Expert Opinion on Drug Delivery

ISSN: 1742-5247 (Print) 1744-7593 (Online) Journal homepage: http://www.tandfonline.com/loi/iedd20

In vitro evaluation of aerosol delivery of aztreonam lysine (azli): an adult mechanical ventilation model

A. Rodríguez, M. Cabrera, LF. Reyes, M. Bodí, S. Trefler, L. Canadell, D. Barahona, S. Ehrmann, I. Martin-Loeches, MI. Restrepo & L. Vecellio

To cite this article: A. Rodríguez, M. Cabrera, LF. Reyes, M. Bodí, S. Trefler, L. Canadell, D. Barahona, S. Ehrmann, I. Martin-Loeches, MI. Restrepo & L. Vecellio (2017): In vitro evaluation of aerosol delivery of aztreonam lysine (azli): an adult mechanical ventilation model, Expert Opinion on Drug Delivery, DOI: 10.1080/17425247.2017.1393411

To link to this article: http://dx.doi.org/10.1080/17425247.2017.1393411

View supplementary material

Accepted author version posted online: 18 Oct 2017.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=iedd20

Download by: [Australian Catholic University] **Date:** 18 October 2017, At: 06:44

Check for updates

Publisher: Taylor & Francis
Journal: *Expert Opinion on Drug Delivery*
DOI: 10.1080/17425247.2017.1393411

In vitro evaluation of aerosol delivery of aztreonam lysine (azli): an adult mechanical ventilation model

A. Rodríguez (1), M. Cabrera (2), LF. Reyes (3), M. Bodí (1), S. Trefler (1), L. Canadell (4), D. Barahona (5), S. Ehrmann (2, 6), I. Martin-Loeches (7), MI. Restrepo (3) and L. Vecellio (2).

AFFILIATIONS:

1) Critical Care Department, Hospital Universitario de Tarragona Joan XXIII, URV, IISPV, CIBERES, Tarragona (Spain). AR: ahr1161@yahoo.es; MB: mbodi.hj23.ics@gencat.cat ; ST: sitrefler@yahoo.es

2) Centre d'Etude des Pathologies Respiratoires INSERM U1100 Equipe 3 Aérosolthérapie et biomedicaments à visée respiratoire. Faculté de Médecine Bâtiment M, Tours Cedex (France). MC: maria.cabrera@univ-tours.fr ; LV: vecellio@med.univ-tours.fr

3) Division of Pulmonary Diseases and Critical Care Medicine University of Texas Health at San Antonio and South Texas Veterans Health Care System, San Antonio, Texas (USA). LFR: reyeslf@utexas.edu ; MIR: restrepom@uthscsa.edu

4) Pharmacology Department, Hospital Universitario de Tarragona Joan XXIII, URV, IISPV, Tarragona (Spain). LC: lcanadell.hj23.ics@gencat.cat

5) Critical Care Department, Hospital Docente de Calderon, Quito (Ecuador) DB: dfbarahona@gmail.com

6) Mêdicine intensive Réanimation. Centre Hospitalier Regional et universitaire de Tours, Tours, France. SE: stephanehrmann@gmail.com

7) Multidisciplinary Intensive Care Research Organization (MICRO). Department of Anaesthesia and Critical Care. St James's University Hospital. Trinity Centre for Health Sciences, Dublin (Ireland). IML: drmartinloeches@gmail.com

CORRESPONDING AUTHOR:
Alejandro Rodríguez, MD, PhD
Critical Care Department
Hospital Univsersitario de Tarragona Joan XXIII
Mallafre Guasch 4 , 43007, Tarragona (Spain)
e-mail: ahr1161@yahoo.es
Tel. +34 977 285 818
Fax. +34 977 214 768

ABSTRACT

Accepted Manuscript

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Background: The delivery profile of Aztreonam lysine (AZLI) during mechanical ventilation (MV) is unknown. We evaluated the amount of AZLI drug delivered using an *in vitro* model of adult MV.

Methods: An adult lung model designed to mimic current clinical practice was used. Both nebulizers were placed before a Y-piece and 4 settings were tested: A) Aeroneb solo® [AS] with a t-piece; B) AS with the spacer; C) M-Neb® [MN] with a t-piece and D) MN with the spacer. Performance was evaluated in terms of: 1) Mass median aerodynamic diameter (MMAD); 2) Geometric standard deviation (GSD), 3) Fine particle dose (FPD), 4) Fine particle fraction (FPF), 5) Inhalable mass (IM), and 6) Recovery rate (RR).

Results: Both devices showed an adequate delivery of AZLI during MV, with MMAD between 2.4-2.5 μm and 87% of FPF. The FPD (38.8 and 31.7), IM (44.8 and 36.1) and RR (30 and 24) were similar for AS and MN respectively. Nebulizer aerosol delivery increased (50% and 70% respectively) for both nebulizers when using the spacer.

Conclusion: Both AS and MN showed a good aerosol delivery profile for AZLI during *in vitro* mechanical ventilation. Better aerosol delivery performance was obtained using the spacer.

