Salbutamol Delivered by Jet Nebulizer: Closed System Design and Impact of a Model Biofilm

Serpil Öcal, Serkan Özen, Emirhan Nemutlu, Didem Kart, Cemil Can Eylem, and Arzu Topeli

BACKGROUND: Aerosol therapy is commonly used by intensivists during invasive mechanical ventilation. More information is needed to optimize outcomes. The first aim of this study was to assess the deposition of salbutamol on components of a closed mechanical ventilation system, both in the presence and absence of biofilm generated by *Acinetobacter baumannii.* The second aim was to evaluate the deposition of salbutamol, using a single dose and a double dose, delivered via a jet nebulizer placed between the flexible tube and the heat and moisture exchanger. METHODS: A mechanical ventilator was connected to a standard system, and a jet nebulizer was placed between the heat and moisture exchanger and the flexible tube. Clinical isolates of *A. baumanii* were used to generate a biofilm layer on the endotracheal tube. Two amounts of salbutamol were delivered via the jet nebulizer. An analytical liquid chromatography tandem mass spectrometry method was developed to evaluate salbutamol deposition. RESULTS: The presence of a biofilm on the endotracheal tube had no impact on salbutamol deposition ($P = .83$). There was no difference in surface deposition of salbutamol on component parts of the closed system in a comparison of a single dose and a double dose delivered via a jet nebulizer. CONCLUSIONS: Our findings indicate that an *A. baumannii* biofilm had no impact on the extent of salbutamol deposition. Salbutamol deposition was comparatively low and could be delivered without removal of the heat and moisture exchanger. *Key words: aerosol therapy; invasive mechanical ventilation; endotracheal tube; salbutamol; nebulizer; heat and moisture exchanger; biofilm.* [Respir Care 2021;66(9):1440–1445. © 2021 Daedalus Enterprises]

Introduction

Invasive mechanical ventilation is applied via a closed system that includes an endotracheal tube (ETT), a flexible tube, a Y-connector, and a dual-limb circuit connected to a mechanical ventilator. By its nature, invasive mechanical ventilation bypasses the upper airway; as such, humidification with a heated humidifier or a heat and moisture exchanger (HME) is routinely included to prevent hypothermia, mucociliary dysfunction, bronchospasm, atelectasis, and airway obstruction that may result from direct inhalation of cold and dry gas. Aerosol therapy, notably the use of bronchodilator drugs, is commonly employed by intensivists; this requires additional devices to be added to the closed system. Many factors influence the efficiency of aerosol therapy during invasive mechanical ventilation, including ventilator- and circuit-related factors, the types of aerosol-generating devices and their configuration, as well as drug- and patient-related factors.[1]

Respiratory viruses such as COVID-19 and influenza are primarily spread through respiratory droplets; as such, it is clear that maintaining a closed system is important during invasive mechanical ventilation. During outbreaks, we recommend the use of a pressurized metered-dose inhaler with a spacer or a mesh or jet nebulizer with a filter for invasively ventilated patients. However, frequent removal and insertion

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The authors have disclosed no conflicts of interest.

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of a heated humidifier or HME to facilitate aerosol therapy will increase the risk of exposure of droplet release. Some intensivists prefer to use HME devices with virus/bacterial filters to provide maximum protection for health care workers. It is critical to recognize that patients with COPD who are undergoing invasive mechanical ventilation are typically treated with double doses of nebulized salbutamol 6 times per day; this schedule was devised on the basis of the observation that airway resistance decreases significantly for 2 h only and then returns to baseline within 4 h of salbutamol delivery via a jet nebulizer.[2] Several investigators have suggested techniques for optimizing aerosol therapy, but these methods have not been formally evaluated and require multiple manipulations by the nursing staff; this increases clinician work load in the ICU and raises the ongoing risk of viral and bacterial infection.

