trudell, Canada); and a vibrating mesh nebulizer with valved chamber (VMN; Aerogen Solo with Ultra, Aerogen Ltd., Galway, Ireland). (Figure 1) The CJN nebulizer consisted of a drool guard formed by extension of the vertical limb into the horizontal limb. This allows liquid to pass through the horizontal limb without drawing into the vertical limb (into the nebulizer). (Figure 2)

Each nebulizer was set up and operated according to manufacturer recommendations with a dose of 3 mL of normal saline placed in the reservoir of each.

Figure 1

Note: (Top left to right) Components for nebulizer testing Monaghan Aeroeclipse (BAN), Pari Sprint (BEN), Allegiance with T-piece with drool guard (CJN) and Aerogen Solo with Ultra (VMN).

Figure 2: *Image of T-pieces*



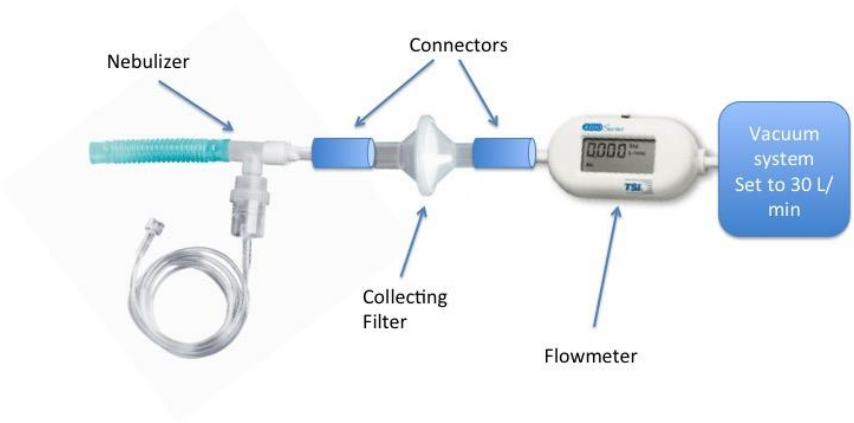
Note: (From left to right) Side view of a T-piece, cross-sectional view of T-piece with drool guard (CJN) and cross-sectional view of a standard T-piece.

Procedures

The test microorganism (Pseudomonas aeruginosa) was prepared according to Pacific Bio Labs (PBL) standard operating procedure (SOP) resulting level was not higher than 10^9 Colony Forming Units (CFL) per mL A suspension of Pseudomonas aeruginosa was diluted with 0.9% sterile saline to a resulting level of > 10^3 colony forming units (CFU) per mL A 2 mL aliquot of broth was pipetted into the mouthpiece of each nebulizer with mouthpiece 10 to 20° angle superior to the nebulizer/adapter, to simulate a patient drooling into the mouthpiece during aerosol administration and allowing the broth to freely flow through the mouthpiece.

A collecting filter with housing and adapters was placed between the mouthpiece and a vacuum system set to provide a continuous draw of 30 L/min as determined with flow sensor (TSI). (Figure 3) Samples were taken prior to instillation (negative control), immediately post instillation of broth into mouthpiece, and again at 4-5 hours after initial contamination.