Keywords: Aztreonam lysine, aerosol delivery, nebulized antibiotic, mechanical ventilation, in vitro model

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

1. INTRODUCTION

Artificial airways such as endotracheal tube (ETT) and tracheostomy tube (TT) are frequently used to provide aerosolized treatments to patients under mechanical ventilation (MV). Aerosolized antibiotics are particularly useful to treat respiratory tract infections in critically ill

patients and as part of long-term airway management in chronically ventilated patients[1–6]. Aerosol delivery efficiency decreases with a reduction of the inner diameter of the ETT, thus, artificial airways contribute to reducing aerosol delivery of antibiotics to the lower respiratory tract [6,7]. Importantly, the smooth inner surface of artificial airways may create a more laminar flow, resulting in better aerosol efficiency delivery[8]. Despite the theoretical added benefit of nebulized administration of antimicrobials, data are still scarce to support the widespread use of this drug delivery procedure in critical ill patients with ventilator-associated lower tract respiratory infection (VA-LRTI) [3,9,10-12] For instance, in a recent international survey on aerosol therapy during MV[2], researchers reported that out of 854 physicians who completed the survey, approximately 30% reported using nebulized antibiotics in more than five patients a year, and in 14% (n=85) of the hospitals, nebulized antibiotics were used in several patients for months (*i.e.,* frequent users).

The administration of aerosolized antibiotics offers the theoretical advantages of achieving high drug concentration at the infection site with low systemic complications and toxicity, due to its low absorption rate[4,13,14]. Nebulized antibiotics have been widely used in patients with cystic fibrosis with promising results[15–18], but data on its utility in patients under MV are scarce and in some cases, controversial. There is growing interest in using nebulized antibiotics for the prevention/treatment of ventilator-associated pneumonia (VAP) in critically ill patients and as co-adjuvant treatment of lung infections caused by highly resistant pathogens [3,19–23]. A phase II clinical trial [24] showed that nebulization and intravenous infusion of ceftazidime and amikacin had similar efficiency in terms of clinical and radiological cure of VAP caused by susceptible *P. aeruginosa*. In addition, nebulization of ceftazidime and amikacin provided more rapid bacterial eradication in distal pulmonary samples than intravenous administration. For instance, the new pneumonia guidelines of the Infectious Diseases Society of America/American Thoracic Society Adults (IDSA/ATS) [25]suggested both inhaled and

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

systemic antibiotics for patients with VAP due to multi-drug resistant gram-negative bacilli (*i.e.,* only susceptible to aminoglycosides or polymyxins). However, aerosol deposition in the lower respiratory tract is affected by several factors including the aerosol-generating system, particle size, properties of the inhaler carrier gas, physicochemical properties of the solution, tidal volume, respiratory rate, mucus, inflammation and pre-treatment with bronchodilators [1,26–28].

Aztreonam lysine solution (AZLI; Gilead Sciences) is an antipseudomonal antibiotic specifically designed to be aerosolized, which decreases respiratory symptoms, delays pulmonary exacerbation, and improves lung function in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*[29–31]. Thus, AZLI has been postulated as a promising option for treating VAP patients. However, the delivery profile of AZLI during mechanical ventilation is unknown. The aim of this study was to evaluate the amount of aerosolized AZLI drug delivered at the tip of the endotracheal tube using an "in vitro" model of adult MV with two different vibrating-mesh nebulizers.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

2. MATERIALS AND METHODS

2.1 Ventilator parameters

Experimental set-up: The bench model used in this study was designed to resemble current clinical practice. To assess the nebulizer's performance and drug delivery under conditions that mimic clinical use, we used a ventilator (Evita XL ventilator, Dräger Medical, Lübeck,

Germany) in volume-controlled mode (Vt= 450mL, f= 15/min, PEEP= 5cmH_2O, ratio between inspiratory and expiratory time=1/2 and a flow rate of 40 L/min) connected to the Dual Adult Training and Test Lung (model 5600i, Michigan Instruments: Resistance=5 cmH_2O/L/s, Compliance=0.05 L/cm H_2O); the modeling of the patient's lung as was previously reported elsewhere[18]. A 7.5 mm endotracheal tube and a right-angle elbow adapter were inserted between the Y-piece and the Test Lung. The humidifier (Fischer and Paykel Healthcare, Auckland, New Zealand) functioning at 37ºC and 100% relative humidity was placed between the ventilator and the Y-piece or spacer (Figure 1). The ventilator circuit tubing was heated and humidified for approximately 15 minutes, until the temperature was stable at 37ºC. During nebulization period, the humidifier was turned off.

2.2. Nebulization and configuration

Nebulization was performed with two vibrating-mesh nebulizers: 1) Aeroneb Solo® [AS] (Aerogen Ltd., Galway, Ireland) powered by the Aeroneb Pro Controller; and 2) M-Neb® [MN] (Nebutec, Germany). We used vibrating mesh nebulizer because of higher lung deposition, negligible residual volumes and faster rate of nebulization than jet nebulizers [8,13,32,33]. AS was chosen due to the widespread use in Spanish ICUs for mechanically ventilated patients. MN has been chosen as a comparator device used in Germany.

Both nebulizers were placed before the Y-piece in the inspiratory limb with a T-adapter piece, as described above. In addition, a spacer (Combihaler® Laboratoire Protec'Som SAS, France) was placed between the inspiratory circuit and the Y-piece. The MV circuit was tested using four different configurations: A) AS with a t-piece; B) AS with the spacer; C) MN with a t-piece and D) MN with the spacer (Figure 1).

The nebulizer reservoir was filled with 150 mg of AZLI diluted in 2 mL of 0.17% sodium chloride (Gilead Sciences International Ltd., Cambridge, United Kingdom). Droplets were

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

generated continuously during the entire breathing cycle. Nebulization started 60 seconds after the onset of MV and stopped when no aerosol was detected on visual inspection. Three repetitions were performed for each condition.

