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Nebulized and intravenous colistin in experimental pneumonia caused by *Pseudomonas aeruginosa*

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Abstract *Purpose:* Emergence of multidrug-resistant strains in intensive care units has renewed interest in colistin, which often remains the only available antimicrobial agent active against resistant *Pseudomonas aeruginosa*. The aim of this study is to compare lung tissue deposition and antibacterial efficiency between nebulized and intravenous administration of colistin in piglets with pneumonia caused by *P. aeruginosa*. *Methods:* In ventilated piglets, colistimethate was administered 24 h following bronchial inoculation of *Pseudomonas aeruginosa* (minimum inhibitory concentration of colistin = 2 μg ml^{-1}) either by nebulization (8 mg kg^{-1} every 12 h, $n = 6$) or by intravenous infusion (3.2 mg kg^{-1} every 8 h, $n = 6$). All piglets were killed 49 h after inoculation. Colistin peak lung tissue concentrations and lung bacterial burden were assessed on multiple post mortem subpleural lung specimens. *Results:* Median colistin peak lung concentration following nebulization was 2.8 μg g^{-1} (25–75% interquartile range = 0.8–13.7 μg g^{-1}). Colistin was undetected in lung tissue following intravenous infusion. In the aerosol group, peak lung tissue concentrations were significantly greater in lung segments with mild pneumonia (median = 10.0 μg g^{-1}, 25–75% interquartile range = 1.8–16.1 μg g^{-1}) than in lung segments with severe pneumonia (median = 1.2 μg g^{-1}, 25–75% interquartile range = 0.5–3.3 μg g^{-1}) ($p < 0.01$). After 24 h of treatment, 67% of pulmonary segments had bacterial counts <10^2 cfu g^{-1} following nebulization and 28% following intravenous administration ($p < 0.001$). In control animals, 12% of lung segments had bacterial counts

$<10^2$ cfu g^{-1} 49 h following bronchial inoculation. *Conclusion:* Nebulized colistin provides rapid and efficient bacterial killing in ventilated piglets with inoculation pneumonia caused by *Pseudomonas aeruginosa*.

Keywords Antibiotic · Colistin · Nebulization · Pneumonia · *Pseudomonas aeruginosa* · Mechanical ventilation

Introduction

Ventilator-associated pneumonia (VAP) is the most frequent nosocomial infection in the critically ill and affects length of stay and cost in the intensive care unit [1, 2]. VAP caused by *Pseudomonas aeruginosa* is a difficult-to-treat infection associated with high rate of recurrence and frequent selection of new resistance to antibiotics despite adequate initial antimicrobial therapy [3–5]. Emergence of multidrug-resistant strains in intensive care units has renewed interest in an old antibiotic—colistin—which often remains the only available antimicrobial agent active against resistant *P. aeruginosa* [6, 7].

Colistin is a cationic and multicomponent lipopeptide characterized by rapid bactericidal activities and low levels of resistance [8]. During the past 50 years, however, because of its renal and cochlear toxicities as well as its poor tissue penetration [9, 10], intravenous colistin has been restricted to systemic infections caused by Gram-negative bacteria resistant to all other antimicrobial agents. Both success and failure of the treatment have been reported in a few observational, retrospective or uncontrolled series [11–14]. Lung tissue penetration of colistin following intravenous infusion is unknown, and pharmacokinetic studies have been reported only recently [15, 16]. Nebulization of antibiotics offers the possibility of generating high drug concentrations at the site of infection [17]: administration of aerosolized amikacin or ceftazidime resulted in higher lung deposition and bactericidal effects than intravenous infusion in ventilated piglets with extensive pneumonia [17–20]. Recent clinical use of nebulized colistin suggests good efficiency for treating VAP caused by multidrug-resistant Gram-negative bacteria [21, 22]. It was also reported that a single nebulization of colistin was associated with long-lasting high sputum concentrations in patients with cystic fibrosis [23]. However, the ability of nebulized colistin to reach high lung tissue concentrations in order to provide rapid and efficient bacterial killing has never been investigated.

The aim of this study is to compare lung deposition and bactericidal efficiency of nebulized and intravenous colistin in a porcine model of inoculation pneumonia caused by *P. aeruginosa*.

Materials and methods

Additional details are provided in the electronic supplement material.