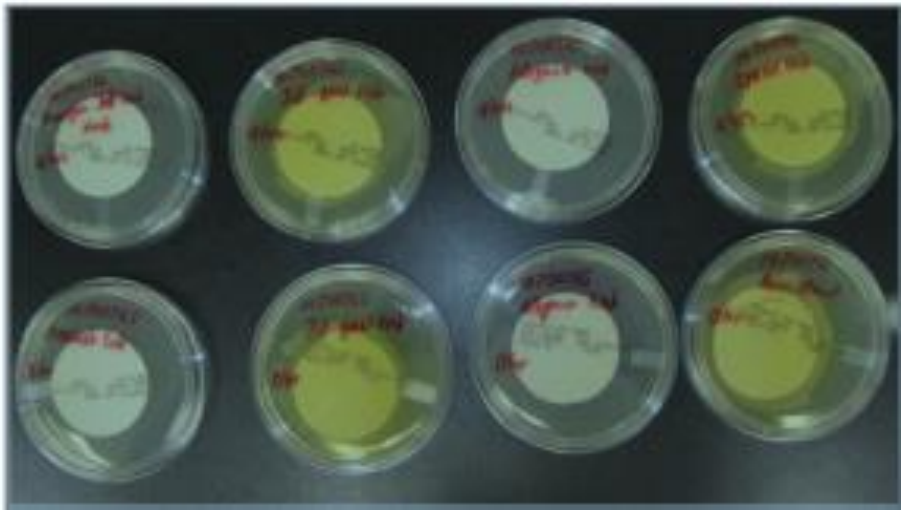
Figure 3: *Schematic of Set Up*



Note: Schematic of basic set up for experiment which consisted of nebulizer with a collecting filter, flow meter and vaccum system.

Aerosol produced continuously with each nebulizer was collected on a filter for 60 seconds.After each run the filter was removed from the housing and placed on a Triptic Soy Agar (TSA) plate and incubated for 3-5 days at 30-35°C followed by colony counts for each plate. At the conclusion of each incubation period, the plates were removed from the incubator and the numbers of colonies on each plate counted. (Figure 4)

Figure 4: *Image of TSA Plates*



Note: Image of TSA plates for all nebulizers at 0 hours and 4 hours post broth instillation.

Results

The actual suspension level for P. aeruginosa was 5.8 x 10^8 CFU/mL. Colony counts from each filter/plate from each of the four nebulizers at the three sampling points: 1) prior to instillation of inoculum; 2) immediately after inocculaton; and 3) four hours after inocculation are presented in Table 1.

Table 1: *Results of Colony Counts at Three Points in Time with Four Nebulizers Tested*

Sample ID	Results		
	Negative Control	0 Hours After Contamination	4 Hours After Contamination
VMN (Aerogen)	0	0	0
CJN (Misty)	0	0	0
BAN (AeroEclipse)	0	110	122
BEN (LC Spirit)	0	TNTC	TNTC

Note: TNTC = Too Numerous to Count

Discussion

This is the first study comparing nebulizer designs and their potential for contamination from contaminated secretions (patient saliva simulated using Psuedamonos broth) introduced through the nebulizer mouthpiece. In contrast to the contamination pattern demonstrated with the BEN and BAN, the CJN with T-piece drool guard and VMN showed no contamination in the emitted aerosol.

Our initial expectation was that all of the jet nebulizers would be contaminated by the bacterial broth due to the position of the open reservoir below the t-piece. However, the lack of contamination with the CJN appears to be based on a design feature of the T-piece with a drool guard in the form of a raised rim around the internal opening surrounding the outlet to the continuous jet nebulizer (Figure 2), which appears to act as a barrier or a "drool guard" diverting the bacterial broth from entering the nebulizer. In our experiment, this feature was sufficient to act as a physical barrier to the 2 mL of broth instilled into the mouthpiece draining into the nebulizer, resulting in no contamination of the emitted aerosol. This design feature could be introduced in the T-piece used with other nebulizers to reduce risk of drool or condensate entering the nebulizer reservoir.

The built-in drool guard in the T-piece of the CJN prevented solution from entering and contaminating the medication reservoir. It should be noted that this feature of the T-piece was not described in the product label, and that T-piece design is no longer used in current version of the CJN.

Despite the valves in the mouthpieces of both BEN and BAN, varying amounts of bacterial broth were able to pass through the mouthpiece and enter the medication reservoir. This suggests that the mouthpiece and T-piece designs pose a potential infection risk for patients and healthcare practiontioners (HCPs).