Another point to consider is the role played by bacterial biofilms. Within hours after intubation, the ETT becomes colonized by microorganisms that form a biofilm on its surface.[3] *Acinetobacter baumannii* is one of the most prevalent pathogens that cause ventilator-associated pneumonia. There are no published studies that address the role of *A. baumannii*-derived biofilms and their impact on aerosol distribution and deposition. The first aim of this study was to assess the impact of inhaled salbutamol and its physical deposition on the ETT both with and without an *A. baumanii*-derived model biofilm in experiments performed in vitro. Our second aim was to determine differences in aerosol deposition using both single and double doses of salbutamol delivered via a jet nebulizer placed between the flexible tube and the HME within a closed system used for invasive mechanical ventilation.

QUICK LOOK

Current knowledge

Many factors influence the efficiency of aerosol therapy during invasive mechanical ventilation. Also, intensivists may prefer to use heat and moisture exchangers (HMEs) with virus/bacterial filters to provide maximum protection for health care workers. Frequent removal and insertion of HME to facilitate aerosol therapy may increase the rate and extent of droplet transmission of respiratory viruses.

What this paper contributes to our knowledge

Biofilm generated from a clinical isolate of *A. baumannii* within the endotracheal tube had no impact on the deposition of nebulized salbutamol. Also, salbutamol deposition did not increase significantly when comparing results from single and double doses delivered via a jet nebulizer. This may be because the inner surface of the endotracheal tube was fully saturated after the first salbutamol dose.

Methods

Experimental Model

For this in vitro study, the experimental model included an ETT (polvinyl chloride, 8 mm inner diameter and 36 cm length; Covidien, Medtronic, Frindley, Minnesota), a flexible tube (22 mm inner diameter and 15 cm length; Medisize, Hillegom, The Netherlands), a jet nebulizer set (Plasti-med, Istanbul, Turkey), an HME with virus/bacteria filtering capabilities (HME1, Phillips-Medisize, Hillegom, The Netherlands), a similar HME filter (HME2, Morton, Los Angeles, California), a dual-limb ventilator circuit with a Y-connector, a viral/bacterial filter (Disposet, Ankara, Turkey), and a mechanical ventilator (GE, Engstorm CS, Datex-Ohmeda, Madison, Wisconsin). The mechanical ventilator was connected to a standard system, and a test lung was included as shown in Figure 1. The jet nebulizer was placed between the flexible tube and the HME. The mechanical ventilator settings included tidal volume 500 mL, PEEP 5 cm H_2O, and breathing frequency 20 breaths/min in volume controlled mode.

Biofilm Formation

An experimental biofilm layer was created on the ETT as described by Raad et al.[4] Briefly, an overnight culture of a clinical isolate of *A. baumannii* was inoculated into sterile tryptic soy broth and incubated at 37°C for 18–24 h. The bacteria were harvested by centrifugation at 5,000 rpm for 5 min; bacterial concentrations were determined via spectrophotometric measurements performed at a wavelength of 590 nm; cells were resuspended at 10^6 colony forming units/mL. Aliquots of this suspension were transferred into 6 sterile ETTs; the entire inner surface of each tube was covered to promote bacterial adherence. After 4 h of static incubation, the tubes were rinsed with continuous-flow sterile tryptic soy broth with a peristaltic pump set at a constant flow of $\sim$ 200 mL/h for 24 h. Bacterial cells in the resulting biofilms were detached via sonication and plated onto tryptic soy agar to determine the number of mature cells on the inner surface of the ETT.

Bronchodilator Therapy

In the first experiment with the HME1 filter as described above, double doses of salbutamol (5,000 μg in 5 mL normal saline) were placed in the reservoir of the jet nebulizer, and gas flow was set at 8 L/min. Nebulization ran continuously until the salbutamol dose was complete. Each experiment was performed either with (no. = 6) or without (no. = 4) the *A. baumannii*-derived biofilm. The second experiment used