2.3. "In vitro" measurement of AZLI

Data for the validation of Aztreonam measurements and Aztreonam extraction coefficient from the filter are shown in appendix 1. An absolute filter (Gelman, Ann Arbor, United States) was placed between the end of the endotracheal tube and the lung model to filter the aerosol delivery to the lung model. The aerosol was not separate from the condensate and was collected by the absolute filter. A second expiratory filter was inserted between the ventilator and the expiratory circuit to protect the ventilator volume and pressure traducers from the nebulized particles. The nebulizer and ventilator circuit were replaced between each experiment. To test the influence of a spacer in the experiments, we performed repetitions with and without the spacer.

To measure the mass of AZLI deposited after nebulization, filters were desorbed in 30 mL of diluents (0.17% sodium chloride in water for injection). The AZLI extracted was then measured by spectrophotometric analysis at 291 nm using the WPA Lightwave II spectrophotometer (Biochrom, England), then, inhalable mass was calculated. Moreover, recovery rate % (RR%) was calculate as the ratio between the mass recovered on the filter and the mass loaded into the nebulizer.

2.4. Particle size determination

The MIPAQ® GS-1E cascade impactor functioning at 1.2 L/min was used to measure the particle size of the aerosol emitted through the endotracheal tube outlet in each configuration (Figure 2). To measure the particle size of aerosolized AZLI emitted at the endotracheal tube

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

outlet, each stage of the cascade impactor was rinsed with 10 mL of 0.17% sodium chloride in water.

The performance of nebulization was evaluated by: 1) Mass median aerodynamic diameter (MMAD); 2) Geometric standard deviation (GSD), 3) Fine particle fraction (FPF) defined as the fraction of particles smaller than 5 μm, 4) Inhalable mass, defined as the mass of total particles which are administered at the extremity of the ETT, 5) RR defined as the percentage of AZLI mass placed in the nebulizer then recovered on the filter, 6) Fine particle dose (FPD) calculated as the product of inhalable mass by the FPF and defined as drug particles smaller than 5 μm administered at the extremity of the ETT (considered to reach the lung)

2.5. Statistical analysis

Discrete variables were expressed as counts (percentages), and continuous variables as means and standard deviation (SD). Differences were assessed using the Chi-squared test or Fisher's exact test for categorical variables, and Student's t-test or the Mann-Whitney U test for continuous and ordinal variables, when appropriate. A p-value <0.05 was considered significant.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

3. RESULTS

3.1. Aerosol particle size and distribution

In vitro aerosol delivery of AZLI during MV showed a good performance with both nebulizers tested, with a MMAD between 2.4 and 2.5 μm and 87% of particles smaller than 5 μm, which are considered to reach the lung. Table 1 presents data comparing the aerosol characteristics of AZLI nebulized with the AS and the MN nebulizers. Both devices produced a polydispersed aerosol with comparable distribution and particles size. The performance of the two devices, determined by particle distribution (MMAD, GSD, FPD), was not statistically different. Additionally, using the spacer did not change any aerosol particle parameters (Table 1). However, the FPD was higher with spacer use for both devices, but was not statistically significant (59.8 which represents an increase of 54% over baseline for the AS device and 52.6 which represent an increase of 66% over baseline for the MN device). We also identified a minor variability in the FPD with the use of the spacer (Figure 3).

3.2.Mass delivered, output efficiency and nebulization time

Table 1 presents the performance of the devices with a simulated adult mechanical ventilation system. With a loading dose of 150mg/2mL of AZLI, the inhalable mass and recovery rate were slightly higher with the AS compared to the MN nebulizer but this difference was not statistically different (Figures 4 A and B). We observed minor variability in both inhalable mass and RR with the use of the spacer. Finally, time to dryness was similar for both devices (Table 1).

4. DISCUSSION

The main finding of the study was that both the AS and the MN nebulizers showed an

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

excellent aerosol delivery profile for AZLI using an *in vitro* adult mechanical ventilation model, and accordingly, high antibiotic doses to the lung are to be expected. Another important finding was that we observed high aerosol delivery using the spacer in addition to the nebulizers. To our knowledge, this is the first study that compares the aerosol delivery performance of AZLI using two currently utilized devices for nebulization in patients under mechanical ventilation.

Ventilator-associated tracheobronchitis (VAT) is a frequent complication in patients under mechanical ventilation, approximately 17% [34]. VAT is associated with higher use of broad spectrum antibiotics, morbidity and increased health care cost [20,34,35]. Importantly, antibiotic treatment for VAT has been discouraged in the new IDSA/ATS guidelines [25]. However, in a recent study, researchers identified that more than 90% of patients with VAT received empirical broad spectrum antibiotic treatment for more than 8 days [34], which could potentially increase the development of antibiotic resistance. Nebulized antibiotics have, in theory, a wide variety of advantages when compared to traditional administration mechanisms [4,14,36,37]. Some of these are; higher drug concentrations at the infection site, fewer systemic side effects [4,14,22,37–39], and probably a reduction of antibiotic resistance[36]. Thus, these potential benefits could be used as a stewardship strategy to optimize antimicrobial usage, prevent antibiotic resistance, and reduce adverse effects during antibiotic treatments. However, there are clinical and technical issues that require solving to make nebulized antibiotics suitable and extensively used in patients under mechanical ventilation[9,10].

An ideal inhaled antibiotic therapy should be a proper formulation for aerosolizing that consistently achieves a high antibiotic concentration at the infection site, which in many cases is nebulizer-dependent[5,10,37]. Currently, only Colistin [3,21,40], tobramycin [18,21] and aztreonan lysine[15,17,29–31] have a formulation specifically developed for nebulization. One of the primary determinants of delivery efficiency and drug deposition is particle size [41]. An optimal particle size (*i.e.,* MMAD 1-5 μm) would generate adequate distribution throughout the

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

lower respiratory tract and prevent deposition within the ventilator circuit. Our data suggest that both devices tested have the potential to generate and deliver AZLI optimally, with appropriate particle size that will ensure adequate deposition throughout the bronchial conducting airways and alveoli. Moreover, under our experimental conditions, using an adult MV model, the utilization of a spacer had no impact on aerosol particle size and distribution.

