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Pharmacokinetic Characteristics of Nebulized Colistimethate Sodium Using Two Different Types of Nebulizers in Critically Ill Patients with Ventilator-Associated Respiratory Infections

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Abstract: *Background:* Rising antimicrobial resistance has led to a revived interest in inhaled colistin treatment in the critically ill patient with ventilator-associated respiratory infection (VARI). Nebulization via vibrating mesh nebulizers (VMNs) is considered the current standard-of-care, yet the use of generic jet nebulizers (JNs) is more widespread. Few data exist on the intrapulmonary pharmacokinetics of colistin when administered through VMNs, while there is a complete paucity regarding the use of JNs. *Methods:* In this study, 18 VARI patients who received 2 million international units of inhaled colistimethate sodium (CMS) through a VMN were pharmacokinetically compared with six VARI patients who received the same drug dose through a JN, in the absence of systemic CMS administration. *Results:* Surprisingly, VMN and JN led to comparable formed colistin exposures in the epithelial lining fluid (ELF) (median (IQR) AUC_{0-24} : 86.2 (46.0–185.9) mg/L·h with VMN and 91.5 (78.1–110.3) mg/L·h with JN). The maximum ELF concentration was 10.4 (4.7–22.6) mg/L and 7.4 (6.2–10.3) mg/L, respectively. *Conclusions:* Based on our results, JN might be considered a viable alternative to the theoretically superior VMN. Therapeutic drug monitoring in the ELF can be advised due to the observed low exposure, high variability, and appreciable systemic absorption.

Keywords: inhaled colistin; colistin pharmacokinetics; ELF colistin concentration; vibrating mesh nebulizer; jet nebulizer; critically ill

1. Introduction

The increasing prevalence of multidrug resistance (MDR) among ventilator-associated Gram-negative infections, and the paucity of available therapeutic options, have led to suggestions of adjunctive strategies with inhaled antibiotics by scientific bodies such as the Infectious Diseases Society of America [1], while the European Society of Clinical Microbiology and Infectious Diseases holds a more restrained approach [2]. In any case, the matter remains highly controversial, especially upon publication of a number of studies that refute the efficacy of inhaled therapy in the setting of MDR infections affecting critically ill patients [3,4].

Polymyxins, namely, polymyxin B and polymyxin E (colistin), are cationic oligopeptide antimicrobials that were revived during the late 1990s and early 2000s for the treatment of Gram-negative infections in the face of rising carbapenem resistance [5,6]. No matter how pressing the need for them, they still possess significant toxicity—neurotoxicity, but

especially nephrotoxicity, which according to several sources might affect a respected minority of patients, especially in the intensive care unit (ICU) setting.

In an effort to mitigate these toxicities, and building on the experience gathered in the field of cystic fibrosis, inhaled colistin treatment—in the form of nebulization of the inactive prodrug, colistimethate sodium (CMS)—emerged as a potential therapy for MDR and extensively drug resistant (XDR) infections [7]. The rationale has been the theoretic ability to achieve sufficient concentration in the infectious milieu of the lung parenchyma while sparing any significant systemic adverse outcome. In contrast to these theoretical considerations, inhaled CMS has only had modest efficacy and in secondary outcomes when tested in a randomized, controlled fashion [8,9]. However, in a meta-analysis of mostly observational data, the performance of adjunctive aerosolized colistin was associated with an approximately 60% increase and 40% decrease in the odds of clinical cure and infection-related mortality, respectively [10]. These discrepancies are reflected in official recommendations, with some guidelines advocating colistin aerosolization in ventilator-associated pneumonia (VAP) as an adjunct to (and not instead of) intravenous therapy [1,11], while some others do not [2].

Currently, inhaled colistin (either in the form of CMS or colistin sulphate) is the most frequently used nebulized antimicrobial treatment in ICUs globally; it is estimated that more than two-thirds of ICUs are actively using this treatment [12]. This is in sharp contrast with the evidence on its efficacy, and lack thereof, and the insufficient knowledge of the pharmacokinetics of CMS/colistin in the lung. Indeed, only a handful of studies have been published that deal with the pharmacokinetics of colistin in the respiratory system, altogether comprising 64 patients [13–16]. Furthermore, published experience only refers to nebulization via vibrating mesh nebulizers (VMNs) with a complete paucity of data on jet nebulization. In the literature, the superiority of VMNs over jet nebulizers (JNs) is supported by in vivo and in vitro studies, indicating advantages of the former consisting of a higher respirable inhaled mass [17], a greater deposition to the lung parenchyma, and a lower residual volume that allows the administration of higher doses in a smaller amount of time [18]. On the other hand, VMNs are not widely available, especially in resource-limited environments, and JNs remain the most frequently used modality in ICUs worldwide [19]. As such, any information on the comparative pharmacokinetic (PK) features of these technologies is of undisputable value and forms the objective of this study.

2. Results

Twenty-five patients were consented for study participation. One patient developed severe bronchospasm upon CMS inhalation completion, which precluded the performance of the lavage procedure. Thus, a total of 24 patients with ventilator-associated tracheobronchitis (VAT, $n = 10$) or VAP ($n = 14$) were enrolled in the study. Six patients received CMS via JN and 18 via VMN. The patient flow chart is depicted in Figure 1. The characteristics of the study population are shown in Table 1. Patients in the VMN group were older, with higher admission Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, however, had a higher $\text{PaO}_2/\text{FiO}_2$ ratio on study entry. Ventilator-associated pneumonia represented 61% and 50% of the diagnosed infections in the VMN and JN groups, respectively.

The achieved colistin concentration in the epithelial lining fluid (ELF) ($C_{\text{colistin, ELF}}$) at consecutive time points with the two modalities under comparison are shown in Figure 2. Median (IQR) values of $C_{\text{colistin, ELF}}$ obtained with VMN were 0.7 (0.4–1.3) mg/L, 2.1 (1.0–8.5) mg/L, 8.6 (4.8–18.9) mg/L, and 1.42 (0.5–2.5) mg/L, at 1, 4, 6 and 8 h post-nebulization, respectively. Median values of $C_{\text{colistin, ELF}}$ achieved with JN at the same time points were 1.2 (1.0–1.7) mg/L, 6.42 (5.9–8.3) mg/L, 3.3 (2.5–4.0) mg/L and 1.3 (1.2–1.3) mg/L, respectively.