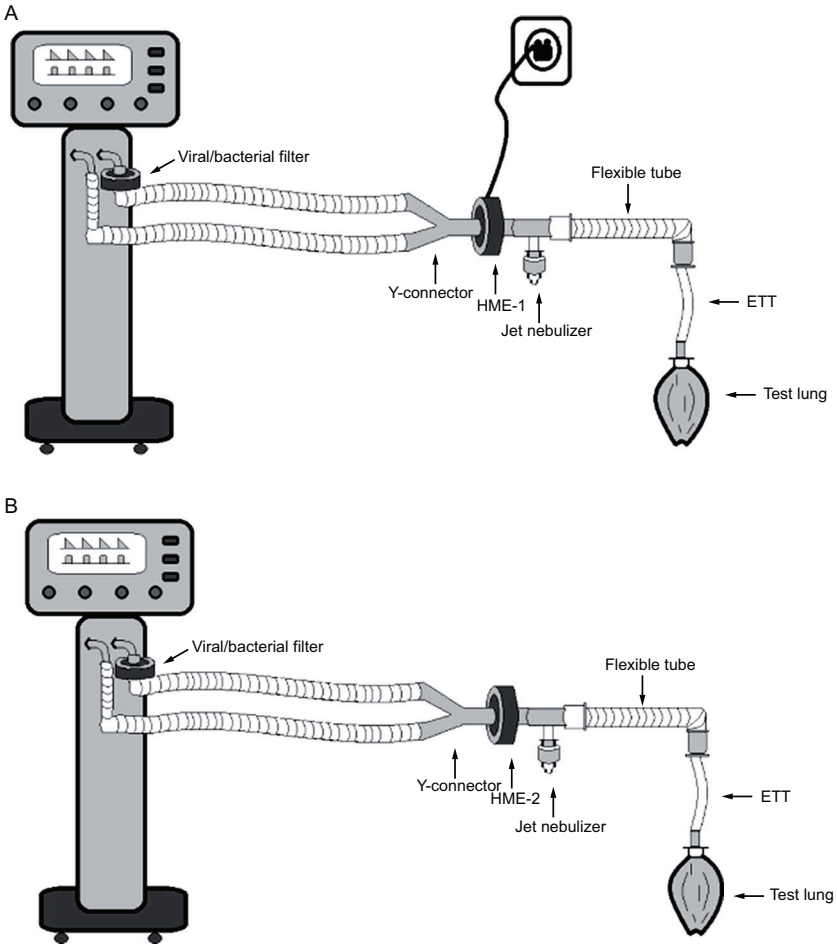

Fig. 1. Model of salbutamol delivery to be used during invasive mechanical ventilation. Experimental setup of the study: a test lung, endotracheal tube (ETT), flexible tube, jet nebulizer, heat and moisture exchanger (HME), a dual-limb ventilator circuit with a Y-connector, viral/bacterial filter, and mechanical ventilator. A: HME1 filter with heating element and a water source adapter (HME booster), and B: HME2 filter.

the HME2 filter; single doses of salbutamol (2,500 μg in 2.5 mL normal saline) were placed in the reservoir of the jet nebulizer with gas flow set to 8 L/min. Nebulization ran continuously until the salbutamol dose was complete. After all parts of the closed system were changed, the same experiment was performed with double doses of salbutamol (5,000 μg in 5 mL normal saline). After nebulization was complete, all parts of the closed system were stored at –80°C to determine salbutamol deposition on the surface at a later date. Each experiment was performed 3 times with both single doses (no. = 3) and double doses (no. = 3) of salbutamol.

Quantification of Surface Deposition of Salbutamol

Salbutamol deposition was measured on the ETT, flexible tube, Y-connector, HMEs, and the viral/bacterial filter using an analytical liquid chromatography tandem mass spectrometry. Multiple reaction monitoring (positive mode) was used to optimize detection of salbutamol; a 1-ppm standard salbutamol solution was included. The optimization procedure resulted in the identification of 240.10 → 222.10 (m/z) as the fragmentation product of salbutamol at highest relative abundance. Liquid chromatography analyses were carried out at 40°C using a C18 column (100 × 4.6 mm, 3.5 µm inner diameter) and a gradient eluent that included solution A (0.1% formic acid) and solution B (0.1% formic acid) in acetonitrile, at a flow of 0.3000 mL/min over 10 min. Under these conditions, the retention time of salbutamol was 4.7 min. Salbutamol was extracted by immersion of each component individually in 100 mL absolute methanol and sonicated for 15 min; 1 mL of each sonicated sample was transferred to a vial and analyzed with liquid chromatography tandem mass spectrometry.