The inhalable mass is defined as the drug mass, in the form of an aerosol, produced by the nebulizer that reaches the patient's mouth [41]. It predicts the amount of drug that would reach the patient's airways. Within the vibrating mesh nebulizer category, there are significant variations in delivery efficiency [33,37,39]. Our results showed good delivery efficiency with both devices tested. Approximately 30% and 25% of nominal drug dose might be delivered to the lung using the AS and the MN nebulizers respectively. For the AS, the delivery dose is higher than reported by the manufacturer (13%)[37] but similar to what is observed in another study[42]. This observed discrepancy could be due to differences in the model (*e.g.,* infant animal model) and delivery dose determination used (*i.e.,* quantification of radiolabeled aerosol) in the studies that supported this manufacturer's data[43]. In addition, we found that aerosolized AZLI delivery was higher (>50%) when the vibrating mesh nebulizer was used with a spacer, instead of the T adapter, which is in concordance with recently published studies [27,44]. This increase in nebulization efficiency may be related with aerosol homogenization within the spacer. The larger volume of the spacer may limit the deposition of wet aerosol to the wall by condensation and may also help to limit deposition by sedimentation due to increased residence time during expiration[27]. Heated and humidified inspiratory gas coming from the ventilator increases MMAD of particles, increases deposition in the ventilator circuit, and reduces distal lung deposition [42]. The effect of humidification on MMAD is variable and seems to be related to the type of nebulizer used[44]. Our model was heated (37ºC) and humidified (100% RH) because is it a "real" condition in mechanically ventilated patients. Even under these conditions,

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

the particle size and estimated lung dose were optimal with both devices. We can hypothesize, that aerosol delivery performance would have been even better without gas humidification, but this situation was not tested and is unusual in ventilated critically ill patients. Finally, with a loading dose of 2 mL, similar time to dryness was observed for both devices tested (Table 1).

Our study has some limitations that should be acknowledged. First, results are from an artificial adult mechanical ventilation model, with standard ventilator settings and under controlled laboratory conditions. Therefore, potential problems with patients' coordination or irregular breathing patterns were not considered. Second, the use of nebulizers requires careful attention to the ventilator settings. These adjustments to the ventilator configurations could be complex in critically ill patients due to the wide variety of ventilator modes or settings available. However, testing *in vivo* aerosol performance would not be feasible for practical reasons. In this context, our model was constructed to determine the particle size and the amount of aerosol at the exit of the endotracheal tube as well as the amount of AZLI deposited elsewhere in the system, under conditions that mimicked clinical practice. Third, we used a standard ventilator setting with an inspiratory flow of 40 L/min. Delivery of aerosol to the end of the endotracheal tube decreased with an increasing flow [44]. During mechanical ventilation in critically ill patients, inspiratory flow rate varies but often exceeds the setting used in the present *in vitro* study and subsequently, a reduction in the AZLI inhalable mass might be observed. Finally, we only tested two specific devices and our findings cannot be generalized to other vibrating mesh nebulizers.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

5. CONCLUSION

Both the AS and the MN showed an excellent aerosol delivery profile for Aztreonam lysine (AZLI) in an *in vitro* model of mechanical ventilation with short drug-delivery time.

Better aerosol delivery performance was obtained using the spacer. Inhalable mass and recovery rate observed were high in our study, and might be sufficient to be considered effective for the treatment of microorganisms which require high antibiotic concentrations. However, further studies are needed to determine the clinical efficacy of AZLI nebulized in adult patients under mechanical ventilation.

Funding

This study was supported by grants from Gilead Science.

Declaration of Interest

A Rodríguez has received a Gilead Science Grant for the development of the present study. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

REFERENCES

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Ari A, Harwood RJ, Sheard MM,et al. An In Vitro Evaluation of Aerosol Delivery Through Tracheostomy and Endotracheal Tubes Using Different Interfaces. Respir Care . 2012;57(7):1066–70.

2. Ehrmann S, Roche-Campo F, Sferrazza Papa GF,et al. Aerosol therapy during mechanical ventilation: An international survey. Intensive Care Med. 2013;39(6):1048–56.

* An international survey that reports a high use of aerosol therapy during mechanical ventilation and frequently for antibiotics nebulized.

3. Kwa ALH, Loh C, Low JGH,et al.Nebulized Colistin in the Treatment of Pneumonia Due to Multidrug- Resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.Clin Infec Dis 2005;41:754–7.

4. Palmer LB. Ventilator-associated Infection : The Role for Inhaled Antibiotics. Curr Opin Pulm Med. 2015;21(3):239–49.

**Review on the rationale for inhaled therapy examining the delivery and clinical efficacy of inhaled antibiotics, and the potential role for this mode of delivery actually decreasing antibiotic resistance in the respiratory tract

5. Rodríguez A, Barcenilla F. Nebulized antibiotics. An adequate option for treating ventilator-associated respiratory infection? Med intensiva 2015;39(2):97–100.

6. Ahrens RC, Ries RA, Popendorf W WJ. The delivery of therapeutic aerosols through endotracheal tubes. Pediatr Pulmonol. 1986;2(1):19–26.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

7. Takaya T, Takeyama K TM. The efficiency of beta 2-agonist delivery through tracheal tubes with the metered-dose inhaler: an in vitro study. J Anesth. 2002;16(4):284–8.