In contrast, the VMN design has a closed reservoir placed above the mesh which acts as a physical barrier to gas and liquid entering the nebulizer medication reservoir. In this case, the mouthpiece used with the closed reservoir VMN is a component of a valved chamber further isolating the VMN from secretions or condensate contacting external surfaces of the mesh. As the nebulizer is attached at the lateral wall of the chamber, contaminated condensate that settles in the chamber does not have direct contact with aerosol generator, thereby greatly reducing the risk of contamination of either the mesh or the medication reservoir beyond. This is the first study to confirm that contaminated fluid entering the mouthpiece does not contaminate the mesh nebulizer. Nebulizer designs that minimize the risk of potential contamination with pathogens by patients should be considered in the selection of aerosol delivery devices.[6]

It is important to make the distinction between medical aerosols which do not contain pathogens, and bioaerosols which are generated by patients and possibly contain bacteria or viruses. Medical aerosols which start with sterile solutions in clean nebulizers therefore pose no added infection risk unless they are contaminated by the HCP handing the drug and device, and/or with secretions or droplets from an infected patients.[6] Proper aspetic

technique should be used to prevent contamination of the nebulizer and medication reservoir by the HCP administering the aerosol treatment. Washing hands and using clean gloves while handling and loading the nebulizer with medications are critical. If the gloves become soiled by contact with patient or contaminated surfaces prior to handling and loading the medication into the nebulizer they should be removed, followed by handwashing and replacement with a pair of clean gloves prior to loading the medication. This will help limit risk of inadvertent contamination of the medication and the nebulizer reservoir.

Reducing the risk of crosscontamination is an important factor to consider with selection of aerosol delivery devices. Practices such as use of multidose vials of medication have been implicated in crosscontamination between patients.[7] This has been attributed to contamination of the medication dropper used with a multidose vial, whether through contact with contaminated gloves or hand, or surface of medication reservoir during the medication loading process. If a nebulizer reservoir is contaminated with pathogens from patient derived secretions then use of multidose vials, lack of proper hygiene and poor aspetic technique between patients may lead to crosscontamination between patients potentially resulting in a hospital acquired infection.[7] These concerns suggest that large volume multi-dose bottles of medication for aerosol administration should not be used between patients, unless doses are drawn up aseptically by a pharmacist or clinician not at the bedside, Although we did not study crosscontamination in our study, it makes sense that use of a disposable single patient use device with a physical barrier between the patient interface and closed medication reservoir could be considered an additional safety measure to prevent crosscontamination. Single patient use disposable nebulizer designs with physical barriers to patient derived secretions and a closed medication reservoir are preferred.

Surveillance cultures of nebulizers have historically been done by swabbing the surfaces of suspected contaminated devices and allowing them to culture on a medium. These methods are prone to swab contamination on the sides or walls of the nebulizer, and possible contamination of the reservoir and medication, especially with serial measurements of the same nebulizer.[6] Although, swabs can be informative, we felt that the culture of collected aerosol emitted from each nebulizer would be more relevant to the clinical setting. In addition, this method required minimal manipulation of the nebulizer between samplings thereby reducing potential risk of sample contamination from the researcher.

These findings lead us to believe that current recommendations for infection prevention with JN may not be sufficient in protecting patients from potential infection associated with some nebulizer designs. Current Infection Prevention and Control (IPC) recommendations for nebulizer care in the home and in the hospital identify inconsistencies in ICP guidelines, lack of standard of practice by institutions and respiratory therapists, and manufacturer instructions are not always in line with either recommendations or current practice.[7] Based on the above O'Mally recommends that jet nebulizers should be replaced every 24 hours.[7] The results of this study suggest that jet nebulizers can be contaminated during a single aerosol treatment. The risk of infectious contamination of medical aerosols, is that they may spread pathogenic agents throughout the lung, and possibly disperse them into the surrounding environment. Based on our results even CDC recommendations that jet nebulizers be changed, rinsed, air dried, washed or sterilized between treatments[8] would not protect from the potential translocation of pathogens from upper airway secretions to deep lung distribution. Replacing the jet nebulizer device every 24 hours or after each treatment may not prevent contamination of the nebulizer reservoir and subsequently produced medical aerosols. Aerosol devices that reduce risk of patient contamination of the medication reservoir may be a better choice for infection control and prevention.

