Efficacy of High-dose Nebulized Colistin in Ventilator-associated Pneumonia Caused by Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Qin Lu, M.D., Ph.D.,* Rubin Luo M.D.,† Liliane Bodin, M.D.,* Jianxin Yang, M.D.,† Noël Zahr, Pharm.D.,‡ Alexandra Aubry, M.D., Ph.D.,§ Jean-Louis Golmard, M.D., Ph.D.,‖ Jean-Jacques Rouby, M.D., Ph.D.#; and the Nebulized Antibiotics Study Group**

ABSTRACT

Background: Colistin often remains the only active agent against multidrug-resistant Gram-negative pathogens. The aim of the study was to assess efficacy of nebulized colistin for treating ventilator-associated pneumonia (VAP) caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Methods: One hundred and sixty-five patients with VAP caused by *P. aeruginosa* and *A. baumannii* were enrolled in a prospective, observational, and comparative study. The sensitive strain group included 122 patients with VAP caused by *P. aeruginosa* and *A. baumannii* susceptible to β-lactams, aminoglycosides, or quinolones and treated with intravenous antibiotics for 14 days. The multidrug-resistant strain group included 43 patients with VAP caused by multidrug-resistant *P. aeruginosa* and *A. baumannii* and treated with nebulized colistin (5 million international units every 8 h) either in monotherapy (n = 28) or combined to a 3-day intravenous aminoglycosides for 7–19 days. The primary endpoint was clinical cure rate. Aerosol was delivered using vibrating plate nebulizer.

Results: After treatment, clinical cure rate was 66% in sensitive strain group and 67% in multidrug-resistant strain group (difference −1%, lower limit of 95% CI for difference −12.6%). Mortality was not different between groups (23 *vs.* 16%). Among 16 patients with persisting or recurrent *P. aeruginosa* infection, colistin minimum inhibitory concentration increased in two patients.

Conclusion: Nebulization of high-dose colistin was effective to treat VAP caused by multidrug-resistant *P. aeruginosa or A. baumannii*. Its therapeutic effect was noninferior to intravenous β-lactams associated with aminoglycosides or quinolones for treating VAP caused by susceptible *P. aeruginosa* and *A. baumannii*.

What We Already Know about This Topic

- Colistin is often the only active antibiotic for multidrug-resistant (MDR) gram negative pathogens

What This Article Tells Us That Is New

- Using 5 million IU every 8 h of nebulized colistin in patients with ventilator-associated pneumonia caused by MDR organisms or intravenous β-lactams to patients with sensitive organisms, the clinical cure rate was similar in patients with sensitive (66%) or MDR (67%) *P. aeruginosa and A. baumannii*

VENTILATOR-ASSOCIATED pneumonia (VAP) caused by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is characterized by high rate of recurrence and frequent selection of new resistance to antibiotics despite adequate initial antimicrobial therapy. The decreased susceptibility of these strains to antibiotics has become a major health problem worldwide. Nowadays, few antimicrobials are available to treat Gram-negative multidrug-resistant VAP, and often, colistin remains the only active agent.[1]

* Praticiens Hospitaliers, # Professor of Anesthesiology and Critical Care and Medical Director of the Intensive Care Unit, Department of Anesthesiology and Critical Care Medicine, Multidisciplinary Intensive Care Unit Pierre Viars, La Pitié-Salpêtrière Hospital, Assistance-Publique-Hôpitaux-de-Paris, UPMC Univ, Paris, France. † Research Assistant, Department of Emergency Medicine, Second Affiliated Hospital, Zhejiang University, School of Medicine, Hangzhou, China. ‡ Praticien Hospitalier, Department of Pharmacology, La Pitié-Salpêtrière Hospital, UPMC Univ, Paris, France. § Maitre de Conférence des Universités-Praticien Hospitalier, Department of Bacteriology, La Pitié-Salpêtrière Hospital, UPMC Univ. ‖ Maitre de Conférence des Universités-Praticien Hospitalier, ER4 "Modélisation en recherche clinique" Université Pierre et Marie Curie et UF de Biostatistique, La Pitié-Salpêtrière Hospital, UPMC Univ ** See the appendix for members of the Nebulized Antibiotics Study Group.

Received from the Multidisciplinary Intensive Care Unit, Department of Anesthesiology and Critical Care Medicine, La Pitié-Salpêtrière Hospital, Assistance-Publique-Hôpitaux-de-Paris, UPMC Univ, Paris, France. Submitted for publication June 17, 2012. Accepted for publication September 13, 2012. Support was provided from institutional and/or departmental sources. Dr. Luo was the recipient of a research scholarship from the Association pour la Recherche Clinique et Expérimentale en Anesthésie-Réanimation (ARCEAR) of the Department of Anesthesiology and Critical Care Medicine, La Pitié-Salpêtrière Hospital, Paris, France. Dr. Yang was the recipient of a research scholarship from the Department of Emergency Medicine of the Second Affiliated Hospital of Hangzhou, China. Drs. Lu and Luo contributed equally to this work.

Address correspondence to Dr. Lu: Département d'Anesthésie-Réanimation, Réanimation Polyvalente Pierre Viars, Hôpital Pitié-Salpêtrière, 47–83 boulevard de l'Hôpital, 75013 Paris, France. qin.lu@psl.aphp.fr. Anesthesiology 2012; 117:1335-47

Although synergistic antibiotic activity with colistin combined to rifampicin or carbapenems has been shown *in vitro*,[2,3] the superiority of such combination therapy has never been demonstrated in patients.[4–6] Intravenous colistin monotherapy is therefore often used as salvage therapy in the treatment of patients with VAP caused by multidrug-resistant Gram-negative pathogens susceptible only to colistin.[7–9] Because of its poor lung tissue penetration,[10,11] the effectiveness of intravenous administration is highly questionable. Both success and failure of the treatment have been reported in a few observational, retrospective, or uncontrolled series. Another major concern regarding intravenous colistin monotherapy is the emergency of resistance.[12,13] The risk of acquisition of colistin resistance could be even higher if its lung tissue concentration is insufficient compared with minimal inhibitory concentration (MIC).[14]

Nebulization of antibiotics offers the possibility of generating high drug concentrations at the site of infection.[15–17] In a previous experimental study, nebulized colistin monotherapy resulted in high lung deposition and bactericidal effects in ventilated piglets with inoculation pneumonia caused by *P. aeruginosa* intermediate to ceftazidime.[10] A recent clinical study performed in patients with VAP reported that combined nebulization of ceftazidime and amikacin was effective against *P. aeruginosa* intermediate to ceftazidime and/or amikacin and may prevent per-treatment acquisition of antibiotic resistance.[18]

The objective of the study was to evaluate the efficacy of high-dose nebulized colistin for treating VAP caused by multidrug-resistant *P. aeruginosa* and *Acinetobacter baumannii*. A group of patients with VAP caused by susceptible *P. aeruginosa* or *A. baumannii* treated with conventional intravenous antibiotics served as controls. The second objective was to assess the risk of developing colistin resistance after administration of nebulized colistin in patients with recurrent VAP.

Materials and Methods

Study Design and Patients

This prospective, observational study was conducted from January 1, 2006, to December 31, 2010, in the 26-bed multidisciplinary intensive care unit (ICU) of La Pitié-Salpêtrière Hospital. The institutional review board of La Pitié-Salpêtrière Hospital, Paris, France, approved the study protocol. Because nothing more than routine diagnostic tests, monitoring, and treatment was performed during the study, informed consent from patients or their relatives was waived.

The eligible criteria for the study were age older than 18 yr, mechanical ventilation required for more than 48 h, and VAP caused by *P. aeruginosa* or *A. baumannii*. VAP was defined as the presence of new and persistent infiltrates on chest radiography and bedside lung ultrasound highly evocative of lung infection[19,20] associated with one of the following clinical features: (1) temperature ≥ 38.4°C or < 36.5°C; (2) leukocyte count > 11.10^3/ml; and (3) purulent bronchial secretions. *P. aeruginosa or A. baumannii* was confirmed in lower respiratory tract specimens sampled either by fiberoptic bronchoscopy with nonprotected bronchoalveolar lavage or protected mini-bronchoalveolar lavage.[21] A positive sample was defined as more than or equal to 10^4 colony-forming unit (CFU)/ml for nonprotected bronchoalveolar lavage and more than or equal to 10^3 CFU/ml for protected mini-bronchoalveolar lavage. Exclusion criteria were (1) severe immunosuppression, defined as leukocyte counts less than 1000 cells/ml or neutrophils less than 500 cells/ml; (2) extrapulmonary infection such as bacteremia, urinary infection, peritonitis, catheter-related infection, mediastinitis, meningitis, endocarditis, or skin infections; (3) patients treated with intravenous colistin; (4) patients treated with a combination of nebulized colistin and intravenous antibiotics other than aminoglycosides; and (5) allergy to colistin. Before obtaining the result of the bronchoalveolar lavage culture, each patient included in the study received either an empirical antimicrobial therapy based on the bacterial ecology of the ICU during a mean time of 1.6 ± 0.5 days or an antimicrobial therapy for treating the previous episode of VAP.