8. Dhand R. Special problems in aerosol delivery: artificial airways. Respir Care. 2000;45(6):636–45.

** Review about several factors that influencing aerosol deposition during mechanical ventilation with special interest in the type of aerosol generator used, the ventilator settings, endotracheal tube size and in the place of location of the nebulizer and spacers use.

9. Russell CJ, Shiroishi MS, Siantz E,et al.The use of inhaled antibiotic therapy in the treatment of ventilator-associated pneumonia and tracheobronchitis: a systematic review. BMC Pulmonary Medicine; 2016;16(1):40.

10. Zampieri FG, Nassar AP, Gusmao-Flores D,et al. Nebulized antibiotics for ventilator-associated pneumonia: a systematic review and meta-analysis. Crit Care 2015;19:150.

** A systematic review and meta-analysis that report that nebulized antibiotics seem to be associated with higher rates of clinical cure in the treatment of ventilator-associated pneumonia.

11. Montgomery AB, Vallance S, Abuan T, et al. A Randomized Double-Blind Placebo-Controlled Dose-Escalation Phase 1 Study of Aerosolized Amikacin and Fosfomycin Delivered via the PARI Investigational eFlow ® Inline Nebulizer System in Mechanically Ventilated Patients. J Aerosol Med Pulm Drug Deliv 2014;27(6):441–8.

12. Ruijgrok EJ, Vulto AG, Van Etten EW. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromised rats. J Antimicrob Chemother 2001;48(1) :89–95.

13. Palmer LB. Aerosolized antibiotic in the Intensive Care Unit. Clin Chest Med.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

2011;32:559–74.

14. Kollef MH, Hamilton CW, Montgomery BA. Aerosolized antibiotics: do they add to the treatmen t of pneumonia? Respir Care 2015;60(6):762–70.

15. Gibson RL, Retsch-Bogart GZ, Oermann C, et al. Microbiology, safety, and pharmacokinetics of aztreonam lysinate for inhalation in patients with cystic fibrosis. Pediatr Pulmonol. 2006;41(7):656–65.

**A double-blind, placebo-controlled, dose- escalation study for to determine pharmacokinetics and tolerability of nebulized AZLI in subjects with Cystic Fibrosis. AZLI nebulized was safe and well tolerated and provided sputum AZLI concentration that exceeds the drug concentrations required to inhibit bacterial growth of *P. aeruginosa*

16. Okusanya ÓO, Bhavnani SM, Hammel J, et al. Pharmacokinetic and pharmacodynamic evaluation of liposomal amikacin for inhalation in cystic fibrosis patients with chronic pseudomonal infection. Antimicrob Agents Chemother. 2009;53(9):3847–54.

17. Assael BM, Pressler T, Bilton D, et al. Inhaled aztreonam lysine vs. inhaled tobramycin in cystic fibrosis: A comparative efficacy trial. J Cyst Fibros 2013;12(2):130–40.

18. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. N Engl J Med. 1999;340:23–30.

19. Abu-Salah T, Dhand R. Inhaled antibiotic therapy for ventilator-associated tracheobronchitis and ventilator-associated pneumonia: An update. Adv Ther. 2011;28(9) :728–47.

20. Craven DE, Chroneou A, Zias N,et al. Ventilator-associated tracheobronchitis; the impact of targeted antibiotic therapy on patient outcomes. Chest. 2009;135(2):521–8.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

21. Hodson ME, Gallagher CG, Govan JRW. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. Eur Respir J. 2002;20(3):658–64.

22. Palmer LB. Aerosolized antibiotic in critically ill ventilated patients. Curr Opin Crit Care. 2009;15:413–8.

23. Lu Q, Luo R BL, Yang J,et al. Efficacy of High-dose Nebulized Colistin in Ventilator-associated Pneumonia Caused by Multidrug-resistant. Anesthesiology. 2012;117(6):1335–47.

24. Lu Q, Yang J, Liu Z, Gutierrez C, et al. Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. Am J Respir Crit Care Med. 2011;184(1):106–15.

25. Kalil AC, Metersky ML, Klompas M,et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 2016;63 (5):e61–111.

26. Ari AFJB. Factors affectig bronchdilator delivery in mechanically ventilated adults. Nurs Crit Care. 2010;15(4):192–203.

27. Boukhettala N, Porée T, Diot P, et al. In Vitro Performance of Spacers for Aerosol Delivery during Adult Mechanical Ventilation. J Aerosol Med Pulm Drug Deliv 2014; 27:1–7.

28. Dugernier J, Wittebole X, Roeseler J, et al. Influence of inspiratory flow pattern and nebulizer position on aerosol delivery with a vibrating-mesh nebulizer during invasive mechanical ventilation: an in vitro analysis. J Aerosol Med Pulm Drug Deliv 2015;28 (3):229–36.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

29. Oermann CM, Retsch-bogart GZ, Quittner AL et al. An 18-Month Study of the Safety and Efficacy of Repeated Courses of Inhaled Aztreonam Lysine in Cystic Fibrosis r. Pediatr Pulmonol. 2010;45(11):1121–34.

* Study in children population with Cystic Fibrosis (CF) that reported that hospitalization rates, FEV1 and scores on the Cystic Fibrosis Questionnaire-Revised Respiratory Symptom scale improved and bacterial density in sputum was reduced with each course of AZLI.

30. McCoy KS, Quittner AL, Oermann CM, et al. Inhaled aztreonam lysine for chronic airway Pseudomonas aeruginosa in cystic fibrosis. Am J Respir Crit Care Med. 2008;178(9):921–8.

** Study that report safety and efficacy of nebulized aztreonam lysine (AZLI) and it may be an alternative treatment option for patients with cystic fibrosis and chronic *P. aeruginosa* infection.