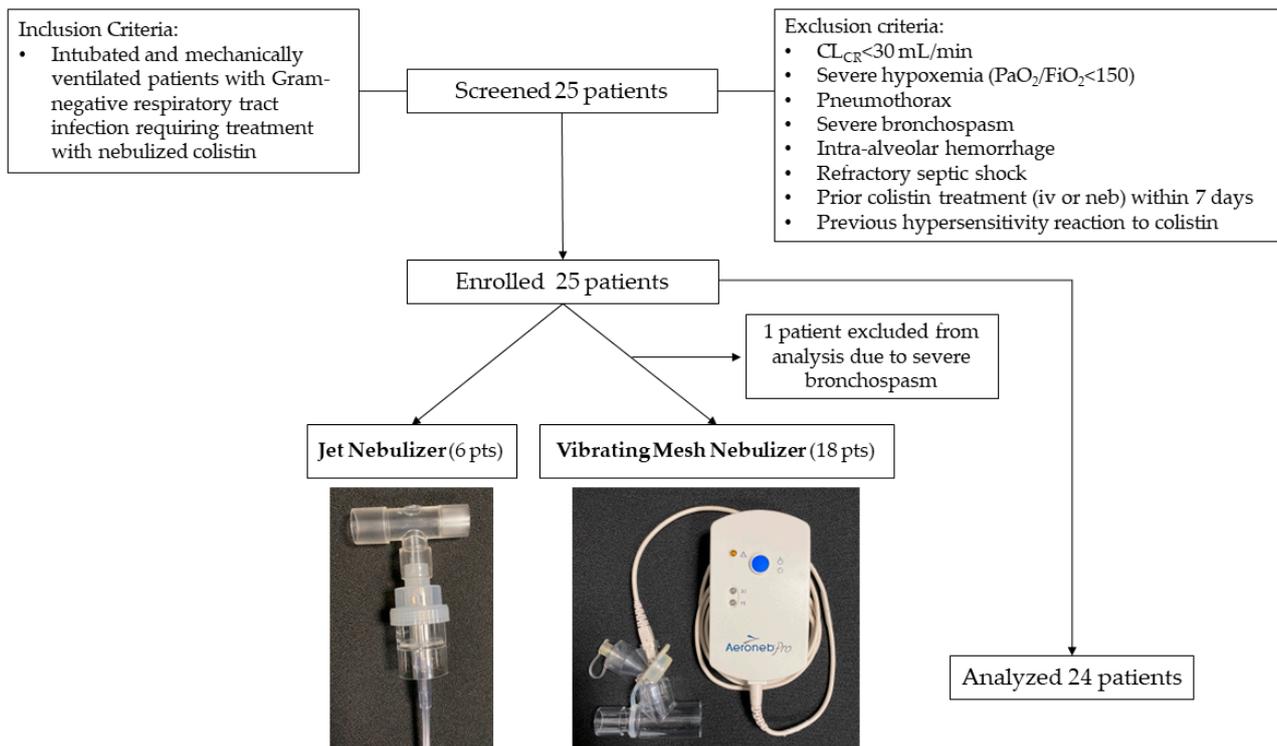


Figure 1. Patient flow chart. iv: intravenous, neb: nebulized.

Table 1. Patient baseline characteristics. VMN: vibrating mesh nebulizer; JN: jet nebulizer; IQR: interquartile range; APACHE II: Acute Physiology and Chronic Health Evaluation II score; VAP: ventilator-associated pneumonia; VAT: ventilator-associated tracheobronchitis.

Characteristic		VMN (n = 18)	JN (n = 6)
Median age in years (IQR)		69.5 (56.8–83.0)	57.5 (55.5–67.3)
Female gender, n (%)		5 (27.8)	1 (16.7)
Caucasian race, n (%)		18 (100)	6 (100)
Mean admission APACHE II (SD)		21.4 (6.3)	18.2 (6.0)
Type of infection (%)	VAP	11 (61.1%)	3 (50%)
	VAT	7 (38.9%)	3 (50%)
Isolated pathogen (%)	<i>A.baumannii</i>	11 (61.1%)	3 (50%)
	<i>K.pneumoniae</i>	2 (11.1%)	
	Both	2 (11.1%)	
	Culture negative	3 (16.7%)	3 (50%)
Median creatinine clearance, mL/min (IQR)		53.0 (40–65.5)	86 (67.5–122.0)
Mean PaO_2/FiO_2 (SD)		258.7 (88.0)	190.3 (68.1)

As shown in Figure 2, both nebulizers achieved concentrations in the ELF higher than the European Committee on Antimicrobial Susceptibility Testing (EUCAST) minimum inhibitory concentration (MIC) breakpoint for *Enterobacterales* and *Acinetobacter* spp. [20], with the effect being more pronounced for the VMN device and the 4 h and 6 h timepoints.

The median (IQR) values of colistin concentration in plasma ($C_{colistin, plasma}$) using VMN nebulization were 0.6 (0.4–0.7) mg/L, 2.3 (1.4–2.7) mg/L, 1.7 (1.1–2.0) mg/L, 0.8 (0.7–0.9) mg/L, 0.4 (0.3–0.6) mg/L, and 0.2 (0.2–0.4) mg/L, at 0.5, 1, 2, 4, 6 and 8 h post-nebulization. The corresponding values with JN were 0.1 (0.1–0.1) mg/L, 0.1 (0.1–0.2) mg/L, 0.3 (0.3–0.4) mg/L, 0.2 (0.2–0.2) mg/L, 0.1 (0.1–0.1) mg/L, and 0.1 (0.1–0.1) mg/L (Figure 3). Median $C_{colistin, plasma}$

was greater than 2 mg/L at 1 h post-nebulization, a value which is the average concentration, in steady-state conditions, that predicts acute renal failure [21,22]. Such an occurrence was not evident for nebulization via the JN device (Figure 3).