Two groups of patients were distinguished from the cohort patients: the sensitive strain group including patients with VAP caused by *A. baumannii* or *P. aeruginosa* susceptible to β-lactams and the multidrug-resistant strain group including patients with VAP caused by *P. aeruginosa* or *A. baumannii* resistant to all β-lactams and susceptible to colistin. When the first episode of VAP was caused by susceptible *P. aeruginosa or A. baumannii* and followed by a second episode caused by multidrug-resistant *P. aeruginosa or A. baumannii,* patients were included in the multidrug-resistant strain group rather than in the sensitive strain group. Patients' flowchart is shown in figure 1.

Patients in the sensitive strain group received for 14 days intravenous β-lactam (ticarcillin/piperacillin, ceftazidime, or imipenem) combined either with aminoglycoside (78% of patients) or quinolone (22% of patients) for 3 days. This 14-day treatment regimen was selected to avoid a high pulmonary infection recurrence rate.[22]

Patients in the multidrug-resistant strain group received an aerosol of 5 million international units (IU) (400 mg) of colistimethate every 8 h for 7–19 days. The duration of aerosol was maintained for 14 days or until successful weaning from mechanical ventilation. After extubation, aerosol of antibiotics cannot be properly delivered through the natural airways during spontaneous breathing because of inspiratory flow turbulence precluding reaching the deep lung. Aerosolized colistimethate dose was calculated according to a 40% extrapulmonary deposition as previously described.[10] Therefore, the resulting fraction of colistimethate reaching the respiratory tract was 60% of the initial dose placed in the nebulizer chamber, representing a daily dose equivalent to 3 million IU delivered to the respiratory tract every 8 h.

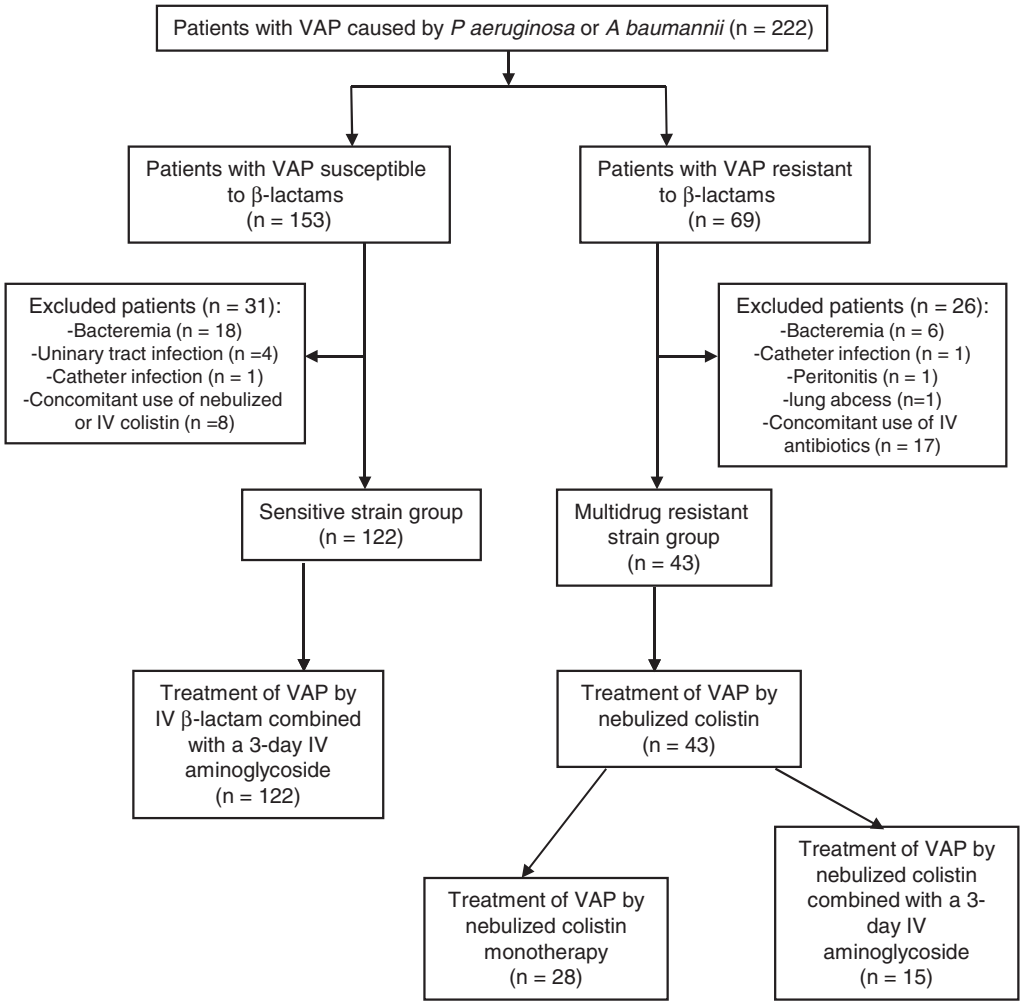

Fig. 1. Patients' flowchart. *A. baumannii* = *Acinetobacter baumannii*; IV = intravenous; *P. aeruginosa* = *Pseudomonas aeruginosa*; VAP = ventilator-associated pneumonia.

Colistin plasma concentrations were measured at day 2 and day 3 using high-performance liquid chromatography method.[23,24] Peak and trough serum concentrations were measured 30 min after nebulization and immediately before the next nebulization, respectively. All blood samples were immediately centrifuged for 15 min (4,000*g*) at 4°C, and plasma samples were stored at −40°C for later analysis.

Serum creatinine was recorded on days 0, 3, 7, and 14 in both groups. Colistin-induced renal function impairment was defined as an increase in serum creatinine level more than or equal to 1.5 times the pretreatment value.[25,26]

Aerosol Generation

In the multidrug-resistant strain group, nebulization was performed with a vibrating plate nebulizer (Aeroneb Pro®, Aerogen Nektar Corporation, Galway, Ireland) positioned on the inspiratory limb 10 cm proximal to the Y-piece.[27] After inserting 5 million IU of colistimethate powder diluted in 10 ml of sterile water into the nebulizer chamber, each nebulization was delivered over 60 min. Specific ventilator settings were used during the nebulization period to reduce flow turbulences and thereby extrapulmonary deposition.[17] They included removal of heat and moisture exchanger or conventional humidifier, volume controlled mode, administration of constant inspiratory flow, respiratory rate of 12 breaths/min, inspiratory–expiratory ratio of 50%, tidal volume of 8 ml/kg, and an end-inspiratory pause representing 20% of the duty cycle. During the nebulization period, expired aerosolized particles were collected in a filter with pore size equal to 0.2 μm positioned on the distal part of the expiratory limb (Hygrobac; Mallinckrodt Medical, Mirandola, Italy). Strict coordination between the patient and the ventilator was demanded to avoid inspiratory turbulences and optimize distal lung deposition of aerosolized particles. In case of discoordination, 2 mg/kg of propofol was infused. After each aerosol, the filter was removed and heat and moisture exchanger or conventional humidifier repositioned. To standardize the procedure of aerosol administration, a checklist form was completed by the nurse in charge of the patient as previously described.[18]

Clinical and Microbiological Assessments

Clinical responses were classified at the end of treatment at day 14 by independent physicians as cure, persisting VAP, recurrence, and superinfection. Cure of VAP was defined as resolution of clinical and biological signs of infection, clinical pulmonary infection score (CPIS) less than 6 and negative culture of lower respiratory tract specimens if available. Persisting VAP was defined as lack of improvement of clinical and biological signs of infection, CPIS greater than 6, and significant concentrations of *P. aeruginosa* and *A. baumannii* persisting in the lower respiratory tract. Recurrence was defined as initial cure of VAP with antimicrobial treatment at day 14 followed by the reappearance of clinical and biological signs of infection, CPIS greater than 6, and significant concentrations of *P. aeruginosa* and *A. baumannii* in lower respiratory tract specimens. Superinfection was defined as reappearance of VAP caused by pathogens other than *P. aeruginosa* or *A. baumannii* isolated from lower respiratory tract specimens.[18]

All causative microorganisms were identified using routine microbiological methods. The disk diffusion method was used for antibiotic susceptibility testing, except for colistin. Mueller–Hinton agar and disks of antibiotics were purchased from Sanofi Diagnostics Pasteur (Marne la Coquette, France) and were used according to the guidelines of the Antibiogram Committee of the French Society for Microbiology. Susceptibility of the isolates to colistin was determined using Etest strips following the manufacturer's guidelines (AB bioMérieux, Basingstoke, United Kingdom) and the guidelines of the Antibiogram Committee of the French Society for Microbiology. The strains with MIC ≤ 2 mg/l were defined as susceptible to colistin for *P. aeruginosa* and *A. baumannii*.