31. Retsch-Bogart GZ, Quittner AL, Gibson RL,et al. Efficacy and safety of inhaled aztreonam lysine for airway pseudomonas in cystic fibrosis. Chest. 2009;135(5):1223–32.

* This study report that 28-day treatment with AZLI significantly improved respiratory symptoms and pulmonary function in patients with Cystic Fibrosis with *P. aeruginosa* infection.

32. Dolovich MB, Dhand R. Aerosol drug delivery: Developments in device design and clinical use. Lancet 2011;377(9770):1032–45.

** This review discuss recent developments in the design and clinical use of aerosol devices over the past 10–15 years with an emphasis on the treatment of respiratory disorders

33. Rouby J-J. Aerosolized antibiotics for Ventilator-associated Pneumonia. Anesthesiology . 2012;117(6):1364–80.

34. Martin-Loeches I, Povoa P, Rodríguez A, et al. Incidence and prognosis of ventilator-

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

associated tracheobronchitis (TAVeM): a multicentre, prospective, observational study. Lancet Respir Med 2015 ;3(11):859–68.

35. Nseir S, Di Pompeo C, Pronnier P,et al. Nosocomial tracheobronchitis in mechanically ventilated patients: Incidence, aetiology and outcome. Eur Respir J. 2002;20(6):1483–9.

36. Palmer LB, Smaldone GC. Reduction of bacterial resistance with inhaled antibiotics in the intensive care unit. Am J Respir Crit Care Med. 2014;189(10):1225–33.

** A double-blind placebo-controlled study in critically ill intubated patients with signs of respiratory infection reported that aerosolized antibiotics successfully eradicated existing multidrug-resistant organisms and reduced the pressure from systemic agents for new respiratory resistance

37. Bassetti M, Luyt C-E, Nicolau DP,et al. Characteristics of an ideal nebulized antibiotic for the treatment of pneumonia in the intubated patient. Ann Intensive Care 2016;6(1):35.

* The authors review inhaled antibiotic options currently in development for the treatment of pneumonia in patients who are intubated and mechanically ventilated

38. Palmer LB, Smaldone GC, Chen JJ, et al. Aerosolized antibiotics and ventilator-associated tracheobronchitis in the intensive care unit. Crit Care Med. 2008;36(7):2008–13.

39. Restrepo MI, Keyt H, Reyes LF.Aerosolized Antibiotics. Respir Care. 2015;60(6):762–73.

40. Metersky ML, O'Donnell AE. Nebulized colistin for non-cystic fibrosis bronchiectasis: Déjà vu all over again? Am J Respir Crit Care Med. 2014;189(10):1151–2.

41. Brown JS, Gordon T, Price O,et al.Thoracic and respirable particle definitions for human health risk assessment. Part Fibre Toxicol 2013;10:12.

** This study provide estimates of the thoracic and respirable fractions for adults and children during typical activities with both nasal and oral inhalation, that may be used in the design of experimental studies and interpretation of health effects evidence

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

42. Ehrmann S, Mercier E, Vecellio L,et al. Pharmacokinetics of high-dose nebulized amikacin in mechanically ventilated healthy subjects. Intensive Care Med. 2008;34(4):755–62.

43. Dubus JC, Vecellio L, De Monte M, et al.Aerosol deposition in neonatal ventilation. Pediatr Res. 2005;58:10–4.

44. Pedersen KM, Handlos VN, Heslet L KH. Factors influencing the in vitro deposition of Tobramycin aerosol: A comparison of an ultrasonic nebulizer and a high-frequency vibrating mesh nebulizer. J Aerosol Med. 2006;19(2):175–83.

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

FIGURE LEGENDS

Figure 1: Schematic drawing of the adult ventilator model under humidified conditions used for the experiment. Both the Aeroneb Solo® and the M-Neb® were connected to the Y-piece on the inspiratory limb with a t-piece and the spacer (A, B, C and D).

Figure 2: Schematic drawing of the adult ventilator model with the cascade impactor for particle size measurement experiments.

Figure 3: Nebulization performance under humidified conditions expressed as fine particle dose according to four different configurations used in the experiment. Three cycles were performed for each configuration. Data are shown as mean with standard deviation (SD).

Figure 4: Aerosol performance by nebulization under humidified conditions expressed as inhalable mass (A) and recovery rate (B) according to four different configurations used in the experiment. Three cycles were performed for each configuration. Data are shown as mean with standard deviation (SD).