Study design

Sixteen Large White−Landrace piglets (20 ± 2 kg) were anesthetized, ventilated (tidal volume = 10 ml kg^{-1}, positive end-expiratory pressure = 5 cmH$_2$O, prone position), and instrumented [18, 19]. In 12 animals, 40 ml suspension containing 10^6 colony forming units (cfu) ml^{-1} *P. aeruginosa* isolated from an immunocompetent patient with VAP (without concurrent bacteremia) was bronchoscopically inoculated in both lungs (10 ml in upper lobes, 10 ml in middle lobes, and 20 ml in lower lobes). Minimum inhibitory concentration (MIC) of colistin was 2 mg l^{-1}. All animals were treated according to guidelines of the Department of Experimental Research of Lille University.

Twenty-four hours later, six piglets received an intravenous dose of 40,000 international units (IU) kg^{-1} (3.2 mg kg^{-1}) of colistimethate every 8 h using an electrical infuser (intravenous group), and six an aerosol of 100,000 IU kg^{-1} (8 mg kg^{-1}) of colistimethate diluted in 10 ml sterile water every 12 h, using a vibrating plate nebulizer (aerosol group) (Aeroneb Pro®; Aerogen Ltd., Galway, Ireland). Each administration was performed over 30 min. Nebulization was performed using specific ventilator settings as previously described [20]. One piglet in the intravenous group died of refractory shock after one dose of colistimethate. Mass median aerodynamic diameter of particles (MMAD) was measured using laser velocimeter (Malvern Instrument, Worcestershire, UK) [24]. Extrapulmonary deposition of colistin was determined in vitro as previously described [25]. Blood samples were collected at the end of the antibiotic administration (T0) and then 30 min, 1, 3, 6, and 12 h after T0 in the aerosol group and 30 min, 1, 2, 4, and 8 h in the intravenous group. Animals were killed by exsanguination 1 h after the third aerosol in the aerosol group and 1 h after the fourth intravenous infusion in the intravenous group. Four nontreated inoculated piglets, killed 49 h after inoculation, served as a control group.

Pharmacokinetic and post mortem analysis

Plasma colistin concentrations were measured using high-performance liquid chromatography (HPLC) [26]. The lower limit of quantification for colistin concentration was 0.1 mg l^{-1}. Peak and trough plasma concentrations (C_{max} and C_{min}), elimination half-life ($t_{1/2}$), total area under the

plasma concentration–time curve (AUC), plasma clearance (CL), and volume of distribution were calculated. Following killing, five "subpleural" specimens were excised from upper, middle, and lower lobes. Each was divided in three blocks. On the first cryomixed and homogenized block, colistin tissue concentrations were measured by HPLC. The second and third blocks served for quantitative bacterial analysis and determination of histological severity (mild or severe pneumonia). The lower limit of quantification for bacterial counts in the lungs was 10^2 cfu g^{-1}.

Statistical analysis

All data are expressed as mean ± standard deviation (SD) or as median and 25–75% interquartile range (IQR). Colistin peak lung tissue concentrations measured in the aerosol group in different lung segments were compared by one-way analysis of variance for repeated measures followed by post hoc Fisher least significant difference (LSD) test. Comparison of colistin tissue concentrations between severe and mild pneumonias was performed by Mann–Whitney rank-sum test. Lung tissue bacterial burden was compared between groups using Kruskall–Wallis test. Percentage of infected lung segments and lung segments characterized by bacterial counts ranging between 0 and 10^2 cfu g^{-1} in the three groups were compared by chi-square test. Pharmacokinetic parameters following the first administration of colistin between the two groups of piglets were compared using bilateral unpaired Student's test. Statistical analysis was performed by using Sigmastat 9.0 software (SPSS Inc., San Raphael, CA). A p value <0.05 was considered as significant.

Results

Mass median aerodynamic diameter of aerosolized particles

As shown in Table 1, with tidal volume of 300 ml, MMAD (mean ± geometric SD) was 0.99 ± 1.71 μm at the outlet of the nebulizer and 1.00 ± 1.88 μm at the tip of the endotracheal tube. Eighty-four percent of particle sizes at the tip of the endotracheal tube were below 1.9 μm. MMAD was not influenced by increasing tidal volume to 500 ml.