Computed Tomography Measurements

In nine patients of the multidrug-resistant strain group, computed tomography of the whole lung was obtained before and at the end of antimicrobial treatment according to the demand of the physician in charge. Contiguous axial 5-mm thick computed tomography sections of the whole lung were acquired at end-expiration before and after nebulized colistin. Volumes of gas and tissue and total lung volumes were computed.[18,28] Antibiotic-induced lung reaeration and decrease in lung inflammation after administration of nebulized colistin was measured as the increase in gas volume and decrease in tissue volume in lung regions characterized by multiple and disseminated foci of pneumonia and in lung areas characterized by confluent bronchopneumonia.[19]

Statistical Analysis

The trial was designed to demonstrate the efficacy of nebulized colistin for treating VAP caused by multidrug-resistant *P. aeruginosa* or *A. baumannii*. The primary efficacy analysis assessed the noninferiority of clinical cure rate between multidrug-resistant strain and sensitive strain groups. Noninferiority of nebulized colistin was demonstrated if the lower limit of the one-sided 95% CI for difference in clinical cure rate was more than −16%. The selection of the noninferiority margin was determined by combining statistical reasoning and clinical judgment. The average clinical cure rates of VAP caused by sensitive *P. aeruginosa* and *A. baumannii* in our ICU in the past year were 60 and 85%, respectively. Considering that, in the study population, we would include 75% of patients with VAP caused by *P. aeruginosa* and 25% of patients with VAP caused by *A. baumannii*, the mean clinical cure rate of VAP was estimated to be 66%. Because the multidrug-resistant strain group was a different population with longer length of mechanical ventilation and more episodes of VAP before inclusion, we did consider that 50% of clinical cure rate in these patients was clinically relevant. The rate of 50% is in accordance with the lower limit of cure rate reported in the literature in patients with VAP caused by sensitive *P. aeruginosa* and *A. baumannii* treated with appropriate intravenous antibiotics.[29–32] As a result, the noninferiority margin of 16% was determined (66–50% = 16%). Based on the inclusion ratio of 1:3 (one patient included in the multidrug-resistant strain group for three patients in the sensitive strain group), 32 and 96 patients were needed, respectively, in each group.

Risk for developing antibiotic resistance after administration of nebulized colistin in patients with recurrent pneumonia, assessment of serum colistin concentrations, determination of colistin-induced changes of computed tomography gas and tissue volumes, and assessment of antibiotic-induced nephrotoxicity were statistically analyzed.

Categorical variables were expressed as percentages and continuous variables as mean ± SD or as median and 25%, 75% interquartile range. A two-tailed hypothesis was tested in the statistical methods. Differences between sensitive strain and multidrug-resistant strain groups concerning clinical characteristics, duration of mechanical ventilation, length of stay and percentages of persisting VAP, recurrent VAP, and superinfection were compared using the chi-square test, bilateral unpaired Student *t* test, or Mann–Whitney rank-sum test according to the data distributions. Computed tomography changes in gas and tissue volumes before and after nebulized colistin therapy were compared using bilateral paired Student *t* test. Two-way analysis of variance for repeated measures was used to compare changes in CPIS and serum creatinine between groups. Statistical analysis was performed using SPSS 13.0 and SigmaStat 2.03 (SPSS, San Rafael, CA). A *P* value of less than or equal to 0.5 was considered statistically significant.

Results

Patients

Forty-three patients were prospectively included in the multidrug-resistant strain group. Ten patients had VAP

caused by *P. aeruginosa* or *A. baumannii* susceptible only to colistin; 33 patients were infected by *P. aeruginosa* or *A. baumannii* resistant to all β-lactams and susceptible to colistin and aminoglycosides and/or ciprofloxacin. All patients had received inappropriate initial antimicrobial therapy. Twenty-eight patients were treated with nebulized colistin monotherapy. Fifteen patients were treated with nebulized colistin combined with a 3-day administration of intravenous aminoglycosides. The average duration of nebulized colistin administration was 12 days (range 7–19). In the sensitive strain group, 122 patients had VAP caused by *P. aeruginosa* or *A. baumannii* susceptible to at least one β-lactam. Eighty-four percent of patients had received initial appropriate empirical antibiotics. They were treated with intravenous β-lactam for 14 days combined with a 3-day administration of intravenous aminoglycoside or quinolone (fig. 1).

As shown in table 1, clinical characteristics of both groups were similar at ICU admission. At inclusion, VAP caused by *P. aeruginosa* was more frequent in the sensitive strain group than in the multidrug-resistant strain group. In the multidrug-resistant strain group, duration of mechanical ventilation and ICU stay before initiation of antibiotics were significantly longer. In addition, previous administration of antibiotics and tracheostomy was more frequent. One third of patients of the multidrug-resistant strain group had at least two episodes of VAP before inclusion.

Antibiotic Treatment Efficacy

Twenty-nine of the 43 patients (67%) treated with nebulized colistin were clinically cured at the end of treatment compared with 81 of the 122 patients (66%) treated with intravenous β-lactams (difference: –1%, lower limit of 95% CI for the difference between success rate: –12.6%).

Nineteen of the 28 patients treated with nebulized colistin monotherapy and 10 of the 15 patients treated with nebulized colistin combined to a 3-day administration of

Table 1. Patients' Clinical Characteristics at Admission and Inclusion

	Sensitive Strain Group (n = 122)	Multidrug-resistant Strain Group (n = 43)	*P* Value
Admission			
Age, yr, mean ± SD	59 (44 ± 71)	58 (32 ± 62)	0.043
Male, n (%)	88 (72%)	33 (77%)	0.556
COPD, n (%)	37 (30%)	13 (30%)	0.991
SOFA, median (IQR)	9.5 (7–11)	9 (5–11)	0.339
SAPS II, median (IQR)	46 (36–59)	46 (35–58)	0.353
Reason for admission, n (%)			0.511
Multiple trauma	30 (25%)	11 (26%)	
Postoperative complications	75 (61%)	23 (53%)	
Medical disease	17 (14%)	9 (21%)	
Inclusion			
SOFA, median (IQR)	5 (2–8)	6 (3–9)	0.221
Causative pathogen, n (%)			0.002
P. aeruginosa	113 (93%)	32 (74%)	
A. baumannii	9 (7%)	11 (26%)	
Duration of MV before inclusion, median (IQR)	9 (6–14)	18 (8–35)	<0.001
Length of stay in ICU before inclusion, median (IQR)	8 (4–13)	17 (6–32)	<0.001
Previous antibiotic use, n (%)	87 (71%)	41 (95%)	0.001
Tracheostomy, n (%)	34 (28%)	35 (81%)	<0.001
No. previous VAP, n (%)			0.013
None	54 (44%)	17 (39%)	
1	56 (46%)	14 (33%)	
≥2	12 (10%)	12 (28%)	0.018

Patients of the sensitive stain group were treated with intravenous β-lactams combined with a 3-day intravenous administration of aminoglycoside or quinolone; patients of the multidrug-resistant stain group were treated with nebulized colistin either in monotherapy (n = 28) or combined with a 3-day intravenous administration of aminoglycoside (n = 15).

A. baumannii = *Acinetobacter baumannii*; COPD = chronic obstructive pulmonary disease; ICU = intensive care unit; IQR = interquartile range (25–75%); MV = mechanical ventilation; *P. aeruginosa* = *Pseudomonas aeruginosa*; SAPS II = Simplified Acute Physiology Score II; ; SOFA = Sequential Organ Failure Assessment; VAP = ventilator-associated pneumonia.