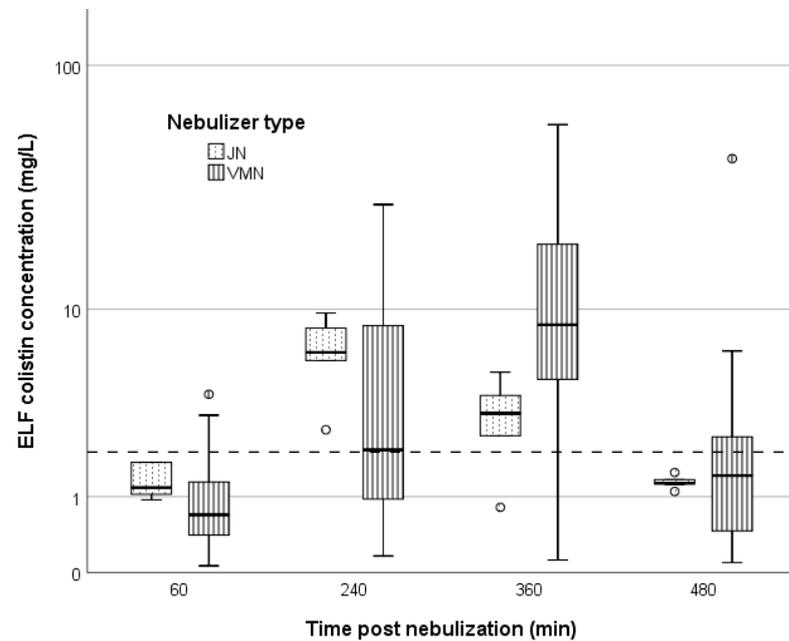


Figure 2. Colistin concentration in the ELF at 60, 240, 360 and 480 min after inhalation of 2 million international units of colistimethate sodium via jet nebulizers (dotted boxplots) or vibrating mesh nebulizers (vertical line boxplots). The dashed line represents the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for susceptible *Enterobacterales* and *Acinetobacter* spp. (2 mg/L). Circles represent outliers. ELF: epithelial lining fluid, JN: jet nebulizers, VMN: vibrating mesh nebulizers.

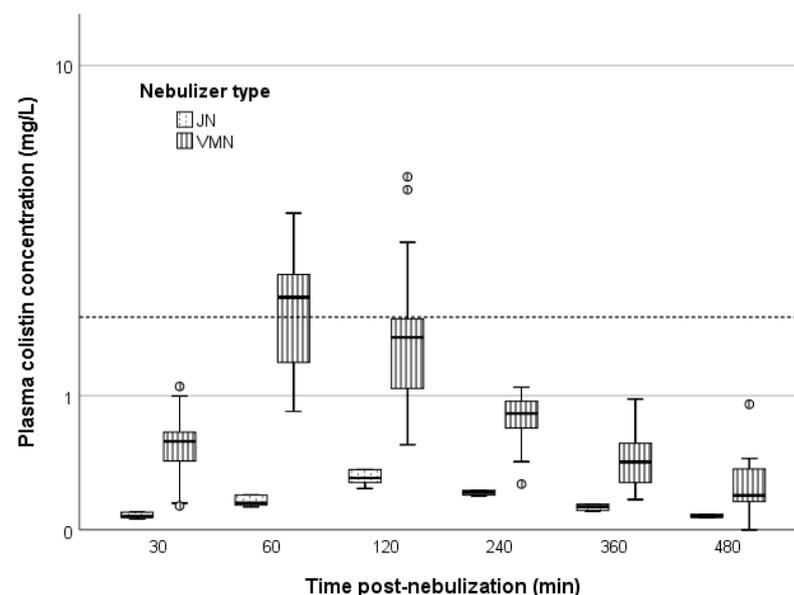


Figure 3. Colistin plasma concentration at 30, 60, 120, 240, 360 and 480 min after inhalation of 2 million international units of colistimethate sodium via jet nebulizers (dotted boxplot) and vibrating mesh nebulizers (vertical line boxplot). The dashed line represents the average steady-state plasma colistin concentration (2 mg/L) associated with the occurrence of acute renal failure according to [21]. Circles represent outliers. JN: jet nebulizers, VMN: vibrating mesh nebulizers.

Pharmacokinetic parameters of colistin in the ELF and plasma are shown in Table 2. Median maximum concentration (C_{max}) in the ELF was arithmetically greater in the VMN group than in the JN group (10.4 vs 7.4 mg/L, respectively), however the difference was not statistically significant (p : 0.549). A later peak (by approximately 2 h) was observed with the VMN device. Accordingly, while at 2 h from the index nebulization, a greater percentage of JN patients had ELF concentrations over 2 mg/L (the EUCAST breakpoint for colistin susceptibility among *Acinetobacter* spp. and *Enterobacteriales*), this was reversed at 6 and 8 h. Considering the EUCAST breakpoint for *Pseudomonas* spp. (4 mg/L), JN-treated patients had more frequently at 4 h, and less frequently at 6 h post-nebulization, exceeding ELF concentrations. Overall, the ELF exposure achieved with the two devices was comparable, and their difference was not statistically significant (p : 0.871).

Table 2. Pharmacokinetic/pharmacodynamic (PK/PD) parameters in epithelial lining fluid (ELF) and in plasma after inhalation of 2 million international units of colistimethate sodium via jet nebulizer (JN) or vibrating mesh nebulizer (VMN). Values are expressed as frequencies or medians and interquartile ranges, unless otherwise specified. Comparisons with statistically significant differences are depicted in bold.

PK/PD parameter	ELF			Plasma		
	VMN	JN	p Value	VMN	JN	p Value
AUC _{0-8h} , mg/L·h	28.7 (15.1–68.1)	30.5 (26.0–36.8)	0.871	7.5 (5.5–8.8)	1.3 (1.3–6.4)	0.039
AUC ₀₋₂₄ , mg/L·h	86.2 (46.0–185.9)	91.5 (78.1–110.3)	0.871	22.45 (±9.1)	4.0 (3.8–19.3)	0.039
$C_{colistin}$ over 2 mg/L						
30 min, %				0	0	
60 min, %	16.7	16.7	1.0	61.1	16.7	0.155
120 min, %				22.2	16.7	1.0
240 min, %	50	100	0.052	5.6	16.7	0.446
360 min, %	88.9	83.3	1.0	0	0	
480 min, %	41.2	0	0.124	0	0	
$C_{colistin}$ over 4 mg/L						
60 min, %	5.6	16.7	0.446			
240 min, %	27.8	83.3	0.050			
360 min, %	83.3	33.3	0.038			
480 min, %	17.6	0	0.539			
C_{max} , mg/L	10.4 (4.7–22.6)	7.4 (6.2–10.3)	0.549	2.6 (2.0–3.5)	0.3 (0.3–1.6)	0.016
C_{min} , mg/L	0.4 (0.1–0.8)	1.1 (0.9–1.2)	0.006	0.2 (0.1–0.3)	0.1 (0.1–0.2)	0.016
T_{max} , h	6.5 (6.3–6.6)	4.3 (3.5–4.3)	0.003	1.5 (1.4–1.7)	2.2 (2.1–2.2)	0.013
Volume of distribution/ fraction of dose absorbed (L)	1.7 (0.8–5.2)	4.5 (3.5–5.1)	0.141	22.8 (16.8–33.0)	129.8 (95.6–151.9)	0.016
Clearance/fraction of dose absorbed (L/h)	1.4 (0.8–3.6)	1.8 (1.5–2.1)	0.779	7.3 (6.0–9.1)	37.6 (23.2–39.9)	0.016
Mean $T_{1/2}$, h (SD)	-	-		2.4 (1.1)	2.4 (0.5)	
Mean ELF/Plasma ratio (SD)	5.5 (13.2)	4.8 (1.4)				

Regarding the plasma compartment, a greater amount of formed colistin was observed in the VMN group, as evidenced by both the higher area under the curve (AUC) and C_{max} . At various timepoints, a considerable percentage of patients had plasma concentrations over 2 mg/L, a value associated with acute renal failure [21,22], with the effect being almost exclusively present in the VMN group. The ELF/plasma mean concentration ratio was comparable between the two groups (5.5 vs 4.8 for VMN and JN, respectively).