Extrapulmonary deposition of colistin

Of the initial amount of colistimethate inserted into the nebulizer, 3 ± 1% was retained in the nebulizer's chamber, 26 ± 8% in the inspiratory limb of the

Table 1 Mass median aerodynamic diameter of colistin aerosol

	Outlet of NEB		Tip of ET	
	M1/M2/M3	Mean	M1/M2/M3	Mean
TV 300 ml				
D50 (μm)	0.98/1.06/0.92	0.99	1.04/1/0.98	1.00
σg (μm)	1.76/1.76/1.61	1.71	2.06/1.85/1.75	1.88
D84 (μm)	1.73/1.86/1.48	1.69	2.14/1.86/1.72	1.90
TV 500 ml				
D50 (μm)	0.99/1.00	0.99	0.99/0.89/0.87	0.92
σg (μm)	1.92/1.87	1.89	2.14/1.69/1.41	1.74
D84 (μm)	1.9/1.87	1.88	2.11/1.50/2.11	1.90

M1 first measure; *M2* second measure; *M3* third measure; *mean* mean value of the three measures; *D50* mass median of aerodynamic diameter; *σg* geometric standard deviation; *D84* 84% of particles sizes are below this diameter. *NEB* nebulizer; *ET* endotracheal tube

respiratory circuit, 1 ± 1% in the endotracheal tube, and 10 ± 3% in the expiratory filter. Total extrapulmonary deposition was 40%. The resulting fraction of colistimethate reaching the respiratory tract was 60% of the initial dose of 8 mg kg^{-1} placed in the nebulizer chamber, representing a daily dose equivalent to 9.6 mg kg^{-1} delivered to the respiratory tract.

Colistin lung tissue deposition

Mild pneumonia characterized 52% of secondary pulmonary lobules in the aerosol group versus 73% in the intravenous group. Severe pneumonia characterized 37% of secondary pulmonary lobules in the aerosol group versus 20% in the intravenous group. In the control group, 45% of lung segments were characterized by mild pneumonia and 30% by severe pneumonia.

In the aerosol group, median peak lung tissue concentration of colistin was 2.8 μg g^{-1} (25–75% IQR: 0.8–13.7 μg g^{-1}). Figure 1 shows the regional distribution of colistin lung tissue concentrations within the infected lung parenchyma in the aerosol group. Colistin peak lung tissue concentrations were significantly lower in lung segments S3 and S10 than in segment S8. Peak lung tissue concentrations were significantly lower in lung areas with severe pneumonia than in lung areas with mild pneumonia (Fig. 2).

In the intravenous group, colistin could not be detected in any of the analyzed lung segments.

Bactericidal activity of colistin

After 24 h of colistin administration, median (25–75% IQR) *P. aeruginosa* lung tissue bacterial burdens in the aerosol, intravenous, and control groups were, respectively, 0 (0–5 × 10^2) cfu g^{-1}, 4.10^2 (0–2 × 10^3) cfu g^{-1},
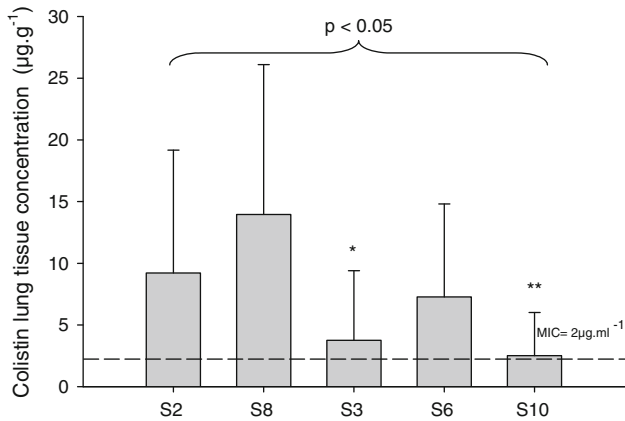
Fig. 1 Regional distribution of colistin peak lung tissue concentrations measured 1 h after the third aerosol of colistin on lung specimens obtained in different lung segments (*S*) representative of each lobe: upper lobe (*S2*), middle lobe (*S3*), and lower lobe (*S6*, *S8*, and *S10*). *MIC* Minimal inhibitory concentration. $p < 0.05$ at the top of the figure indicates statistically significant difference existing between lung segments compared using one-way analysis of variance for repeated measures. Comparisons between two lung segments were performed using post hoc Fisher LSD test: *S3 versus S8, $p < 0.05$, **S10 versus S8, $p < 0.05$

and 5×10^3 cfu g^{-1} (5.8×10^2–3.5×10^6) ($p < 0.001$). As shown in Fig. 3, in the aerosol group, 67% of lung segments were characterized by bacterial counts ranging between 0 and 10^2 cfu g^{-1}, versus 28% in intravenous and 12% in control group. The difference of segments characterized by bacterial counts ranging between 0 and 10^2 cfu g^{-1} was statistically significant between aerosol and intravenous groups and between aerosol and control groups.