Data Analysis

Statistical analyses were performed using the SPSS 22 (IBM, Armonk, New York). The variables were investigated

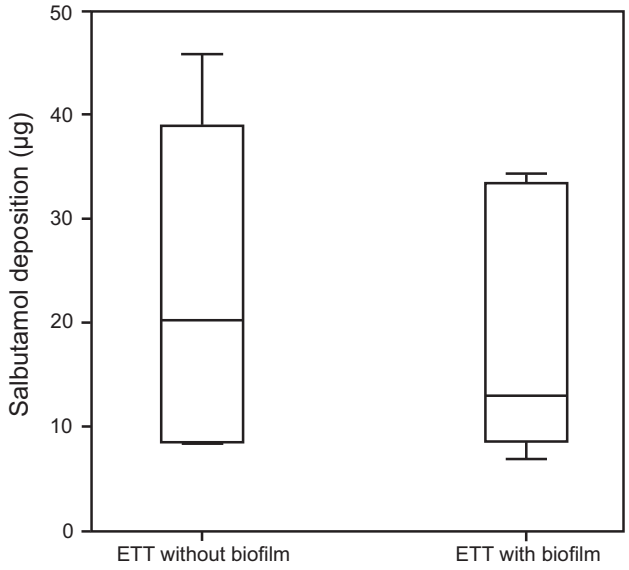

Fig. 2. Box plot of median (range) salbutamol deposition detected on the endotracheal tube (ETT) in the presence of absence of an *A. baumannii*-derived biofilm via jet nebulizer placed between the HME1 and the flexible tube that delivered double doses (5,000 µg) ($P = .83$). The box plot represents the median and interquartile range of the values in each group.

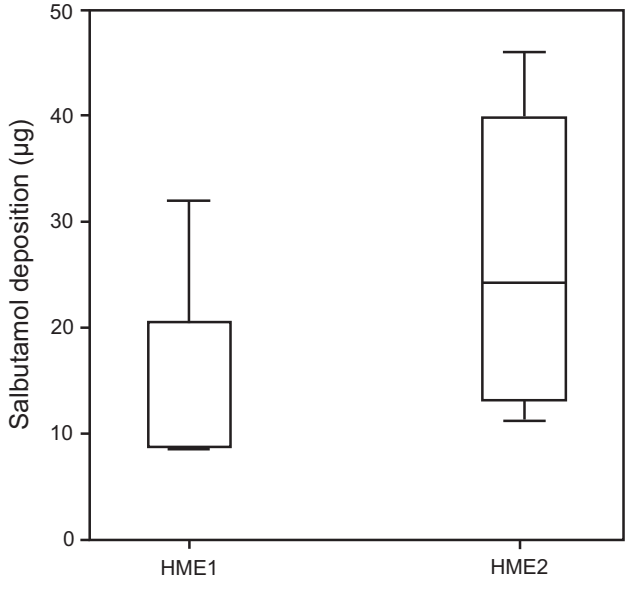

Fig. 3. Box plot of median (range) salbutamol deposition detected on the HME1 and HME2 in response to double doses (5,000 µg) delivered via jet nebulizer placed between the flexible tube and HME ($P = .28$). The box plot represents the median and interquartile range of the values in each group. HME = heat and moisture exchanger.

using analytical methods (Kolmogorov-Simirnov and Shapiro-Wilk tests) to identify normal distributions. Descriptive analysis was presented using median and range for the non-normally distributed data. Friedman tests were conducted to identify significant differences with respect to single and double doses due to violations of parametric test assumptions (ie, non-normal distributions and low numbers). The Wilcoxon rank sum test was performed to test the significance of pairwise differences using the Dunn test. An overall 5% type-1 error level was used to assess statistical significance.