Safety

There were no instances of clinically important deterioration of oxygenation during the nebulization. One patient developed bronchospasm upon completion of CMS inhalation via VMN and was treated promptly by inhaled β_2 -agonists and ipratropium, which led to a rapid recovery.

3. Discussion

In this single-center study, we compared the pharmacokinetic properties of formed colistin in the ELF and the plasma compartment when inhaled CMS, at a typical dose,

was administered either via a VMN or a generic JN. According to our results, the two devices were associated with comparable exposures in the lung, of a level, however, that will be doubtful in leading to desirable clinical results. Indeed, the median total (as opposed to unbound) AUC_{0-24} in the ELF for the two modalities were 86.2 and 91.5 mg/L·h for VMN and JN, respectively. Assuming an unbound fraction of colistin in the ELF of 0.05 to 0.1 [16], this translates to a free colistin exposure that would be 10 to 20-fold lower and in the range of 5–10 mg/L·h for both devices. Relatively recent evidence suggests that the optimal pharmacokinetic/pharmacodynamic index for colistin is the area under the unbound colistin concentration curve divided by the pathogen MIC (fAUC/MIC) [23,24]. At typical plasma maximum concentrations, an fAUC/MIC of 25 has been linked to 2-log killing in in vitro experiments for *Klebsiella pneumoniae* [23], while values of 12.2–22.8 and 7–42 have been found to predict 1-log killing in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* animal thigh and lung infection models [24]. Assuming that these considerations and hypotheses are true, and given the rising MICs to colistin among critically ill patients in endemic environments [25] (where this inhalational antimicrobial treatment is needed the most), it is highly unlikely that for the “median” patient the AUCs measured herein will have any positive impact on the infection outcome. Indeed, suboptimal exposure might lie behind reported failures of inhaled polymyxins to objectively improve clinical outcomes [8,26].

The equivalence of exposure of the two devices came as a surprise, since evidence has suggested that VMNs are linked to higher exposures for several reasons [27]. The number of patients that received JN-delivered treatment was small, however these results provide a basis for JN nebulization of CMS in areas where VMNs are not available.

JN nebulization led to an earlier peak of ELF concentration, a similar exposure and a slightly lower C_{max} . Indeed, at 4 h post-nebulization, six out of six JN-treated patients had ELF concentrations over 2 mg/L, while five out of six had values over 4 mg/L. The speed of peak concentration attainment might be relevant in critical settings. If these findings are verified in a larger population, then higher doses and shorter between-dose intervals in JN-treated patients (as opposed to VMN-treated) might be worth considering.

Our results were in concordance with a previous study in a similar ICU setting, where administration of 1 mIU of CMS via VMN led to a median (IQR) C_{max} of 6.7 (4.8–10.1) mg/L [13], while inhalation of the double dose in our study led to a median C_{max} of approximately 1.5-fold. The same pattern was evident in systemic absorption and plasma levels (median C_{max} was 2.6 mg/L in our study vs. 1.6 mg/L in the Athanassa et al. paper [13]). However, the ELF exposure was similar, despite the higher dose, probably due to a higher clearance from the lung compartment in our study, as evidenced by the lower C_{min} . The reason for this discrepancy is not straightforward; although, it might be due to the inter-patient variability of CMS/colistin pharmacokinetics, disease characteristics, the mode of the mechanical ventilation application, or differences in the manufacturing process of CMS over the years.

Contrarily, our results were only marginally compatible with the results of Boisson et al. [14] and Gkoufa et al. [16]. In the former study, the range of colistin concentration in the ELF following a 2 mIU inhalation spanned from 9.53 to 1137 mg/L [14], while it was between 0.8 mg/L and 57.9 mg/L in the present study. In the latter study, the exact values were not reported, however in the figured data it was shown that after an inhalation of 3 to 5 mIU of CMS, formed colistin concentrations were in the range of 10^2 to 10^3 mg/L [16]. Again, it is not known whether these differences were due to pre-analytical/analytical procedures or were the result of variability of colistin pharmacokinetics, which has been observed in repeated instances [14,16]. Furthermore, despite the higher colistin ELF concentration in these studies, the systemic absorption was far less compared with our observations. Indeed, the plasma concentrations in the two papers were generally below 1 mg/L [14,16], whereas in our study 50% of the VMN-treated population had plasma C_{max} over 2.6 mg/L, and 25% had values over 3.5 mg/L. In any case, if such values are substantiated in future research, then this should alert clinicians to the danger of acute renal failure in nebulized CMS-treated patients, since formed colistin plasma values over 2–2.5 mg/L have been linked to the occurrence of nephrotoxicity [21,22].

Nevertheless, in a piglet model of ventilated *P. aeruginosa* inoculation pneumonia, Lu et al. observed a serum maximum colistin concentration of 1.6 ± 1.4 mg/L after VMN aerosolization of 0.1 mIU/kg of CMS [28]. Upon animal sacrifice, 1 h after the third inhaled dose, it was noted that the median peak lung tissue concentration was 2.8 μ g/g, suggesting that a certain level of systemic absorption is required for a sufficient colistin amount to reach the extracellular space of lung parenchyma [28]. Post-nebulization colistin tissue levels have never been measured in humans; if the results above are reproducible in the human host, and given that Lu et al. observed a positive infection outcome in their animal model with inhaled treatment, one should acknowledge not only the risks of systemic absorption, but also the benefit of it as a side effect of desirable tissue penetration.

The inter-patient variability in colistin ELF exposure that we and others have observed [14–16], and the importance of the exposure to the infection outcome [23], probably mandates that every effort should be made for therapeutic drug monitoring (TDM) to be applied in difficult-to-treat infections with demanding pathogens. It is worth noting that this variability was more pronounced in VMN-treated patients. With a similar rationale, TDM has been advocated for systemic CMS administration [11,23,29], and given the safety and costs of the mini-bronchoalveolar lavage (BAL) procedure, “respiratory TDM” for colistin should be considered as an option.