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

ABBREVIATIONS

AZLI: Aztreonam lysine

MV: Mechanical ventilation

ETT: Endotracheal tube

TT: Tracheostomy tube

MMAD: Mass median aerodynamic diameter

GSD: Geometric standard deviation

FPF: Fine particle fraction

FPD: Fine particle dose

RR: Recovery rate

VAP: Ventilator-associated pneumonia

VAT: Ventilator-associated tracheobronchitis

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

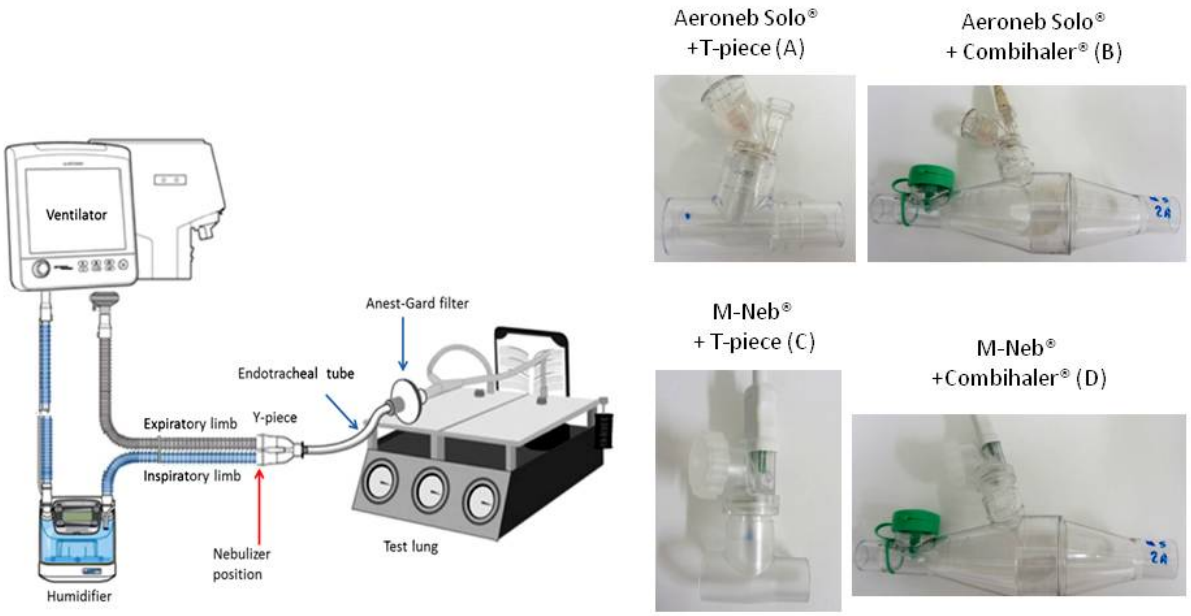
Accepted Manuscript

Acknowledgements

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions: AR, MIR, MB, DB and LFR wrote and edited the manuscript. MC, LV and SE designed the experiments. MC and LV performed the experiments. LV, SE, ST, MIR and LFR provided experimental technical support. AR, MB, LFR, ST, DB, MIR, IML, LC, SE, MC, and LC contributed intellectually.