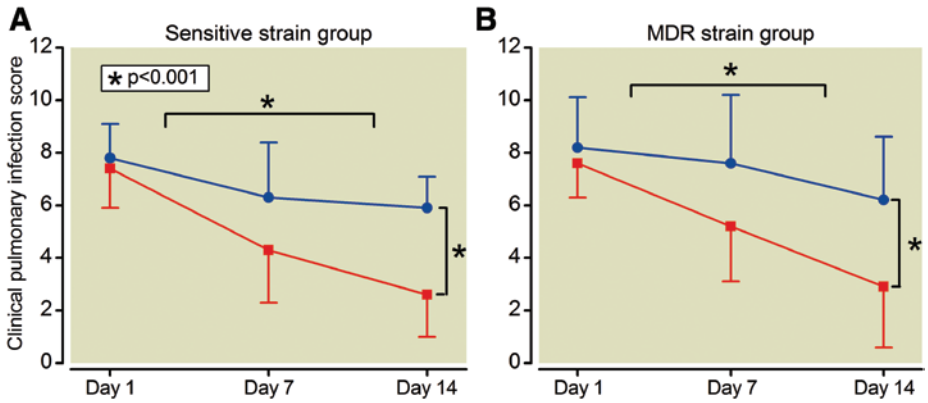

Fig. 2. Evolution of clinical pulmonary infection score in patients of the sensitive strain group treated with intravenous β-lactam combined with a 3-day intravenous administration of aminoglycoside or quinolone (*A*) and in patients of the multidrug-resistant strain group treated with nebulized colistin either in monotherapy (n = 28) or combined with a 3-day intravenous administration of aminoglycoside (n = 15) (*B*). *Red square* = clinical cure of ventilator-associated pneumonia; *blue circle* = clinical failure of antibiotics for treating ventilator-associated pneumonia (VAP). *P* values indicate statistical significance for repeated measures (time effect) and significant interaction between patients with clinical cure of VAP and patients in whom antibiotics failed to cure VAP. MDR = multidrug resistant.

intravenous aminoglycoside were clinically cured at the end of treatment. Clinical cure rate was not different between the patients treated with nebulized colistin monotherapy and those treated with nebulized colistin associated with a 3-day administration of intravenous aminoglycoside (67 *vs.* 68%, $P = 0.94$).

CPIS decreased significantly in patients successfully treated with antibiotics in both groups (fig. 2). As shown in table 2,

Table 2. Antibiotic Treatment Efficacy in Both Groups of Patients

	Sensitive Strain Group (n = 122)	Multidrug-resistant Strain Group (n = 43)	*P* Value
Cure of VAP at day 14, overall	81/122 (66.4%)	29/43 (67.4%)	
Cure of VAP caused by *P. aeruginosa*	72/113 (64%)	19/32 (59.3%)	0.654
Cure of VAP caused by *A. baumannii*	9/9 (100%)	10/11 (91%)	0.353
Persisting VAP at day 14, n (%)			
VAP caused by *P. aeruginosa*	21/113 (19%)	10/32 (31%)	0.122
VAP caused by *A. baumannii*	0/9	0/11	
VAP caused by superinfection at day 14, n (%)	16/122 (13%)	2/43 (6%)	0.126
Per-treatment death, n (%)	4/122 (3%)	2/43 (5%)	0.679
Recurrence of VAP after day 14, n (%)			
VAP caused by *P. aeruginosa*	11/113 (10%)	6/32 (26%)	0.162
VAP caused by *A. baumannii*	1/8 (11%)	0/11 (0)	
VAP caused by superinfection after day 14, n(%)	8/122 (6.6%)	4/43 (9%)	0.551
Duration of MV after inclusion, media (IQR)	8 (2–21)	15 (6–24)	0.031
Duration of MV, median (IQR)	18 (12–33)	38 (23–54)	<0.001
Length of stay in ICU, median (IQR)	25 (16–46)	54 (32–73)	<0.001
All-cause ICU mortality	28 (23%)	7 (16%)	0.357

Patients of the sensitive stain group were treated with intravenous β-lactams combined with a 3-day intravenous administration of aminoglycoside or quinolone; patients of the multidrug-resistant stain group were treated with nebulized colistin either in monotherapy (n = 28) or combined with a 3-day intravenous administration of aminoglycoside (n = 15). "Cure" of VAP: resolution of clinical and biological signs of infection, CPIS less than 6 and negative culture of lower respiratory tract specimens. IQR range = 25–75%. Persisting VAP: lack of improvement of clinical and biological signs of infection and CPIS greater than 6 with significant concentrations of *P. aeruginosa* and *A. baumannii* persisting in lower respiratory tract specimens. Recurrence: initial cure of VAP after antimicrobial therapy followed by post-treatment relapse of VAP caused by *P. aeruginosa* or *A. baumannii* isolated in lower respiratory tract specimens. Superinfection: post-treatment relapse of VAP caused by pathogens other than *P. aeruginosa* or *A. baumannii* isolated in lower respiratory tract specimens.

*A. bau*mannii = *Acinetobacter baumannii*; CPIS = clinical pulmonary infection score; ICU = intensive care unit; IQR = interquartile range; MV = mechanical ventilation; *P. aeruginosa* = *Pseudomonas aeruginosa*; VAP = ventilator-associated pneumonia.

Table 3. Pseudomonas aeruginosa Susceptibility in Patients with Persisting or Recurrent Ventilator-associated Pneumonia

	Sensitive Strain Group (n = 32)		Multidrug-rsesistant Strain Group (n = 16)	
	Before Treatment	After Treatment	Before Treatment	After Treatment
Susceptible to all β-lactam antibiotics, n (%)	21 (65.6%)	8 (25.0%)	0	0
Resistant to piperacillin and/or ceftazidime, n (%)	6 (18.8%)	11 (34.3%)	0	2 (12.5%)
Resistant to carbapenem, n (%)	5 (15.6%)	7 (21.9%)	0	2 (12.5%)
Resistant to all β-lactam antibiotics, n (%)	0	6 (18.8%)	16 (100%)	12 (75%)

91% of patients with VAP caused by multidrug-resistant *A. baumannii* were cured by nebulized colistin. All patients with VAP caused by sensitive *A. baumannii* were cured by intravenous β-lactams. Treatment failure with persisting VAP caused by *P. aeruginosa* was not statistically different between groups ($P = 0.122$). Recurrence of *P. aeruginosa* VAP and VAP caused by superinfection was similar in both groups. The duration of mechanical ventilation after inclusion was longer in patients of the multidrug-resistant strain group. All-cause ICU mortality was similar between groups.

Microbiological Response and Acquisition of Antibiotic Resistance

In the 122 patients of the sensitive strain group, 62% of initial *P. aeruginosa* isolates were susceptible to all antipseudomonal antibiotics, 22% were resistant to carbapenems, and 16% were resistant to piperacillin and/or ceftazidime. Among 32 patients with persisting and recurrent *P. aeruginosa* VAP despite antibiotic treatment, the resistance of *P. aeruginosa* increased significantly: six strains became resistant to all β-lactams (table 3).

In the 43 patients of the multidrug-resistant strain group, colistin MIC at inclusion was 1.5 (0.75–2) mg/l for *P. aeruginosa* strains and 0.12 (0.09–0.19) mg/l for *A. baumannii*. Among 16 patients with persisting or recurrent VAP caused by *P. aeruginosa*, four strains became susceptible to β-lactams. Two patients had been treated with nebulized colistin monotherapy and two by nebulized colistin combined to a 3-day administration of intravenous aminoglycosides. In one patient treated with nebulized colistin monotherapy, colistin MIC increased from 0.75 to 3 mg/l. In another patient treated with nebulized colistin combined with 3-day intravenous aminoglycoside, colistin MIC did not change after the first VAP recurrence but increased from 1.5 to 3 mg/ml during the second VAP recurrence, after an additional 14-day administration of nebulized colistin.

Computed Tomography Changes in Lung Aeration and Inflammation after Nebulized Colistin

In seven patients successfully treated with nebulized colistin, gas volume increased and tissue volume decreased significantly in regions with confluent pneumonia (fig. 3). They remained unchanged in regions with normal lung and/or disseminated foci of pneumonia. In two patients in whom nebulized colistin failed to treat VAP, tissue volume increased in lung regions with normal lung and/or disseminated foci of pneumonia (+177 and +198 ml) and decreased in regions of confluent pneumonia (−73 and −65 ml). Illustrative examples are shown in fig. 4.

Serum Pharmacokinetics and Kidney Function

Colistin serum concentrations were measured in 16 patients. Peak colistin concentrations were not different between day 2 and day 3. Trough colistin concentrations were significantly higher at day 3 compared with day 2 (fig. 5) and were not different between patients treated with nebulized colistin monotherapy and patients treated with nebulized colistin combined to a 3-day administration of intravenous aminoglycoside.

Serum creatinine remained stable within the treatment period in both groups of patients (fig. 6). At the end of treatment, increase of serum creatinine more than 1.5 times its baseline value was found in 8% of patients treated with intravenous β-lactam combined with a 3-day intravenous administration of aminoglycoside or quinolone and 12% in patients treated with nebulized colistin ($P = 0.47$). Per-treatment changes in serum creatinine were not different between patients treated with nebulized colistin monotherapy and patients treated with nebulized colistin combined to a 3-day administration of intravenous aminoglycoside.

Discussion

Main results of the study are nebulized colistin is effective to treat VAP caused by multidrug-resistant *P. aeruginosa and A. baumannii*; the clinical cure rate is noninferior to that obtained in VAP caused by susceptible *P. aeruginosa and A. baumannii*; the risk of developing colistin resistance after nebulization is low; and nebulized colistin does not increase the risk of kidney failure, although repeated nebulization induces systemic accumulation.