The strengths of our study are the strict clinical, pre-analytical and analytical protocol that vouch for the internal validity of our findings. Furthermore, patients were naïve of systemic CMS administration, so confounding by recent or remote receipt of the drug was totally avoided. One limitation of our research was the relatively smaller size of the JN population, owing to the pragmatic design of the study. Additional weaknesses were the decision to forfeit collection of outcome data and the imbalances between the two groups. An example of the latter was the non-statistically significant (p : 0.177) 12 year difference in median age between the two groups. However, the modest expected decline in pulmonary function in such a duration [30] and the strict nebulization process in patients that were mechanically ventilated argue against a significant effect on our results. All these shortcomings were partially justified by logistical issues and by our specific aim of comparing the pharmacokinetics associated with the two devices. Additionally, we opted for using mini-BAL to approach lung pharmacokinetics. It is well-known that the lavage might be contaminated by tracheal secretions [31,32], however until microdialysis techniques become widely available [33], bronchoalveolar lavage is the only, logistically feasible, option. Nevertheless, the results presented herein should ideally be verified by *in vivo* microdialysis studies. Furthermore, we did not measure CMS, which could have helped us elucidate the differences with other studies that we noted [14,16]; and, lastly, we did not build a population pharmacokinetic model.

4. Materials and Methods

4.1. Study Population

The study population consisted of 24 critically ill adult patients, admitted to the intensive care unit of the First Department of Pulmonology of the National and Kapodistrian University of Athens between June 2014 and November 2017. All patients were intubated and mechanically ventilated, and received inhaled CMS as part of their treatment for VAP or VAT caused by Gram-negative pathogens. Subjects were studied around the first dose of nebulized treatment, which always preceded intravenous treatment, to avoid confounding by colistin (or CMS) reaching the ELF via the systemic circulation. We excluded patients that received a dose different to the institution’s standard of 2 million international units (mIU) (~160 mg) of CMS and patients who were planned to receive intravenous colistin within 8 h of nebulization initiation. We also excluded patients with: (i) a creatinine clearance (CR_{CL}) of less than 30 mL/min, (ii) severe hypoxemia defined as PaO_2/FiO_2 of less than 150, (iii) pneumothorax, (iv) severe bronchospasm, (v) intra-alveolar hemorrhage, (vi) refractory septic shock, (vii) prior colistin treatment, either intravenously or by inhala-

tion, within 7 days prior to index nebulization and, (viii) previous hypersensitivity reaction to colistin (Figure 1).

Demographic, clinical, and microbiological characteristics of the study population were recorded on the first day of nebulized CMS administration.

4.2. Study Procedures

A dose of 2 mIU of CMS (Colistin[®], Norma Hellas, Athens, Greece), corresponding to approximately 160 mg CMS or 68 mg colistin-base activity, was nebulized over a 10–30 min period via either VMN (Aeroneb Solo with Aeroneb Pro controller, Aerogen, Galway, Ireland) or generic JN. This was not a randomized trial, and the nebulizer selection was exclusively a matter of availability of VMN consumables in the ICU at the time. The intended dose was dissolved in 4 mL of normal saline 0.9%. The nebulizer was placed at the inspiratory limb of the ventilator circuit, at 15 cm from the Y-piece, and the heat and moisture exchanger was removed. During nebulization, the patients were sedated and ventilated using assist volume controlled mode, with tidal volume set at 8 mL/kg of ideal body weight, a constant squared-wave inspiratory flow, a positive end expiratory pressure (PEEP) of less than or equal to 10 cm H₂O, an inspiratory to expiratory time ratio equal to 1, an inspiratory pause of 10%, and a respiratory rate set at 15/min [31].

Mini BAL was performed just before, and at 1, 4, and 8 h after the index nebulization, via a 65 cm long, 12 F diameter sterile catheter (Kimberly-Clark, Roswell, GA, USA). The catheter was inserted through the endotracheal tube. The lavage was performed with instillation of 2 syringes of 20 mL sterile normal saline at room temperature through the catheter. The aspirate from the first syringe was considered representative of bronchial washing and was, therefore, discarded. The aspirate from the second syringe was collected and centrifuged in frigid centrifuge (3500 × *g* for 10 min at 4 °C) immediately (<2 min) to avoid falsely elevated levels of urea in BAL [34] and CMS hydrolysis to formed colistin. The supernatant liquid was stored in plastic micro test tubes (Safe-lock; Eppendorf AG; Hamburg, Germany) and frozen at −80 °C, until analysis.

Blood samples (5 mL) were collected through a peripheral arterial line just before and at 0.5, 1, 2, 4, and 8 h post-nebulization initiation. The specimens were immediately centrifuged (3500 × *g* for 10 min) in a frigid centrifuge (at 4 °C) and supernatants were collected in Eppendorfs and were frozen at −80 °C until analysis.

4.3. Colistin Measurement Assay in Plasma and BAL Fluid

Colistin concentrations in plasma ($C_{\text{colistin,plasma}}$) and epithelial lining fluid ($C_{\text{colistin,ELF}}$) were measured through high performance liquid chromatography (HPLC)-fluorescence analysis.

4.3.1. Instrumentation

The liquid chromatographic system consisted of a Shimadzu LC-20 AD pump with a DGU-20 A_{5R} degasser, a SIL-HT thermostated auto sampler, a CTO-20 AC oven and an RF-20 A prominence fluorescence detector.

4.3.2. Plasma and BAL Fluid Assay

The samples were rapidly centrifuged, and plasma and BAL supernatant were separated and kept frozen (−80 °C) before analysis, as previously described. An HPLC-RF assay was used for colistin concentration measurements in plasma and BAL fluid samples, based on the method of Li et al. [35], as modified by Markou et al. [36] and Ratjen et al. [37]. Briefly, netilmicin was used as the internal standard and the method consisted of a protein precipitation step with trifluoroacetic acid (TFA), followed by colistin derivatization with 9-fluorenylmethyl chloroformate on a solid phase extraction C18 cartridge. The eluted fluid was dried under vacuum and the solid residue was reconstituted in mobile phase for subsequent reversed-phase HPLC with fluorescence detection (excitation: 260 nm; emission: 316 nm). The analytical column was a Phenomenex Luna C18, 3 μm, 50 mm × 3 mm with 100 Å particle size, while the mobile phase consisted of ACN/THF/H₂O (80/7.7/12.3%)

with a flow rate of 0.5 mL/min. The injection volume was 20 μ L. The method was linear at a concentration range of 0.10–4.8 mg/L ($R^2 = 0.999$). The limit of detection (LOD) and lower limit of quantification (LLOQ) were 0.01 and 0.04 mg/L, respectively, while the intra- and inter-assay variabilities were less than 16%. Representative chromatograms and calibration curves in plasma and BAL are given in the Supplementary Material (Figures S1 and S2).