There has been speculation by some that aerosol delivery with metered dose inhaler (pMDI) are less likely than nebulizers to become contaminated during mechanical ventilation resulting in less incidence of VAP and may be a more practical choice. Dubosky and colleagues evaluated VAP occurance, days on mechanical ventilation and in-hospital mortality with the use of pMDI compared with VMN during mechanical ventilation.[9] Two hundred twenty-eight subjects were included between August 2011 and August 2013.[9] They found no difference in VAP, days on mechanical ventilation, and in-hospital mortality associated with the use of a pMDI or VMN in mechanically ventilated subjects. These findings are in contrast to recommendations that pMDI may be a more prudent choice from an infection prevention perspective.[10] In addition, efforts to reduce costs such sharing the same pMDI cannister between patients should be discouraged since there is a high

possibility of crosscontamination between patients in the absence of good handwashing and aspectic technique.[7] It is important to note that speculation that nebulizers may increase the risk of nosocomial infection and/or VAP may be due to the design of JN which sit below the ventilator circuit with medication reservoirs that are open to contamination from rainout and patient secretions in circuits. Vibrating mesh nebulizers are designed such that the medication reservoir and aerosol producing mechanism is not open to potential contamination with both in line and hand-held applications. This could at least partially account for the results seen by Dubosky and colleagues. The VMN may offer a viable and safe alternative for aerosolized medications especially with formulations not available in pMDI form.[11]

In a separate study Dubosky and colleagues conducted a RCT in mechanically ventilated patients compared bacterial growth in the nebulizer reservoir, ventilator circuit and sputum between JNs and VMNs.[12] They followed 120 patients randomly assigned either VMN (removed to capture culture and placed back in line for duration of therapy via MV) or JN (changed every 3 days and discarded after capturing cultures). Despite the desparity between handling of the nebulizers to capture cultures and duration of use between devices, they found no statistical difference in overall bacteria growth between the aerosol produced by the nebulizers.[12] A VMN left in line for duration of therapy may be a convienent option for busy respiratory therapists compared to frequent JN changes, especially if there is no associated increased risk of infection. Another interesting finding from this study was that overall there was no match between patient, circuit and nebulizer pathogens.[12] One would suspect that if a nebulizer is implicated in a ventilator associated infection that there would need to be similar pathogens captured from all three sites (patient sputum, circuit and nebulizer).

While there are infection concerns surrounding aerosols in the clinical environment, there is no conclusive evidence that nebulizers increase the risk of transmission of infection.[1,13] It is also important to note that patients with respiratory infections benefit from aerosolized medication and stopping their use could negatively impact patient outcomes.[14] Current CDC and WHO recommendations for infection prevention precautions in addition to utilizing nebulizers that minimize the potential for patient contaminated emissions can allow safer delivery of aerosolized medications to patients in need of medically necessary treatment.

Limitations of the Study

This was a limited feasibility study to determine the ability to contaminate medical nebulizers through the mouthpiece and quantify aerosol collected on a filter medium placed on agar plates to differentiate and quantify bacterial colony counts. Normal cleaning procedures were not considered in the design of the study because this was a bench test of the nebulizer design and not a study of the clinical process or application.

Conclusions

Nebulizer type and design influence the impact of pathogen containing fluids passing through the mouthpiece and contaminating the aerosol produced by the device. Use of aerosol delivery devices such as JN with open reservoirs and the potential for contamination and production of bioaerosols should be avoided. Fugitive aerosols from contaminated devices is a crucial issue in the presence of infectious respiratory diseases. Use of nebulizers that prevent contamination of patient generated secretions with physical barriers between the patient interface, the medication reservoir and aerosol producing mechanisms such as VMNs or JNs with effective T-piece drool gaurds would be the preferred choice. Further studies in patients are required to better understand incidence and impact of such contamination.