Results

The *A. baumannii* model biofilm had no impact on salbutamol deposition on the ETT ($P = .83$; Fig. 2); likewise, the choice of HME had no impact on salbutamol deposition ($P = .28$; Fig. 3). Salbutamol deposition on the ETT, flexible tube, HME2, Y-connector, viral/bacterial filter, and the test lung after single and double doses delivered via jet nebulizer is presented in Table 1. No significant differences in deposition were observed when comparing single versus double doses of salbutamol via jet nebulizer.

Discussion

To our knowledge, this is the first study to demonstrate that an *A. baumannii* biofilm generated on an ETT had no impact on the deposition of salbutamol delivered via jet nebulizer. Our system featured a jet nebulizer placed between the flexible tube and the HME, using a system design that is analogous to that used during invasive mechanical ventilation. Although many in vivo and in vitro aerosol therapy studies have been published, the impact of biofilms on the function of ETTs has not been fully explored. Many researchers have recommended removing the HME or heated humidifier during aerosol therapy with invasive mechanical ventilation. Ari et al[5] reported that aerosols can be delivered via systems that include an HME; however, the authors recommended that one should avoid placing an HME with filtering capacity between the nebulizers and ETT. In accordance with this recommendation, the jet nebulizer was placed between the HME and the flexible tube in our model.

It has become clear that bacterial biofilms can form on ETTs. Gil-Perotin et al[6] reported that *A. baumannii* and *Pseudomonas aeruginosa* are among the most prevalent bacteria in ETT biofilms. Vandelvelde et al[7] reported that ipratropium bromide and, to a lesser extent, salbutamol may work together with antibiotics to promote bacterial clearance and disassembly of *Streptococcus pneumoniae* biofilms in vitro. By contrast, Kenia et al[8] reported that salbutamol facilitated the formation of *P. aeruginosa* biofilms in subjects with cystic fibrosis. Biofilm bacteria produce proteins and carbohydrates that facilitate their own attachment and promote adherence of other bacteria to natural and artificial surfaces. High molecular weight extracellular

Table 1. Deposition of Salbutamol on Model Components

	Salbutamol		*P*
	Single Dose = 2,500 μg	Double Dose = 5,000 μg	
Endotracheal tube, μg	22.1 (13.9–71.2)	76.5 (16.2–115.2)	.28
Flexible tube, μg	28.8 (13.4–139.8)	30.2(7.3–633.4)	.28
Heat and moisture exchanger, μg	45.1 (11.0–526.8)	403.8 (11.0–1533.5)	.83
Y connector, μg	3.9 (2.2–9.2)	20. (1.8–13.2)	.51
Viral/bacterial filter, μg	14.7 (6.6–109.6)	30.19 (7.3–633.4)	.51
Test lung, μg	35.6 (28.3–216.6)	85.3 (51.9–231.8)	.28

Values are expressed as median (range).

polysaccharide substances from bacteria form elaborate 3-dimensional structures that stabilize cells within the biofilm.[9] As such, we hypothesized that these substances might also facilitate deposition of nebulized drugs, including salbutamol, to the irregular and adhesive surface generated on the ETT. Interestingly, a biofilm generated from a clinical isolate of *A. baumannii* biofilm had no impact on deposition of salbutamol in our model.

Respiratory viruses are primarily spread through respiratory droplets. As such, protection of the closed system is important during invasive mechanical ventilation; repetitive disruption of the closed system for insertion and removal of a heated humidifier or HME device will increase droplet dispersion. It is clear that this should be avoided, especially during virus outbreaks; patients with COPD are often those most critically affected by respiratory viruses. We believe that a jet nebulizer with a filter should be positioned between the ETT and the HME to reduce the potential for droplet spread in the ICU setting.