4.3.3. Urea Plasma and BAL Assay

Urea concentration in plasma (Urea_{plasma}) and BAL (Urea_{BAL}) of all patients were also measured, applying an enzymatic UV method, and the ratio of Urea_{plasma}/Urea_{BAL} was used as a dilution factor to correct measured colistin concentration in BAL and calculate actual ELF concentrations as:

$$C_{\text{ELF}} = C_{\text{BAL}} \times \frac{\text{Urea}_{\text{plasma}}}{\text{Urea}_{\text{BAL}}} \quad (1)$$

4.3.4. PK Analysis

PK analysis was performed on measured serum and ELF concentrations using the Phoenix[®] version 8.3 PK/PD software package (Certara, Princeton, NJ, USA).

Noncompartmental analysis (NCA) was applied to plasma and ELF colistin concentration–time curves and basic PK parameters were calculated, namely, the maximum observed plasma and ELF colistin concentration (C_{max}) and the time C_{max} was observed (T_{max}) for each patient. The area under the concentration–time curves from time zero to the last experimental point (AUC_{0-t}) was obtained by the log-linear trapezoidal method. The last observed concentration divided by the terminal slope λ (k_{el}), was used to calculate the area under the concentration–time curve extrapolated to infinity (AUC_{inf}). The terminal slope was estimated by linear regression analysis on the last three points of the log-transformed concentrations vs. time plot. The elimination half-life was calculated as $t_{1/2} = 0.693/\lambda$. Total apparent clearance (CL/F) and volume of distribution (V_d/F) were evaluated as $\text{dose}/\text{AUC}_{\text{inf}}$, and $\text{dose}/(\lambda \times \text{AUC}_{\text{inf}})$, respectively. AUC_{0-24} was calculated as $\text{AUC}_{\text{inf}} \times 3$ (for thrice daily administration).

4.4. Safety

Subjects were medically followed during the nebulization phase and the post-nebulization period for the development of side effects, especially those related to the respiratory system. In cases of significant hypoxemia and/or bronchospasm, the nebulization and lavage processes were suspended, and the patient was treated as clinically indicated.

4.5. Statistical Analysis

Statistical analysis was performed using IBM's SPSS statistics v.24.0. Data were summarized as frequencies or as the mean value \pm standard deviation (SD), or as the median and interquartile range (IQR), as appropriate. A Mann–Whitney non-parametric U-test was performed to compare differences between groups, and Fisher's exact test was employed for the comparisons between frequencies. A p-value of 0.05 was considered as the threshold of statistical significance.

4.6. Protocol Approval and Ethical Considerations

The clinical protocol was reviewed and approved by the Institutional Review Board (IRB) of the General Hospital for the Diseases of the Chest "I Sotiria" (Approval numbers: 6764/15.03.2013, 04155/26.05.2015 and 1132/18.01.2017—original protocol and subsequent amendments). The IRB consists of nine health professionals (physicians and nurses) with discrete levels of hierarchy, and is imbued with the role of an ethics committee. Approval was also provided by the Special Research Account of the National and Kapodistrian University of Athens (ELKE, Approval Number 12483) which was also responsible for the financial management of the study, in accordance with local regulations. All patients were mechanically ventilated and unconscious and, thus, written informed consent was obtained from their legally acceptable representatives, as per local legislation. The study was sponsored by Norma

Hellas, however the conduct of the study, the decision to submit the manuscript and the content of this report were exclusively a responsibility of the investigators.

This work was presented, in part, in the American Thoracic Society International Congress held in Philadelphia, TN, USA. between 15 and 20 May 2020 [38].

5. Conclusions

Given the assumptions and limitations of the current study, inhalation of 2 mIU of CMS led to similar exposures when administered either via VMN or JN nebulization. The latter resulted in lower colistin plasma levels, below the average steady-state plasma colistin concentration (2 mg/L) associated with the occurrence of acute renal failure. Accordingly, JN appears as a viable substitute of VMN in cases of availability issues, however a different dose and interval might be considered. Apart from the comparison between devices, the measured ELF exposure casts doubt on whether the selected dose might be relied upon for difficult-to-treat infections, while, contrary to most published evidence, systemic colistin absorption was not negligible, especially with the vibrating mesh technology.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/antibiotics11111528/s1>: Figure S1. Representative chromatograms of Colistin and Netilmicin (I.S.) in plasma (A) and BAL (B). Figure S2. Representative calibration curves in plasma (A) and BAL (B).

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the General Hospital for the Diseases of the Chest “I Sotiria” (approval numbers: 6764/15.03.2013, 04155/26.05.2015 and 1132/18.01.2017—original protocol and subsequent amendments) and by the Special Research Account of the National and Kapodistrian University of Athens (ELKE, Approval Number 12483) for studies involving humans.

Informed Consent Statement: Informed consent was obtained from the legally acceptable representatives of all subjects involved in the study.

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References

1. Kalil, A.C.; Metersky, M.L.; Klompas, M.; Muscedere, J.; Sweeney, D.A.; Palmer, L.B.; Napolitano, L.M.; O'Grady, N.P.; Bartlett, J.G.; Carratalà, J.; 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*, e61–e111. [CrossRef]
2. Rello, J.; Solé-Lleonart, C.; Rouby, J.J.; Chastre, J.; Blot, S.; Poulakou, G.; Luyt, C.E.; Riera, J.; Palmer, L.B.; Pereira, J.M.; et al. Use of nebulized antimicrobials for the treatment of respiratory infections in invasively mechanically ventilated adults: A position paper from the European Society of Clinical Microbiology and Infectious Diseases. *Clin. Microbiol. Infect.* **2017**, *23*, 629–639. [CrossRef]