Two additional microorganisms were identified in lung tissue specimens: *Pasteurella multocida* (37% and 36% of lung specimens in aerosol and intravenous groups, respectively) and *Streptococcus suis* (30% and 20% of lung specimens in aerosol and intravenous groups, respectively). Differences in lung tissue bacterial burden were not statistically significant between the groups.

Serum pharmacokinetics

As shown in Table 2, following the first administration, colistin C_{max}, C_{min}, and AUC were significantly lower and elimination half-life longer in the aerosol group than in the intravenous group. C_{max}/MIC and AUC/MIC ratios were 3.0 ± 0.7 and 6.7 ± 1.0, respectively, in the intravenous piglets. According to a 40% extrapulmonary deposition, 4.8 mg kg^{-1} colistin entered the lungs by the tracheobronchial tree, whereas in the intravenous group, 3.2 mg kg^{-1} mg colistin was infused intravenously. Therefore, the antibiotic volume of distribution and plasma clearance corrected for the relative bioavailability of aerosolized colistin were similar in aerosol and intravenous groups.

Discussion

This study, performed in anesthetized and ventilated piglets with inoculation pneumonia caused by *P. aeruginosa*, shows high colistin lung tissue deposition following nebulization, contrasting with the absence of any detectable deposition following intravenous administration. In the
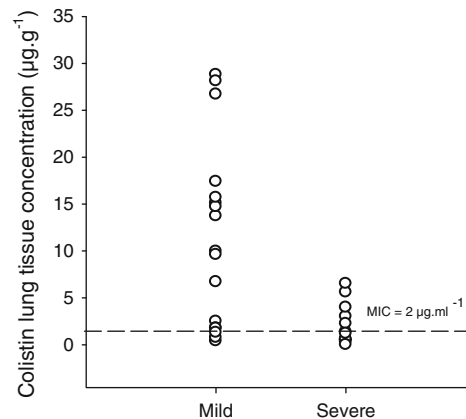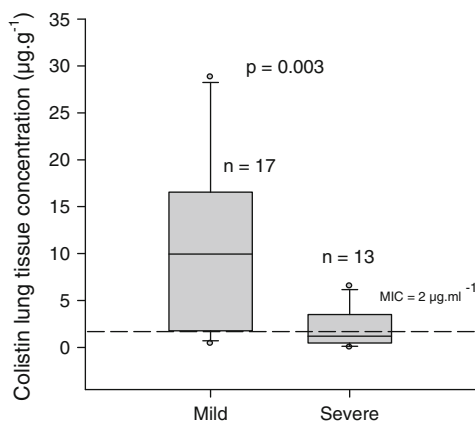

Fig. 2 Colistin peak lung tissue concentrations according to histological severity of pneumonia following 24-h treatment by nebulized colistin. *Left panel* shows median and 25–75% inter-quartile values; *right panel* shows individual values. *Dashed lines* represent minimum inhibitory concentration (*MIC*) of inoculated

Pseudomonas aeruginosa. Mild mild pneumonia, *Severe* severe pneumonia. *n* number of lung segments in each histological category. Peak colistin lung tissue concentrations were significantly greater in lung segments with mild pneumonia
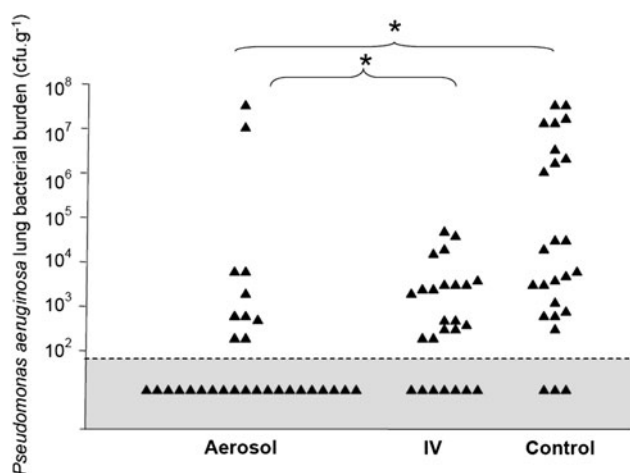
Fig. 3 Lung bacterial burden of *Pseudomonas aeruginosa* after 24 h of colistin administration. Lung segments (*triangles*) were sampled 1 h after the third aerosol in the aerosol group and after the fourth infusion in the intravenous group (*IV*) and 49 h after the bacterial inoculation in the untreated control group. The *grey area* indicates the lower limit of quantification for bacterial counts. *Asterisk* at the top of the figure indicates statistically significant difference existing between the percentage of lung segments characterized by bacterial counts ranging between 0 and 10^2 cfu g^{-1} in aerosol and intravenous groups and in aerosol and control groups