Salbutamol deposition did not increase significantly when comparing results from single and double doses delivered via jet nebulizer. This may be because the inner surface of the ETT was fully saturated after the first salbutamol dose. This observation may be related to surface area as opposed to the specific drug dose. However, as the nebulizer chamber was at its maximum volume (ie, 5 mL) for the administration of double doses, we were unable to evaluate the impact of 3 doses at one time on the extent of aerosol deposition. When both single doses and double doses of salbutamol were delivered via jet nebulizer, more salbutamol was detected on the flexible tube than was associated with the ETT. Among the potential explanations for this observation, the flexible tube has a tubular structure, and turbulent flow that develops in this section of the closed system may result in increased salbutamol deposition. Likewise, the flexible tube has more dead space and overall surface area than the ETT.

Three different types of commercially available nebulizers are currently used in clinical practice, including mesh, ultrasonic, and jet nebulizers; there are advantages and disadvantages presented by each type of nebulizer. Harvey et al[10] reported that an ultrasonic nebulizer, as opposed to a jet pneumatic nebulizer, was superior for aerosol delivery to the lung, with lower aerosol deposition in the trachea and ETT in an in vivo study. However, ultrasonic nebulizers are heavier than jet pneumatic nebulizers; furthermore, transport of this device between patients increases the risk of infection, increasing the work load in the ICU. Although mesh nebulizers are more efficient for aerosol therapy than jet nebulizers, jet nebulizers are most commonly used for the treatment of patients with COPD and asthma due to their simplicity and durability; they are easy to use and significantly less expensive.[11] Jet nebulizers have no impact on drug stability and concentration and are therefore suitable for use in the ICU.

When an HME with viral/bacterial filtering capabilities is placed between the ETT and the aerosol device, it quickly becomes saturated and generates increased airway resistance and work of breathing. Ari et al[12] reported that the amount of albuterol delivered from a jet nebulizer was greater when it was assembled with 15-cm large-bore tubing within 15 cm of the ventilator than when it was adjacent to the Y-connector, regardless of whether the ventilator circuit included a heated humidifier. Our study featured a different design; we believe that our design presents an alternative to removing and replacing the HME and the need to include 15-cm large-bore tubing to generate a connection near the mechanical ventilator for each salbutamol administration.

ETTs are typically made of polyvinyl chloride; we recognize that ETT design and manufacture may have an impact on aerosol deposition. Research should ultimately evaluate different brands of mechanical ventilators, nebulizers, and HMEs used in more sophisticated systems. Furthermore, some in vitro studies using closed systems do not include a flexible tube. The flexible tube has minimal compliance with low dead space volume; it is not clear whether it is a spacer or dead space with respect to aerosol therapy.

Among the limitations, we note that our study was performed using an in vitro model and a closed system. As such, we cannot comment on the extent of aerosol deposition

in in vivo models and in routine clinical practice. Likewise, mixed biofilms harboring microbial pathogens are formed on the ETT comparatively quickly after intubation, while the ETT model biofilm was generated using a clinical isolate of *A. baumannii* only. Likewise, as the nebulizer chamber was at its maximum 5 mL when administering double doses of salbutamol, we could not evaluate the impact of 3 doses at a time. Finally, our study featured only 2 types of HME and one type of nebulizer; our findings should not be generalized to other types of HMEs and related devices that we have not yet had the opportunity to evaluate.

Conclusions

In this study, we examined the impact of a laboratory-generated biofilm and various HME filters on the deposition of nebulized salbutamol on components of a closed invasive mechanical ventilation system. An ETT biofilm generated with a clinical isolate of *A. baumannii* had no impact on deposition of salbutamol delivered via jet nebulizer. Likewise, introducing a second dose (ie, a double dose) had no impact on the deposition of nebulized salbutamol. These results suggest that the first dose saturated the available surfaces within the closed system. Our findings suggest a feasible method for aerosol therapy with salbutamol.

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