Rationale for Using Nebulized Colistin

Most multidrug-resistant Gram-negative pathogens exhibit resistance to almost all antibiotics except colistin. Experimental

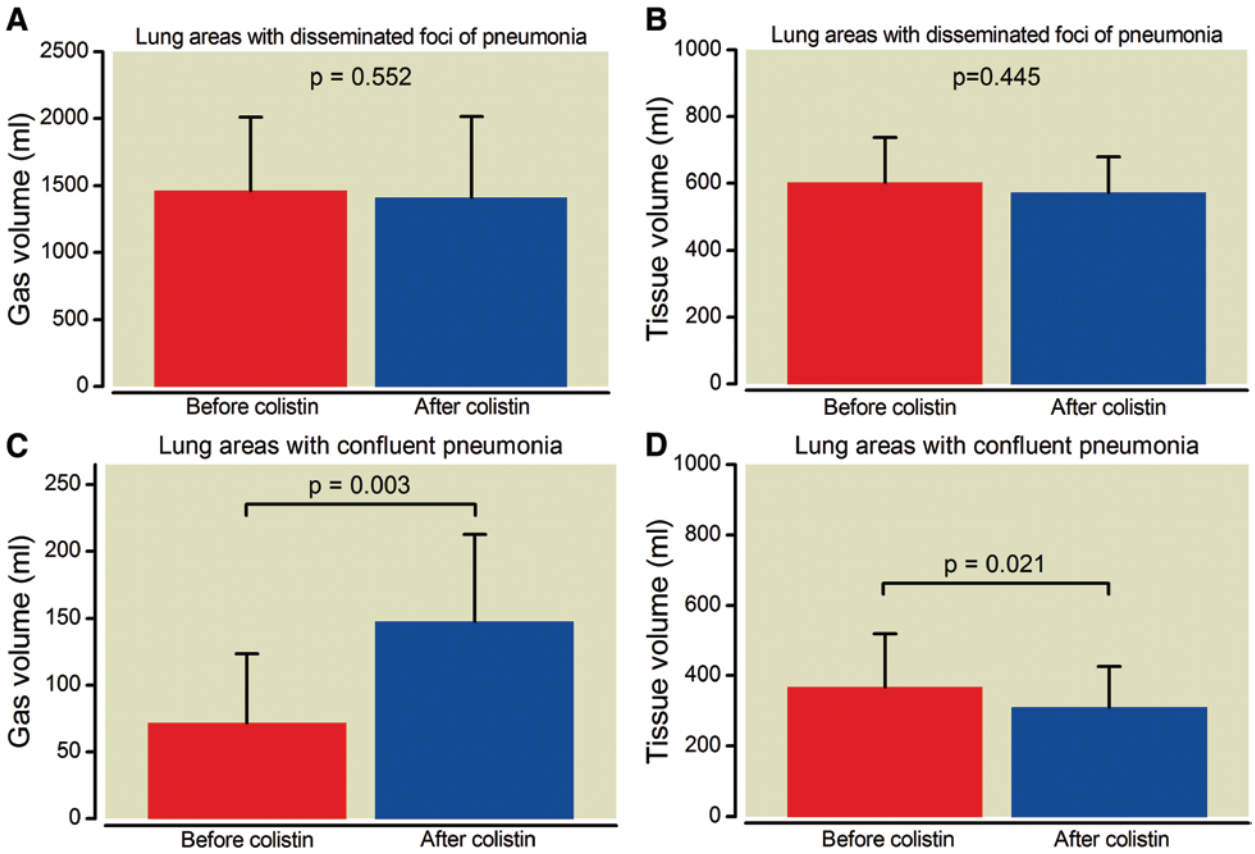

Fig. 3. Computed tomography assessment of gas and tissue volumes in lung regions characterized by normal lung and/or disseminated foci of pneumonia (*A* and *B*) and in lung areas of confluent pneumonia (*C* and *D*) in seven patients of the multidrug-resistant strain group successfully treated with nebulized colistin (six treated with monotherapy and one patient treated with monotherapy combined with a 3-day intravenous administration of aminoglycoside). *Red bar* = before nebulized colistin; *blue bar* = after nebulized colistin.

studies provide evidence for high antibiotic lung deposition and rapid bacterial killing after nebulization of antibiotics.[10,15,16] Therefore, the rationale for treating VAP caused by multidrug-resistant pathogens by nebulized colistin is strong. Nebulized colistin either as adjunct to systemic antibiotics[32–34] or as monotherapy[35,36] was previously reported in patients with VAP or tracheobronchitis. In these retrospective studies, neither the dose nor the conditions of nebulization were optimized. Our prospective study is the first to report the effectiveness of high-dose nebulized colistin as a treatment of VAP caused by multidrug-resistant *P. aeruginosa* or *A. baumannii*.

Methodological Limitations

Patients with VAP caused by susceptible strains and treated with a combination of intravenous β-lactams and aminoglycosides served as a control group. An *a priori* hypothesis of a noninferior clinical cure rate of nebulized colistin compared with conventional bitherapy was made and defined as more than −16% difference of cure rate. Our aim was to prove that nebulized colistin can kill multidrug strains and cure lung infection. Recurrence after initial cure of VAP was therefore not regarded as a failure of antimicrobial therapy, considering that persisting reservoirs are inaccessible to either intravenous or nebulized colistin. Anyway, late recurrence of VAP was not different between groups.

In the multidrug-resistant strain group, nebulized colistin was given either alone (n = 28) or in combination with a 3-day administration of intravenous aminoglycosides (n = 15), based on clinical decision of attending physician. Many experimental and clinical studies suggest a poor efficiency of intravenous aminoglycosides for treating VAP. After intravenous administration, aminoglycosides tissue concentrations equal to or below MIC have been reported in animals with normal or infected lungs.[15,37–39] Aminoglycosides bronchial concentrations equal to or below MIC have been reported in patients with cystic fibrosis or VAP.[40–43] Two meta-analysis have demonstrated the lack of superiority of β-lactam–aminoglycoside combination to β-lactam monotherapy for treating various causes of sepsis.[44,45] These meta-analyses however suffer from important limitations: heterogeneity of sepsis in which the comparison was made; randomized clinical trials performed more than 15 yr ago; and comparison of different β-lactams in the tested treatment arms. As a consequence, many physicians still believe that patients with VAP might benefit from β-lactam–aminoglycoside combination. The same clinical cure rate was obtained in the 28 patients

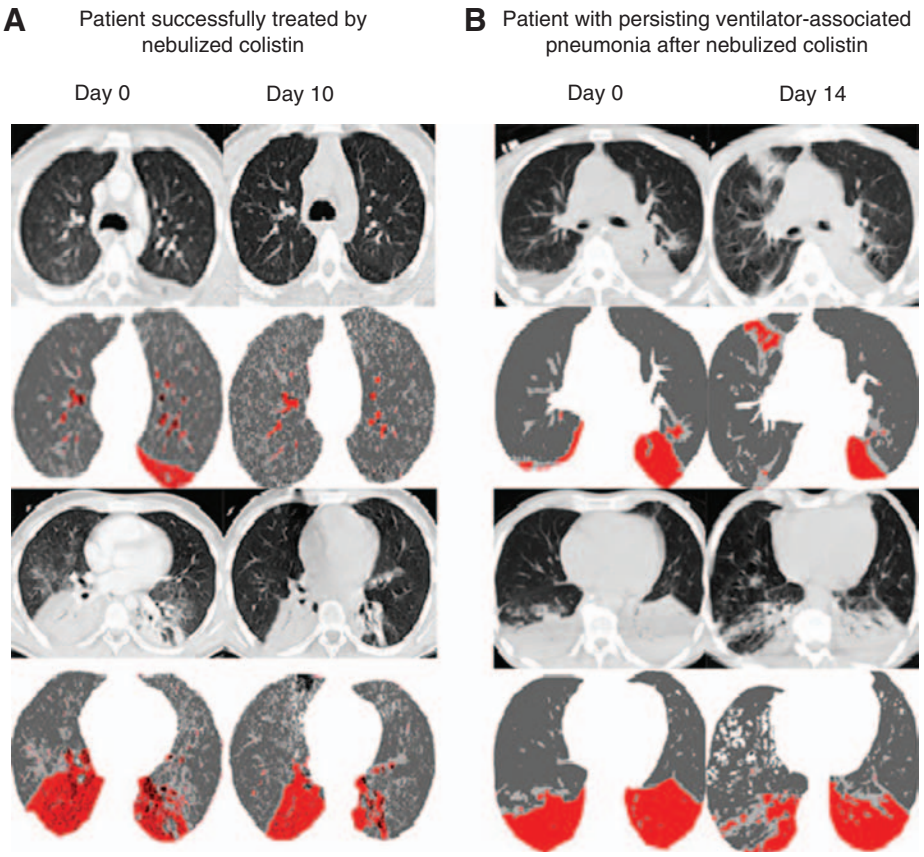

Fig. 4. Representative computed tomography images obtained in two patients of the multidrug-resistant strain group infected by *Pseudomonas aeruginosa*. Computed tomography sections were obtained before and after nebulization of colistin in a patient successfully treated with nebulized colistin monotherapy (*A*) and in another patient with failure of nebulized colistin combined with a 3-day intravenous administration of aminoglycoside (*B*).

treated with nebulized colistin monotherapy and in the 15 patients treated with nebulized colistin–aminoglycoside combination. This result suggests that clinical outcome of VAP could be attributed to nebulized colistin rather than intravenous aminoglycosides.