Table 2 Serum colistin pharmacokinetic parameters measured after the first administration in five piglets of the intravenous group (IV) and six piglets of the aerosol group (mean ± SD)

	IV	Aerosol	*p*-Value
Dose (mg kg^{-1})	3.2	4.8*	
C_{max} (mg L^{-1})	6.0 ± 1.5	1.6 ± 1.4	0.01
C_{min} (mg L^{-1})	0.2 ± 0.1	0.0 ± 0.1	0.001
$T_{1/2}$ (h)	1.7 ± 0.4	3.2 ± 1.1	0.02
AUC (mg h L^{-1})	13.5 ± 2.0	7.5 ± 1.8	<0.001
F (%)	–	37	
V_d (l)	14.9 ± 7.8	22.5 ± 7.3	ns
CL (L h^{-1})	5.9 ± 2.0	5.0 ± 1.3	ns

C_{max} peak plasma concentration; C_{min} trough plasma concentration; $T_{1/2}$ elimination half-life; *AUC* area under the plasma concentration–time curve, V_d volume of distribution; *CL* plasma clearance; *F* relative bioavailability of aerosol, calculated as (AUC aerosol/AUC IV) × (dose IV/dose aerosol) × 100. *Dose reaching the respiratory system after 40% extrapulmonary deposition; *ns* not significant

aerosol group, the high lung deposition was associated with rapid and efficient bactericidal activity. Confirming previous experiments on nebulized amikacin [19] and ceftazidime [20], colistin distal lung deposition decreased with severity of pneumonia and aeration loss. Of particular interest, colistin nebulization was associated with decreased systemic exposure compared with intravenous administration.

Lung infection in aerosol and intravenous piglets

Forty-nine hours after inoculation of *P. aeruginosa*, around 90% of secondary pulmonary lobules were infected in both groups. Two additional microorganisms (*P. multocida* and *S. suis*) were isolated from one-third of lung specimens. This result is not surprising, because healthy piglets rapidly develop VAP caused by *P. multocida* and *S. suis* after prolonged mechanical ventilation [27, 28]. Therefore, our experimental model is a severe lung infection caused by bronchial inoculation of *P. aeruginosa* and aggravated by VAP. In both groups, different grades of severity were unevenly distributed through both lungs, with predominance and higher severity in dependent lung segments [27, 28].

Intravenous colistin-induced lung tissue deposition and bactericidal activity

Although recent studies suggest lower toxicity of intravenous colistin than previously reported [14, 29], its clinical use for treating VAP remains controversial. As colistin is a concentration-dependent antibiotic on Gram-negative bacteria, its bactericidal activity depends on peak tissue concentrations reached in infected lung parenchyma [30, 31]. Following three doses of intravenous colistin, lung tissue concentrations were under the detectable threshold in all subpleural specimens, suggesting lack of significant lung deposition. This result is in accordance with a recent study performed in mice with pneumonia caused by multidrug-resistant *P. aeruginosa* [32]: following intravenous colistin, peak concentrations measured in homogenized lungs were 50-fold less than after intranasal administration, resulting in lack of survival protection. Concentrations measured from homogenized lung represent the total amount of colistin present in interstitial and cell compartments. *Pseudomonas aeruginosa* does not penetrate into cells and remains in the interstitial space, where colistin exerts its bactericidal activity by binding to bacterial cell membrane. Therefore, colistin concentrations measured from homogenized lung biopsies underestimate "effective" interstitial concentrations, due to a dilution factor caused by intracellular components [33], and the absence of detectable colistin concentration in our study could be partially artifactual. The lack of significant bactericidal effect, however, clearly suggests the lack of significant colistin lung deposition. Another result that may explain the lack of bactericidal effects is the colistin C^{max}/MIC ratio of ~3 in the intravenous group despite high dose of intravenous colistimethate administered. This result confirms data obtained in patients with multidrug-resistant infections (C_{max}/MIC ratio of ~1.5) [34]. Such ratio is inadequate for providing bactericidal effects.