Conflict of Interest: Patricia Dailey is currently a Senior Medical Science Liaison for Aerogen, Ltd. Contact information: Dr. Fink is Chief Science Officer for Aerogen Pharma Corporation.

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References

1. Tran K, Simon K, Severn M, Pessoa-Silva CL, Conly J. Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: A systematic review. *PloS One*. 2012;7(4):e35797.
2. Siegel JD, Rhinehart E, Jackson M, Chiarello L. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. *Am J of Infect Control*. 2007;35(10 Suppl2):S65-S164. Doi:10.1016/i.ajic.2007.10.007
3. WHO. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. World Health Organization. https://www.who.int/publications-detail-redirect/10665-332299. Published 2020. Accessed May 6, 2022.
4. Srikanth P, Sudharsanam S, Steinberg R, Bio-aerosols in indoor environment: composition, health effects and analysis. *Infiain J Med Microbiol*.2008;26(4):302-312. Doi:10.4103/0255-857.4355
5. Craven, D. E., Lichtenberg, D. A., Goularte, T. A., Make, B. J., & McCabe, W. R. (1984). Contaminated medication nebulizers in mechanical ventilator circuits. Source of bacterial aerosols. *Am J Med*. 1984 *77*(5):834-838. Doi:10.1016/0002-9343(84)90520-5
6. Fink JB, Ehrmann S, Li J, et al. Reducing Aerosol-Related Risk of Transmission in the Era of COVID-19: An Interim Guidance Endorsed by the International Society of Aerosols in Medicine. *J Aerosol Med Pulm Drug Deliv*. 2020;33(6):300-304. doi:10.1089/jamp.2020.1615
7. O'Malley CA. Device cleaning and infection control in aerosol therapy [published correction appears in Respir Care. 2015 Aug;60(8):e150]. *Respir Care* 2015;60(6):917-930.
8. CDC. Guidelines for prevention of nosocomial pneumonia. Centers for Disease Control and Prevention. MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports. https://pubmed.ncbi.nlm.nih.gov/9036304/. Published 2003. Accessed May 6, 2022.
9. Dubosky MN, Chen Y-FF, Henriksen ME, Vines DL. Vibrating Mesh Nebulizer Compared With Metered-Dose Inhaler in Mechanically Ventilated Subjects. *Respir Care*. 2017; 62(4): 391-395. Doi:10.4187/respcare.04823
10. Branson RD, Hess DR, Kallet R, Robinson L. SARS COV-2 - AARC. AARC. https://www.aarc.org/wp-content/uploads/2020/03/guidance-document-SARS-COVID19.pdf. Published 2020. Accessed May 6, 2022.
11. Gilmore TW. Use of a metered-dose inhaler compared with a vibrating mesh nebulizer during mechanical ventilation: Does it really matter? Respir. Care. 2017;62(4):513-514. doi:10.4187/respcare.05464
12. Dubosky MN, Lamorena E, Jones C, et al. B24. critical care: Acute respiratory failure - mechanical ventilation and beyond. American Thoracic Society International Conference Meetings Abstracts American Thoracic Society International Conference Meetings Abstracts. https://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2017.195.1_MeetingAbstracts.A3025. Published 2017. Accessed May 6, 2022.
13. Goldstein KM, Ghadimi K, Mystakelis H, et al. Risk of Transmitting Coronavirus Disease 2019 During Nebulizer Treatment: A Systematic Review. *J Aerosol Med Pulm Drug Deliv*. 2021;34(3):155-170. Doi:10.1089/jamp.2020.1659
14. Halpin DMG, Criner GJ, Papi A, et al. Global Initiative for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease. The 2020 GOLD Science Committee Report on COVID-19 and Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2021;203(1):24-36. doi:10.1164/rccm.202009-3533SO