Efficacy of Nebulized Colistin in VAP Caused by Multidrug-Resistant P. aeruginosa *and* A. baumannii

In the sensitive strain group, clinical cure rate was observed in two thirds of patients, as previously reported.[18,31,46] In the multidrug-resistant stain group, clinical cure rate was noninferior to that observed in the sensitive strain group. The clinical benefit was associated with radiological improvement as demonstrated in figures 3 and 4. It has to be pointed out that patients infected by multidrug-resistant strains were more severe than patients infected by sensitive strains: they had stayed longer on mechanical ventilation before inclusion; 95% of them *versus* 71% had received multiple antibiotics before inclusion; 28% of them *versus* 10% had two or more previous episodes of VAP, attesting of their inability to activate sufficient host response against infections; and 100% of them *versus* 16% had received inappropriate initial empirical antimicrobial therapy, a condition known to be associated with increased mortality and morbidity.[47] Therefore, the equivalence in terms of clinical cure rate clearly suggests a strong efficacy of nebulized colistin.

Such a benefit was obtained at two conditions. First, factors influencing distal lung deposition were optimized during each nebulization.[18,48] Second, daily high-dose aerosols were administered, taking into consideration that colistin is both a concentration- and time-dependent antibiotic[12,13,49] and has a limited systemic diffusion even in presence of injury of the alveolar–capillary barrier.[10] As previously recommended, nebulization dose of antibiotics should have been calculated as intravenous dose plus extrapulmonary deposition.[48] According to this rationale and considering an intravenous daily dose of 40,000 IU/kg and an extrapulmonary deposition of 40%, a nebulized dose of 56,000 IU/kg of colistimethate should have been administered. Because of its low systemic diffusion and its bactericidal profile, it was decided to increase colistin dose to 200,000 IU/kg, and 5 million IU were nebulized every 8 h, a dosage two to three times higher than previously reported.[32,34] In patients with cystic fibrosis where the target is the bronchial tree, the nebulization of 2 million IU of colistin provides high bronchial concentrations.[50] In patients with VAP where the target is the alveolar space, much higher doses are required to obtain at least fivefold the MIC at the site of infection.

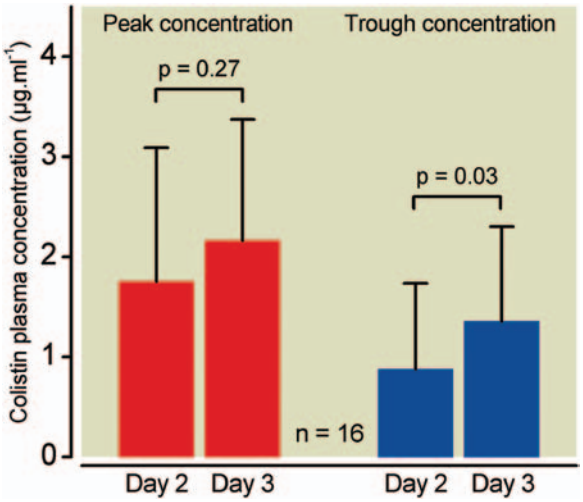

Fig. 5. Colistin peak and trough plasma concentrations measured at day 2 and day 3 of treatment with nebulized colistin either in monotherapy (n = 9) or combined with a 3-day intravenous administration of aminoglycoside (n = 7) in patients of the multidrug-resistant strain group.

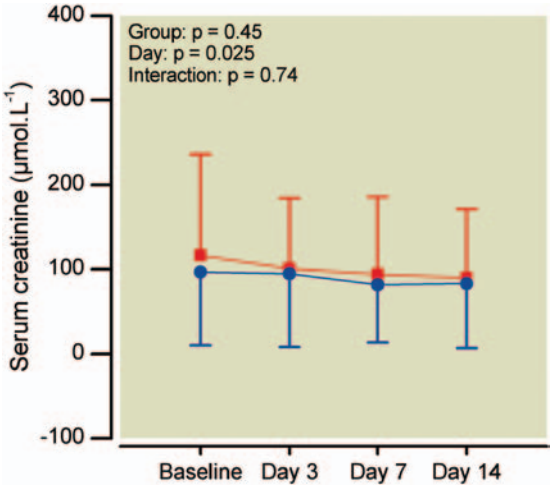

Fig. 6. Evolution of serum creatinine during the treatment period at baseline, day 3, day 7, and day 14 in patients of the sensitive strain group treated with intravenous β-lactams combined with a 3-day intravenous administration of aminoglycoside or quinolone (*red squares*) and those of the multidrug-resistant strain group treated with nebulized colistin either in monotherapy (n = 28) or combined with a 3-day intravenous administration of aminoglycoside (n = 15) (*blue circles*).

Recently, a pharmacokinetic study performed in patients with ventilator-associated tracheobronchitis has shown that a dose of 1 million IU of nebulized colistin every 8 h was not adequate to treat lung infection caused by multidrug-resistant strains.[51] In the current study, no adverse respiratory and bronchial effects were observed after nebulization, confirming a recent experimental study.[10]

Whether using nebulized or intravenous colistin for treating VAP caused by multidrug-resistant Gram-negative bacteria has been a controversial subject.[32,33,46,52] The current study brings convincing evidence that nebulized colistin is clinically efficient, whereas experimental and clinical studies report a poor lung penetration of intravenous colistin.[10,11] Ninety-one percent of patients with VAP caused by multidrug-resistant *A. baumannii* were cured by nebulized colistin. This result is likely explained by high tissue concentration to MIC ratio obtained after administration of nebulized colistin. Only 59% of patients with VAP caused by multidrug-resistant *P. aeruginosa* were cured by nebulized colistin likely because of a less favorable tissue concentration to MIC ratio. In a recent experimental study,[10] MIC of colistin for *P. aeruginosa* was 2 µg/ml, whereas median peak tissue concentrations were 2.8 µg/g (tissue concentration to MIC ratio = 1.8). MIC of colistin for *A. baumannii* is 0.2 µg/ml, and assuming a median colistin peak tissue concentration of 2.8 µg/g (tissue concentration to MIC ratio = 18). Colistin being a concentration-dependent antibiotic, it is easy to understand why *A. baumannii* strains were massively eradicated after the end of treatment. VAP caused by *P. aeruginosa* had a higher incidence of recurrence and superinfection possibly related to the too short duration of treatment resulting from extubation before the 2-week scheduled regimen, as previously reported.[22]

Persisting or Recurrent VAP and Acquisition of Resistance

In the sensitive strain group, 75% of patients whose VAP persisted or relapsed after intravenous antimicrobial therapy acquired resistance to β-lactams. Interestingly, in the multidrug-resistant group, 25% of patients whose VAP caused by *P. aeruginosa* persisted or relapsed after nebulized colistin, recovered susceptibility to β-lactams. These findings are in accordance with two previous studies showing that nebulized antibiotic decreases bacterial resistance in patients with VAP.[18,53]

It has been reported that prolonged use of intravenous colistin predisposes to VAP caused by pandrug-resistant *P. aeruginosa*, likely due to colistin poor lung tissue penetration and low concentrations at the site of infection.[54,55] In patients treated with nebulized antibiotics, much higher tissue concentrations are present in infected lung regions,[10] thereby preventing selection of resistant strains. In the current study, MIC of *P. aeruginosa* strain to colistin increased in two patients after nebulized colistin administered during 14–21 days, indicating a low rate of acquisition of resistance.