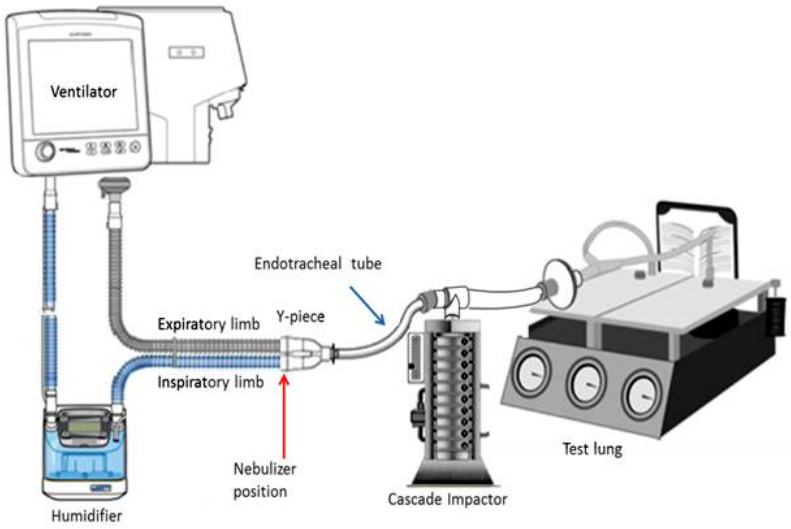
Figure 1



Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted M

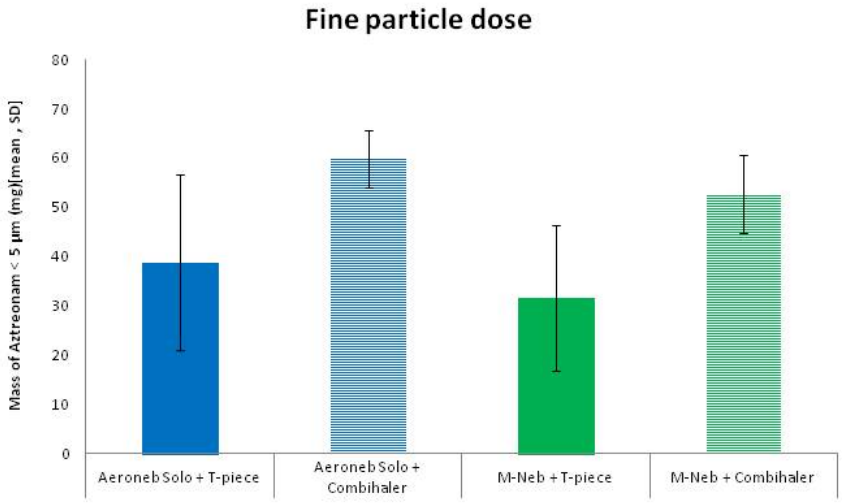
Figure 2



Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted M

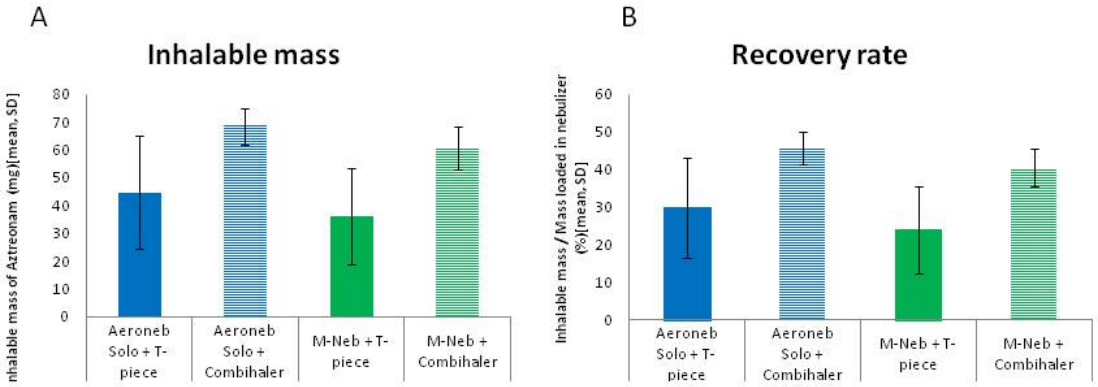
Figure 3



Accepted M

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Figure 4



Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted M

Table 1: Aerosol characteristics of the AZLI nebulized with Aeroneb Solo® and M-Neb® with and without Combihaler® spacer use. (MMAD: Mass median aerodynamic diameter; GSD: Geometric standard deviation; FPD: Fine particle dose)

Variables	Aeroneb Solo® with a T-piece	Aeroneb Solo® with the Combihaler®	M-Neb ® with a T-piece	M-Neb ® with the Combihaler®
Aerosol particle				
MMAD (μm) Mean (SD)	2.4 (0.0)	2.5 (0.1)	2.4 (0.1)	2.4 (0.2)
GSD, Mean (SD)	1.9 (0.1)	2.1 (0.3)	2.0 (0.1)	1.7 (0.1)
Mass delivered				
FPD (%<5 μm) Mean (SD)	38.8 (17.8)	59.8 (5.9)	31.7 (14.8)	52.6 (7.9)
Inhalable mass (mg), Mean (SD)	44.8 (20.2)	68.6 (6.6)	36.1 (17.4)	60.7 (7.5)
Recovery rate (%) Mean (SD)	30 (13)	46 (4)	24 (12)	40 (5)
Time to dryness				
Duration in minutes, Mean (SD)	4.59 (0.3)	4.57 (0.3)	4.22 (1.0)	4.43 (1.1)

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted Manuscript

Appendix 1

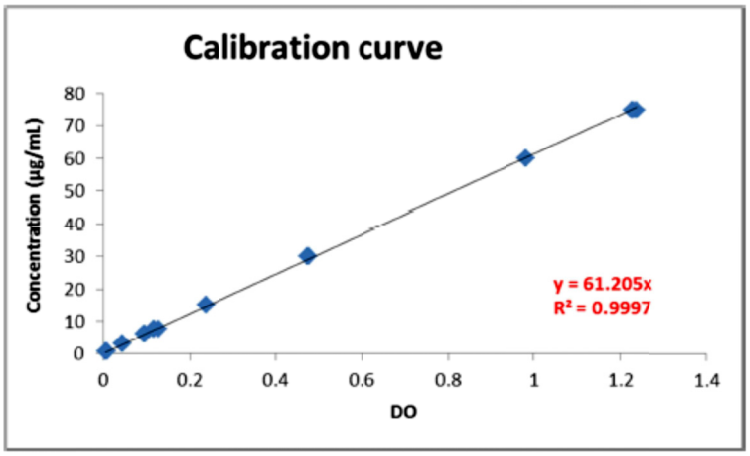
IN VITRO EVALUATION OF AEROSOL DELIVERY OF AZTREONAM LYSINE (AZLI): AN ADULT MECHANICAL VENTILATION MODEL

Validation of Aztreonam lysine (AZLI) measurements (Figure 1-e)

The absorbance spectrum of AZLI was measured, and maximum absorbance was observed at 291nm. All measurements were therefore carried out at this wavelength.

The WPA Lightwave II spectrophotometer (Biochrom, England) was calibrated with dilutions of AZLI solution of known concentration (0.75 to 75 µg/L). The correlation coefficient R^2 of the calibration curve was calculated. The calibration was checked before each series of experiments by measuring the absorbance of samples of known concentrations.

Figure 1-e



Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Accepted manuscript

Nebulized Aztreonam extraction from filters (Table 1-e)

0.5 mL of AZLI (37.5 mg Aztreonam) was nebulized using an Aeroneb Solo® nebulizer with a T-piece.

An Anest-Guard® absolute filter was placed between the nebulizer and a vacuum pump functioning at 15 L/min. After nebulization, the filter and the nebulizer with the T-piece were desorbed in 30 mL of diluent (0.17% sodium chloride in water for injections). The Aztreonam extracted was then assayed by spectrophotometric analysis at 291 nm using the WPA Lightwave II spectrophotometer (Biochrom, England). This experiment was repeated three times.

For each nebulization, the theoretical mass loaded in the filter was calculated by subtracting the residual amount in the nebulizer from the initial mass loaded as follows:

Theoretical mass deposited on the filter = Mass loaded in the nebulizer- Mass remaining in the nebulizer at the end of nebulization

An extraction coefficient was calculated as follows:

Extraction coefficient = (measured mass / theoretical mass) x 100

Table 1-e: Nebulized Aztreonam extraction coefficient from filters

	Mass loaded in the nebulizer (mg)	Mass remaining in the nebulizer and T piece (mg)	Theoretical mass deposited in the filter (mg)	Mass measured in the filter (mg)	**Extraction coefficient (%)**
n1	37.5	1.7	35.8	37.3	104
n2	37.5	3.6	33.9	35.9	106
n3	37.5	1.7	35.8	34.5	97
Mean	37.5	2.4	35.1	35.9	**102**
SD	0	0.9	0.9	1.1	**4**

Downloaded by [Australian Catholic University] at 06:44 18 October 2017

Manuscript