Nebulized colistin-induced lung tissue deposition and bactericidal activity

Most aerosolized particles generated by vibrating-plate nebulizers range between 0.5 and 5 μm, allowing optimal lung deposition. Nebulized colistin is used for treating multidrug-resistant chronic airway infection in patients with cystic fibrosis [35]. In spontaneously breathing patients receiving 2 million IU of nebulized colistin, peak concentrations in sputum were found ten times higher than the colistin MIC breakpoint [36]. Differing from bronchial infection, pneumonia is characterized by distal bronchiolar and alveolar inflammation/infection. In the present study, colistin tissue concentrations were obtained from subpleural lung specimen composed of alveolar structures and distal noncartilaginous bronchioles. Following three consecutive aerosols, peak tissue concentrations were found higher than MIC, indicating significant distal lung deposition. As such concentrations underestimate interstitial concentrations (see above), it is easy to understand why a major bactericidal effect was observed (Fig. 3).

Confirming previous findings, deposition of nebulized colistin was significantly lower in lung segments with severe pneumonia compared with segments with mild pneumonia (Fig. 2). In two dependent lung segments of lower lobe with lung aeration of 3% and 10%, colistin concentrations in lung homogenates were, respectively, 0 and 1.3 μg g^{-1}, likely explaining the lack of bactericidal effect in these segments. Presence of confluent bronchopneumonia with multiple purulent plugs obstructing distal bronchioles markedly reduces lung aeration, thereby explaining decreased colistin lung deposition. Interestingly, the bactericidal effect was observed in 7 of 13 lung segments with severe pneumonia despite more limited distal lung tissue deposition. Very likely, the presence of intraparenchymal pseudocysts and bronchiolar distension in lung areas with severe pneumonia represents one of the routes by which nebulized colistin reaches distal infected lung [19, 37].

Contrasting with previous data showing that nebulization of amikacin does not decrease systemic exposure and risk of toxicity in piglets with severe lung infection [19], colistin plasma concentrations after nebulization were significantly lower than after intravenous administration (Table 2). Colistimethate, which is negatively charged at body pH, is likely confronted with alveolar basement membrane formed also by negative charges [38, 39]. Both electrical charges resulted in slow systemic passage of colistin through alveolar–capillary barrier and therefore increased the colistin elimination half-life in the aerosol group. The smaller AUC also suggests decreased exposure to colistin systemic toxicity. These results confirm a recent study performed in patients with cystic fibrosis treated by inhaled colistin and reporting reduced systemic exposure [23]. In the present study, significant lower concentrations were found in lung areas with severe pneumonia despite the administration of high doses of aerosolized colistimethate (Fig. 2). This finding indicates that an aerosol dose of 8 mg kg^{-1} every 12 h, a dosage exceeding by threefold the dose commonly reported in the literature [21], is required for efficient treatment of pneumonia caused by sensitive *P. aeruginosa*. Moreover, the significantly reduced systemic exposure resulting from nebulized colistin compared with intravenous colistin provides the possibility to increase doses without increasing risk of toxicity.

Limitations of the study

Available therapeutic options for treating VAP caused by multidrug-resistant Gram-negative bacteria are limited. Although recent, uncontrolled studies have reported clinical efficiency of nebulized and intravenous colistin, these two routes of administration have never been compared [40, 41]. Our study, performed in a highly relevant animal model close to human VAP, evidences the superiority of nebulized colistin compared with intravenous colistin as far as lung deposition, bactericidal activity, and systemic toxicity are concerned. However, one should remember that the tracheobronchial tree anatomy of ventilated humans in the supine position is different from that of piglets in the prone position (Fig. 4). As a consequence, the encouraging results obtained from this experimental study cannot be automatically extrapolated to patients with VAP. Another limitation is that nebulized colistin is unable to treat pneumonia when associated with bacteremia.