3. Niederman, M.S.; Alder, J.; Bassetti, M.; Boateng, F.; Cao, B.; Corkery, K.; Dhand, R.; Kaye, K.S.; Lawatscheck, R.; McLeroth, P.; et al. Inhaled amikacin adjunctive to intravenous standard-of-care antibiotics in mechanically ventilated patients with Gram-negative pneumonia (INHALE): A double-blind, randomised, placebo-controlled, phase 3, superiority trial. *Lancet Infect. Dis.* **2020**, *20*, 330–340. [CrossRef]
4. Kollef, M.H.; Ricard, J.-D.; Roux, D.; Francois, B.; Ischaki, E.; Rozgonyi, Z.; Boulain, T.; Ivanyi, Z.; János, G.; Garot, D.; et al. A Randomized Trial of the Amikacin Fosfomycin Inhalation System for the Adjunctive Therapy of Gram-Negative Ventilator-Associated Pneumonia. *Chest* **2017**, *151*, 1239–1246. [CrossRef]
5. Levin, A.S.; Barone, A.A.; Penço, J.; Santos, M.V.; Marinho, I.S.; Arruda, E.A.; Manrique, E.I.; Costa, S.F. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin. Infect. Dis.* **1999**, *28*, 1008–1011. [CrossRef]
6. Falagas, M.E.; Kasiakou, S.K. Colistin: The revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin. Infect. Dis.* **2005**, *40*, 1333–1341. [CrossRef]
7. Michalopoulos, A.; Kasiakou, S.K.; Mastora, Z.; Rellos, K.; Kapaskelis, A.M.; Falagas, M.E. Aerosolized colistin for the treatment of nosocomial pneumonia due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Crit. Care* **2005**, *9*, R53–R59. [CrossRef]
8. Rattanaumpawan, P.; Lorsutthitham, J.; Ungprasert, P.; Angkasekwinai, N.; Thamlikitkul, V. Randomized controlled trial of nebulized colistimethate sodium as adjunctive therapy of ventilator-associated pneumonia caused by Gram-negative bacteria. *J. Antimicrob. Chemother.* **2010**, *65*, 2645–2649. [CrossRef]
9. Abdellatif, S.; Trifi, A.; Daly, F.; Mahjoub, K.; Nasri, R.; Lakhali, S. Ben Efficacy and toxicity of aerosolised colistin in ventilator-associated pneumonia: A prospective, randomised trial. *Ann. Intensive Care* **2016**, *6*, 26. [CrossRef]
10. Valachis, A.; Samonis, G.; Kofteridis, D.P. The role of aerosolized colistin in the treatment of ventilator-associated pneumonia: A systematic review and metaanalysis. *Crit. Care Med.* **2015**, *43*, 527–533. [CrossRef]
11. Tsuji, B.T.; Pogue, J.M.; Zavascki, A.P.; Paul, M.; Daikos, G.L.; Forrest, A.; Giacobbe, D.R.; Viscoli, C.; Giamarellou, H.; Karaiskos, I.; et al. International Consensus Guidelines for the Optimal Use of the Polymyxins: Endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA). *Pharmacotherapy* **2019**, *39*, 10–39. [CrossRef]
12. Solé-Lleonart, C.; Roberts, J.A.; Chastre, J.; Poulakou, G.; Palmer, L.B.; Blot, S.; Felton, T.; Bassetti, M.; Luyt, C.E.; Pereira, J.M.; et al. Global survey on nebulization of antimicrobial agents in mechanically ventilated patients: A call for international guidelines. *Clin. Microbiol. Infect.* **2016**, *22*, 359–364. [CrossRef]
13. Athanassa, Z.E.; Markantonis, S.L.; Fousteri, M.-Z.F.; Myriantsefs, P.M.; Boutzouka, E.G.; Tsakris, A.; Baltopoulos, G.J. Pharmacokinetics of inhaled colistimethate sodium (CMS) in mechanically ventilated critically ill patients. *Intensive Care Med.* **2012**, *38*, 1779–1786. [CrossRef]
14. Boisson, M.; Jacobs, M.; Grégoire, N.; Gobin, P.; Marchand, S.; Couet, W.; Mimos, O. Comparison of Intrapulmonary and Systemic Pharmacokinetics of Colistin Methanesulfonate (CMS) and Colistin after Aerosol Delivery and Intravenous Administration of CMS in Critically Ill Patients. *Antimicrob. Agents Chemother.* **2014**, *58*, 7331–7339. [CrossRef]
15. Boisson, M.; Grégoire, N.; Cormier, M.; Gobin, P.; Marchand, S.; Couet, W.; Mimos, O. Pharmacokinetics of nebulized colistin methanesulfonate in critically ill patients. *J. Antimicrob. Chemother.* **2017**, *72*, 2607–2612. [CrossRef]
16. Gkoufa, A.; Sou, T.; Karaiskos, I.; Routsis, C.; Lin, Y.W.; Psychogiou, M.; Zakyntinos, S.; Giamarellou, H.; Li, J.; Friberg, L.E. Pulmonary and systemic pharmacokinetics of colistin methanesulfonate (CMS) and formed colistin following nebulisation of CMS among patients with ventilator-associated pneumonia. *Int. J. Antimicrob. Agents* **2022**, *59*, 106588. [CrossRef]
17. Galindo-Filho, V.C.; Alcoforado, L.; Rattes, C.; Paiva, D.N.; Brandão, S.C.S.; Fink, J.B.; Dornelas de Andrade, A. A mesh nebulizer is more effective than jet nebulizer to nebulize bronchodilators during non-invasive ventilation of subjects with COPD: A randomized controlled trial with radiolabeled aerosols. *Respir. Med.* **2019**, *153*, 60–67. [CrossRef]
18. Chang, K.H.; Moon, S.H.; Oh, J.Y.; Yoon, Y.S.; Gu, N.; Lim, C.Y.; Park, B.J.; Nam, K.C. Comparison of salbutamol delivery efficiency for jet versus mesh nebulizer using mice. *Pharmaceutics* **2019**, *11*, 192. [CrossRef]
19. Alves, J.; Alp, E.; Koulenti, D.; Zhang, Z.; Ehrmann, S.; Blot, S.; Bassetti, M.; Conway-Morris, A.; Reina, R.; Teran, E.; et al. Nebulization of antimicrobial agents in mechanically ventilated adults in 2017: An international cross-sectional survey. *Eur. J. Clin. Microbiol. Infect. Dis.* **2018**, *37*, 785–794. [CrossRef]
20. European Committee on Antimicrobial Susceptibility Testing. Breakpoints Tables for Interpretation of MICs and Zone Diameters. 2022. Available online: https://www.eucast.org/clinical_breakpoints (accessed on 27 September 2022).
21. Forrest, A.; Garonzik, S.M.; Thamlikitkul, V.; Giamarellos-Bourboulis, E.J.; Paterson, D.L.; Li, J.; Silveira, F.P.; Nation, R.L. Pharmacokinetic/Toxicodynamic Analysis of Colistin-Associated Acute Kidney Injury in Critically Ill Patients. *Antimicrob. Agents Chemother.* **2017**, *61*, e01367-17. [CrossRef]
22. Horcajada, J.P.; Sorlí, L.; Luque, S.; Benito, N.; Segura, C.; Campillo, N.; Montero, M.; Esteve, E.; Mirelis, B.; Pomar, V.; et al. Validation of a colistin plasma concentration breakpoint as a predictor of nephrotoxicity in patients treated with colistin methanesulfonate. *Int. J. Antimicrob. Agents* **2016**, *48*, 725–727. [CrossRef]
23. Tsala, M.; Vourli, S.; Georgiou, P.C.; Pournaras, S.; Tsakris, A.; Daikos, G.L.; Mouton, J.W.; Meletiadis, J. Exploring colistin pharmacodynamics against *Klebsiella pneumoniae*: A need to revise current susceptibility breakpoints. *J. Antimicrob. Chemother.* **2018**, *73*, 953–961. [CrossRef]