Colistin Serum Concentration and Nephrotoxicity

Experimental data suggest a decreased colistin systemic exposure after 24 h of nebulization.[10] The current study shows that colistin trough plasma concentration significantly increased between day 2 and 3, suggesting colistin accumulation with time as a result of slow systemic passage through the alveolar–capillary membrane.[56,57] Renal function impairment was observed in 12% of patients treated with nebulized colistin, and evolution of serum creatinine during the treatment was similar in both groups. Therefore, high doses of nebulized

colistin can be considered as safe when administered during 14 days. We recommend however to measure colistin serum concentration at day 7, particularly in patients with preexisting alterations of renal function.[9,32]

In conclusion, nebulized colistin at high dose is effective and safe for treating VAP caused by multidrug-resistant *P. aeruginosa and A. baumannii*. It provides an attractive alternative in the face of the increasing incidence of VAP caused by multidrug-resistant Gram-negative pathogens in critically ill patients. Further randomized controlled studies comparing intravenous and nebulized colistin for treating VAP caused by multidrug-resistant *P. aeruginosa and A. baumannii* are required.

References

1. Kallel H, Bahloul M, Hergafi L, Akrout M, Ketata W, Chelly H, Hamida CB, Rekik N, Hammami A, Bouaziz M: Colistin as a salvage therapy for nosocomial infections caused by multidrug-resistant bacteria in the ICU. Int J Antimicrob Agents 2006; 28:366–9
2. Rynn C, Wootton M, Bowker KE, Alan Holt H, Reeves DS: *In vitro* assessment of colistin's antipseudomonal antimicrobial interactions with other antibiotics. Clin Microbiol Infect 1999; 5:32–6
3. Yoon J, Urban C, Terzian C, Mariano N, Rahal JJ: *In vitro* double and triple synergistic activities of Polymyxin B, imipenem, and rifampin against multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2004; 48:753–7
4. Linden PK, Kusne S, Coley K, Fontes P, Kramer DJ, Paterson D: Use of parenteral colistin for the treatment of serious infection due to antimicrobial-resistant Pseudomonas aeruginosa. Clin Infect Dis 2003; 37:e154–60
5. Petrosillo N, Ioannidou E, Falagas ME: Colistin monotherapy *vs.* combination therapy: Evidence from microbiological, animal and clinical studies. Clin Microbiol Infect 2008; 14:816–27
6. Tascini C, Gemignani G, Palumbo F, Leonildi A, Tedeschi A, Lambelet P, Lucarini A, Piaggesi A, Menichetti F: Clinical and microbiological efficacy of colistin therapy alone or in combination as treatment for multidrug resistant Pseudomonas aeruginosa diabetic foot infections with or without osteomyelitis. J Chemother 2006; 18:648–51
7. Garnacho-Montero J, Ortiz-Leyba C, Jiménez-Jiménez FJ, Barrero-Almodóvar AE, García-Garmendia JL, Bernabeu-WittelI M, Gallego-Lara SL, Madrazo-Osuna J: Treatment of multidrug-resistant Acinetobacter baumannii ventilator-associated pneumonia (VAP) with intravenous colistin: A comparison with imipenem-susceptible VAP. Clin Infect Dis 2003; 36:1111–8
8. Kallel H, Hergafi L, Bahloul M, Hakim A, Dammak H, Chelly H, Hamida CB, Chaari A, Rekik N, Bouaziz M: Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: A matched case-control study. Intensive Care Med 2007; 33:1162–7
9. Montero M, Horcajada JP, Sorlí L, Alvarez-Lerma F, Grau S, Riu M, Sala M, Knobel H: Effectiveness and safety of colistin for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections. Infection 2009; 37:461–5
10. Lu Q, Girardi C, Zhang M, Bouhemad B, Louchahi K, Petitjean O, Wallet F, Becquemin MH, Le Naour G, Marquette CH, Rouby JJ: Nebulized and intravenous colistin in experimental pneumonia caused by *Pseudomonas aeruginosa*. Intensive Care Med 2010; 36:1147–55
11. Imberti R, Cusato M, Villani P, Carnevale L, Iotti GA, Langer M, Regazzi M: Steady-state pharmacokinetics and BAL concentration of colistin in critically Ill patients after IV colistin methanesulfonate administration. Chest 2010; 138:1333–9
12. Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, Liolios L: Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2006; 50:2946–50
13. Poudyal A, Howden BP, Bell JM, Gao W, Owen RJ, Turnidge JD, Nation RL, Li J: *In vitro* pharmacodynamics of colistin against multidrug-resistant *Klebsiella pneumoniae*. J Antimicrob Chemother 2008; 62:1311–8
14. Li J, Nation RL: Old polymyxins are back: Is resistance close? Clin Infect Dis 2006; 43:663–4
15. Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Marquette CH, Rouby JJ: Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. Am J Respir Crit Care Med 2002; 166:1375–81
16. Ferrari F, Lu Q, Girardi C, Petitjean O, Marquette CH, Wallet F, Rouby JJ; Experimental ICU Study Group: Nebulized ceftazidime in experimental pneumonia caused by partially resistant *Pseudomonas aeruginosa*. Intensive Care Med 2009; 35:1792–800
17. Rouby JJ, Goldstein I, Lu Q: Inhaled Antibiotic Therapy, Principles and Practice Of Mechanical Ventilation, Third Edition. Edited by Tobin MJ. New York, McGraw-Hill Medical Publishing Division, 2012; 1447–56.
18. Lu Q, Yang J, Liu Z, Gutierrez C, Aymard G, Rouby JJ; Nebulized Antibiotics Study Group: Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. Am J Respir Crit Care Med 2011; 184:106–15
19. Bouhemad B, Liu ZH, Arbelot C, Zhang M, Ferarri F, Le-Guen M, Girard M, Lu Q, Rouby JJ: Ultrasound assessment of antibiotic-induced pulmonary reaeration in ventilator-associated pneumonia. Crit Care Med 2010; 38:84–92
20. Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, Kirkpatrick AW, Melniker L, Gargani L, Noble VE, Via G, Dean A, Tsung JW, Soldati G, Copetti R, Bouhemad B, Reissig A, Agricola E, Rouby JJ, Arbelot C, Liteplo A, Sargsyan A, Silva F, Hoppmann R, Breitkreutz R, Seibel A, Neri L, Storti E, Petrovic T; International Liaison Committee on Lung Ultrasound (ILC-LUS) for International Consensus Conference on Lung Ultrasound (ICC-LUS): International evidence-based recommendations for point-of-care lung ultrasound. Intensive Care Med 2012; 38:577–91
21. Rouby JJ, Martin De Lassale E, Poete P, Nicolas MH, Bodin L, Jarlier V, Le Charpentier Y, Grosset J, Viars P: Nosocomial bronchopneumonia in the critically ill. Histologic and bacteriologic aspects. Am Rev Respir Dis 1992; 146:1059–66
22. Chastre J, Wolff M, Fagon JY, Chevret S, Thomas F, Wermert D, Clementi E, Gonzalez J, Jusserand D, Asfar P, Perrin D, Fieux F, Aubas S; PneumA Trial Group: Comparison of 8 *vs* 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: A randomized trial. JAMA 2003; 290:2588–98
23. Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K, Johnson DW: A simple method for the assay of colistin in human plasma, using pre-column derivatization with 9-fluorenylmethyl chloroformate in solid-phase extraction cartridges and reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2001; 761:167–75
24. Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K, Valentine J: Simple method for assaying colistin methanesulfonate in plasma and urine using high-performance liquid chromatography. Antimicrob Agents Chemother 2002; 46:3304–7
25. Cartin-Ceba R, Haugen EN, Iscimen R, Trillo-Alvarez C, Juncos L, Gajic O: Evaluation of "Loss" and "End stage renal disease" after acute kidney injury defined by the Risk, Injury, Failure, Loss and ESRD classification in critically ill patients. Intensive Care Med 2009; 35:2087–95
26. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative workgroup: Acute renal failure - definition, outcome measures, animal models, fluid therapy

and information technology needs: The Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004; 8:R204–12