24. Bergen, P.J.; Landersdorfer, C.B.; Zhang, J.; Zhao, M.; Lee, H.J.; Nation, R.L.; Li, J. Pharmacokinetics and pharmacodynamics of “old” polymyxins: What is new? *Diagn. Microbiol. Infect. Dis.* **2012**, *74*, 213–223. [[CrossRef](#)]
25. Polemis, M.; Mandilara, G.; Pappa, O.; Argyropoulou, A.; Perivolioti, E.; Koudoumnakis, N.; Pournaras, S.; Vasilakopoulou, A.; Vourli, S.; Katsifa, H.; et al. COVID-19 and Antimicrobial Resistance: Data from the Greek Electronic System for the Surveillance of Antimicrobial Resistance—WHONET-Greece (January 2018–March 2021). *Life* **2021**, *11*, 996. [[CrossRef](#)]
26. Rouby, J.-J.; Zhu, Y.; Torres, A.; Rello, J.; Monsel, A. Aerosolized polymyxins for ventilator-associated pneumonia caused by extensive drug resistant Gram negative bacteria: Class, dose and manner should remain the trifecta. *Ann. Intensive Care* **2022**, *12*, 97. [[CrossRef](#)]
27. Lin, H.-L.; Fink, J.B.; Ge, H. Aerosol delivery via invasive ventilation: A narrative review. *Ann. Transl. Med.* **2021**, *9*, 588. [[CrossRef](#)]
28. Lu, Q.; Girardi, C.; Zhang, M.; Bouhemad, B.; Louchahi, K.; Petitjean, O.; Wallet, F.; Becquemin, M.-H.; Le Naour, G.; Marquette, C.-H.; et al. Nebulized and intravenous colistin in experimental pneumonia caused by *Pseudomonas aeruginosa*. *Intensive Care Med.* **2010**, *36*, 1147–1155. [[CrossRef](#)] [[PubMed](#)]
29. Ehrentraut, S.F.; Muenster, S.; Kreyer, S.; Theuerkauf, N.U.; Bode, C.; Steinhagen, F.; Ehrentraut, H.; Schewe, J.C.; Weber, M.; Putensen, C.; et al. Extensive therapeutic drug monitoring of colistin in critically ill patients reveals undetected risks. *Microorganisms* **2020**, *8*, 415. [[CrossRef](#)]
30. Thomas, E.T.; Guppy, M.; Straus, S.E.; Bell, K.J.L.; Glasziou, P. Rate of normal lung function decline in ageing adults: A systematic review of prospective cohort studies. *BMJ Open* **2019**, *9*, e028150. [[CrossRef](#)]
31. Rouby, J.J.; Sole-Lleonart, C.; Rello, J.; Monsel, A.; Constantin, J.-M.; Bouglé, A.; Blot, S.; Poulakou, G.; Pontikis, K.; Kyriakoudi, A.; et al. Ventilator-associated pneumonia caused by multidrug-resistant Gram-negative bacteria: Understanding nebulization of aminoglycosides and colistin. *Intensive Care Med.* **2020**, *46*, 766–770. [[CrossRef](#)]
32. Monsel, A.; Torres, A.; Zhu, Y.; Pugin, J.; Rello, J.; Rouby, J.-J.; European Investigators Network for Nebulized Antibiotics in Ventilator-associated Pneumonia (ENAVAP). Nebulized antibiotics for ventilator-associated pneumonia: Methodological framework for future multicenter randomized controlled trials. *Curr. Opin. Infect. Dis.* **2021**, *34*, 156–168. [[CrossRef](#)] [[PubMed](#)]
33. Rouby, J.J.; Monsel, A.; Leone, M.; Mimoz, O.; Laterre, P.F.; Pugin, J. The IASIS, INHALE and VAPORISE trials. Reasons for a triple failure: Study design, aminoglycosides dosing and technique of nebulisation. *Anaesth. Crit. Care Pain Med.* **2020**, *39*, 179–183. [[CrossRef](#)]
34. Baldwin, D.R.; Honeybourne, D.; Wise, R. Pulmonary disposition of antimicrobial agents: Methodological considerations. *Antimicrob. Agents Chemother.* **1992**, *36*, 1171–1175. [[CrossRef](#)]
35. Li, J.; Milne, R.W.; Nation, R.L.; Turnidge, J.D.; Smeaton, T.C.; Coulthard, K. Use of high-performance liquid chromatography to study the pharmacokinetics of colistin sulfate in rats following intravenous administration. *Antimicrob. Agents Chemother.* **2003**, *47*, 1766–1770. [[CrossRef](#)] [[PubMed](#)]
36. Markou, N.; Markantonis, S.L.; Dimitrakis, E.; Panidis, D.; Boutzouka, E.; Karatzas, S.; Rafailidis, P.; Apostolakos, H.; Baltopoulos, G. Colistin serum concentrations after intravenous administration in critically ill patients with serious multidrug-resistant, gram-negative bacilli infections: A prospective, open-label, uncontrolled study. *Clin. Ther.* **2008**, *30*, 143–151. [[CrossRef](#)] [[PubMed](#)]
37. Ratjen, F.; Rietschel, E.; Kasel, D.; Schwiertz, R.; Starke, K.; Beier, H.; van Koningsbruggen, S.; Grasemann, H. Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. *J. Antimicrob. Chemother.* **2006**, *57*, 306–311. [[CrossRef](#)]
38. Kyriakoudi, A.; Valsami, G.; Pontikis, K.; Avgeropoulou, S.; Neroutsos, E.; Christodoulou, E.; Moraitou, E.; Markantonis, S.; Dekoumetzidis, A.; Koutsoukou, A. Colistin Concentration in Epithelial Lining Fluid (ELF), Following High-Dose Colistimethate Sodium (CMS) Nebulization, Using Two Different Nebulizers. In *A59. Clinical Diagnosis, Prediction and Outcomes of Lung Infections*; American Thoracic Society: New York, NY, USA, 2020; p. A2137. [[CrossRef](#)]