27. Ferrari F, Liu ZH, Lu Q, Becquemin MH, Louchahi K, Aymard G, Marquette CH, Rouby JJ: Comparison of lung tissue concentrations of nebulized ceftazidime in ventilated piglets: Ultrasonic *versus* vibrating plate nebulizers. Intensive Care Med 2008; 34:1718–23
28. Malbouisson LM, Muller JC, Constantin JM, Lu Q, Puybasset L, Rouby JJ; CT Scan ARDS Study Group: Computed tomography assessment of positive end-expiratory pressure-induced alveolar recruitment in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2001; 163:1444–50
29. Combes A, Luyt CE, Fagon JY, Wolff M, Trouillet JL, Chastre J: Impact of piperacillin resistance on the outcome of *Pseudomonas* ventilator-associated pneumonia. Intensive Care Med 2006; 32:1970–8
30. Chastre J, Fagon JY: Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002; 165:867–903
31. Chastre J, Wunderink R, Prokocimer P, Lee M, Kaniga K, Friedland I: Efficacy and safety of intravenous infusion of doripenem versus imipenem in ventilator-associated pneumonia: A multicenter, randomized study. Crit Care Med 2008; 36:1089–96
32. Kofteridis DP, Alexopoulou C, Valachis A, Maraki S, Dimopoulou D, Georgopoulos D, Samonis G: Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: A matched case-control study. Clin Infect Dis 2010; 51:1238–44
33. Korbila IP, Michalopoulos A, Rafailidis PI, Nikita D, Samonis G, Falagas ME: Inhaled colistin as adjunctive therapy to intravenous colistin for the treatment of microbiologically documented ventilator-associated pneumonia: A comparative cohort study. Clin Microbiol Infect 2010; 16:1230–6
34. 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; 65:2645–9
35. Athanassa ZE, Myrianthefs PM, Boutzouka EG, Tsakris A, Baltopoulos GJ: Monotherapy with inhaled colistin for the treatment of patients with ventilator-associated tracheobronchitis due to polymyxin-only-susceptible Gram-negative bacteria. J Hosp Infect 2011; 78:335–6
36. Falagas ME, Siempos II, Rafailidis PI, Korbila IP, Ioannidou E, Michalopoulos A: Inhaled colistin as monotherapy for multidrug-resistant gram (-) nosocomial pneumonia: A case series. Respir Med 2009; 103:707–13
37. Goldstein I, Wallet F, Robert J, Becquemin MH, Marquette CH, Rouby JJ: Lung tissue concentrations of nebulized amikacin during mechanical ventilation in piglets with healthy lungs. Am J Respir Crit Care Med 2002; 165:171–5
38. Makhoul IR, Merzbach D, Lichtig C, Berant M: Antibiotic treatment of experimental *Pseudomonas aeruginosa* pneumonia in guinea pigs: Comparison of aerosol and systemic administration. J Infect Dis 1993; 168:1296–9
39. Berendt RF, Long GG, Walker JS: Treatment of respiratory Klebsiella pneumoniae infection in mice with aerosols of kanamycin. Antimicrob Agents Chemother 1975; 8:585–90
40. Dull WL, Alexander MR, Kasik JE: Bronchial secretion levels of amikacin. Antimicrob Agents Chemother 1979; 16:767–71
41. Panidis D, Markantonis SL, Boutzouka E, Karatzas S, Baltopoulos G: Penetration of gentamicin into the alveolar lining fluid of critically ill patients with ventilator-associated pneumonia. Chest 2005; 128:545–52
42. Santré C, Georges H, Jacquier JM, Leroy O, Beuscart C, Buguin D, Beaucaire G: Amikacin levels in bronchial secretions of 10 pneumonia patients with respiratory support treated once daily *versus* twice daily. Antimicrob Agents Chemother 1995; 39:264–7
43. Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL: Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Respir Dis 1985; 132:761–5
44. Bliziotis IA, Samonis G, Vardakas KZ, Chrysanthopoulou S, Falagas ME: Effect of aminoglycoside and beta-lactam combination therapy *versus* beta-lactam monotherapy on the emergence of antimicrobial resistance: A meta-analysis of randomized, controlled trials. Clin Infect Dis 2005; 41:149–58
45. Paul M, Benuri-Silbiger I, Soares-Weiser K, Leibovici L: Beta lactam monotherapy *versus* beta lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: Systematic review and meta-analysis of randomised trials. BMJ 2004; 328:668
46. Falagas ME, Rafailidis PI, Kasiakou SK, Hatzopoulou P, Michalopoulos A: Effectiveness and nephrotoxicity of colistin monotherapy *vs.* colistin-meropenem combination therapy for multidrug-resistant Gram-negative bacterial infections. Clin Microbiol Infect 2006; 12:1227–30
47. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL; International Surviving Sepsis Campaign Guidelines Committee; American Association of Critical-Care Nurses; American College of Chest Physicians; American College of Emergency Physicians; Canadian Critical Care Society; European Society of Clinical Microbiology and Infectious Diseases; European Society of Intensive Care Medicine; European Respiratory Society; International Sepsis Forum; Japanese Association for Acute Medicine; Japanese Society of Intensive Care Medicine; Society of Critical Care Medicine; Society of Hospital Medicine; Surgical Infection Society; World Federation of Societies of Intensive and Critical Care Medicine: Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008; 36:296–327
48. Rouby JJ, Bouhemad B, Monsel A, Brisson H, Arbelot C, Lu Q; Group tNAS: Aerosolized antibiotics for ventilator-associated pneumonia: Lessons from experimental studies. ANESTHESIOLOGY 2012; 117:1364–80
49. Bergen PJ, Li J, Nation RL, Turnidge JD, Coulthard K, Milne RW: Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: Studies with *Pseudomonas aeruginosa* in an in vitro pharmacodynamic model. J Antimicrob Chemother 2008; 61:636–42
50. 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; 57:306–11
51. Athanassa ZE, Markantonis SL, Fousteri MZ, Myrianthefs PM, Boutzouka EG, Tsakris A, Baltopoulos GJ: Pharmacokinetics of inhaled colistimethate sodium (CMS) in mechanically ventilated critically ill patients. Intensive Care Med 2012 Jul 19. [Epub ahead of print]
52. Koomanachai P, Tiengrim S, Kiratisin P, Thamlikitkul V: Efficacy and safety of colistin (colistimethate sodium) for therapy of infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in Siriraj Hospital, Bangkok, Thailand. Int J Infect Dis 2007; 11:402–6
53. Palmer LB, Smaldone GC, Chen JJ, Baram D, Duan T, Monteforte M, Varela M, Tempone AK, O'Riordan T, Daroowalla F, Richman P: Aerosolized antibiotics and ventilator-associated tracheobronchitis in the intensive care unit. Crit Care Med 2008; 36:2008–13
54. Mentzelopoulos SD, Pratikaki M, Platsouka E, Kraniotaki H, Zervakis D, Koutsoukou A, Nanas S, Paniara O, Roussos C, Giamarellos-Bourboulis E, Routsi C, Zakynthinos SG: Prolonged use of carbapenems and colistin predisposes

to ventilator-associated pneumonia by pandrug-resistant *Pseudomonas aeruginosa*. Intensive Care Med 2007; 33:1524–32

55. Matthaiou DK, Michalopoulos A, Rafailidis PI, Karageorgopoulos DE, Papaioannou V, Ntani G, Samonis G, Falagas ME: Risk factors associated with the isolation of colistin-resistant gram-negative bacteria: A matched case-control study. Crit Care Med 2008; 36:807–11
56. Barrowcliffe MP, Jones JG: Solute permeability of the alveolar capillary barrier. Thorax 1987; 42:1–10
57. Brody JS, Vaccaro CA, Hill NS, Rounds S: Binding of charged ferritin to alveolar wall components and charge selectivity of macromolecular transport in permeability pulmonary edema in rats. Circ Res 1984; 55:155–67

Appendix: Members of the Nebulized Antibiotics Study Group

Guang-Ju Zhou, M.D., Research Assistant, Mao Zhang, M.D., Ph.D., Professor of Emergency Medicine, Medical Director (Department of Emergency Medicine, Second Affiliated Hospital, Zhejiang University, School of Medicine, Hangzhou, China); Charlotte Arbelot M.D., Praticien Hospitalier, Hélène Brisson, M.D., Praticien Hospitalier, Antoine Monsel, M.D., Chef de Clinique Assistant, Ivan Goldstein, M.D., Ph.D., Research Assistant, Alfonso Sartorius, M.D., Research Fellow, Marine Lecorre, M.D., Research Fellow, Fabio Ferrari, M.D., Ph.D., Research Fellow, Corinne Vezinet, M.D., Praticien Hospitalier (Multidisciplinary Intensive Care Unit, Department of Anesthesiology and Critical Care Medicine, La Pitié-Salpêtrière Hospital, Assistance-Publique-Hôpitaux-de-Paris, UPMC Univ Paris 06, France); Claudia Gutierrez, M.D., Research Fellow, Department of Anesthesiology, Faculty of Medicine Federal University from Rio Grande do Sul, Hospital das Clinicas de Porto Alegre, Brazil; Charles-Hugo Marquette, M.D.,Ph.D., Professor of Pneumology, Medical Director of the Department of Respiratory Diseases, Pasteur hospital, University of Nice-Sophia Antipolis, Nice, France; Olivier Petit-Jean, Ph.D., Professor of Pharmacology, Director of the Department of Pharmacology, Avicenne Hospital, Assistance Publique-Hôpitaux de Paris, Bobigny, France; Christine Bernard, Pharm D, Praticien Hospitalier, Jérôme Robert, M.D., Ph.D., Professor of Bacteriology (Department of Bacteriology, La Pitié-Salpêtrière Hospital, Assistance-Publique-Hôpitaux-de-Paris, UPMC Univ Paris 06), Christian Funck-Brentano, M.D., Ph.D., Professor of Pharmacology, Medical Director, Department of Pharmacology, La Pitié-Salpêtrière Hospital, UPMC Univ Paris 06, France.