Many clinicians believe that aerosol therapy as adjunctive to intravenous therapy offers an attractive alternative to intravenous or nebulized therapy alone for treating VAP. The hypothesis that a combination of nebulized and intravenous antibiotics could increase lung tissue deposition and accelerate bacterial killing was previously tested in four experimental piglets whose lungs were infected by massive bronchial inoculation of *Escherichia coli* and that received a combination of nebulized and intravenous amikacin (unpublished data that were part of a previously published study [19]). Unfortunately, no additional increase in lung tissue concentrations were evidenced, whereas increased trough systemic concentrations were observed, increasing the risk of systemic toxicity. In this experimental study, very high amikacin concentrations were found in lymphatic vessels, suggesting large absorption of amikacin into lymphatic vessels of lung interstitial space. It has to be pointed out that intravenous amikacin, in contrast to colistin, diffuses into the alveolar space and that nebulized amikacin diffuses into the systemic circulation in presence of lung infection. The present study provides solid evidence that lung deposition of intravenous colistin is extremely
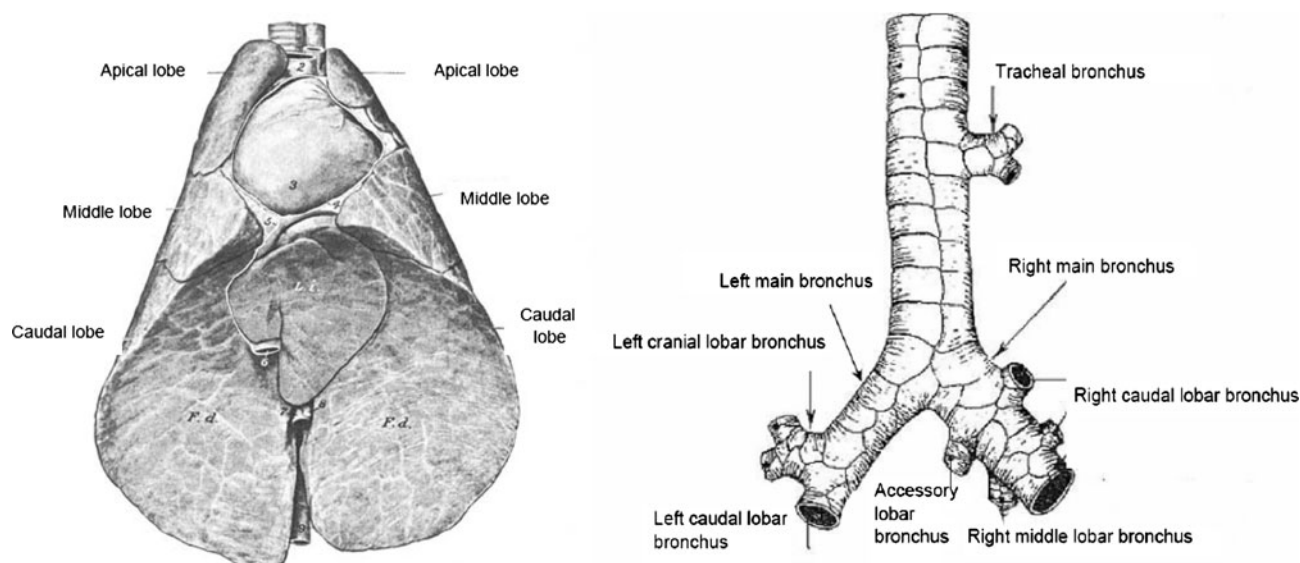
Fig. 4 Lung anatomy of piglet. *Left panel* ventral view of lungs; *right panel* dorsal view of tracheobronchial tree

reduced, if not null. Therefore, it is hazardous to expect that combining nebulized and intravenous colistin might increase colistin lung tissue concentrations. In contrast, increased systemic concentrations resulting from intravenous administration may increase risk of renal toxicity. Intravenous colistin, unlike aminoglycosides and beta-lactams, does not cross the alveolar–capillary barrier of the infected lung parenchyma. Therefore, the rationale for combining nebulized and intravenous colistin as treatment for VAP appears weak. In fact, there is a strong rationale for treating bacteremic VAP caused by resistant *P. aeruginosa* or *Acinetobacter baumanii* with a combination of nebulized and intravenous colistin. Such a combination remains the only therapeutic option.

Unfortunately, our model of inoculation pneumonia is not associated with positive blood cultures and does not allow the assessment of this specific issue. Another experimental study assessing the effects of combined intravenous and nebulized colistin would certainly provide relevant information regarding treatment of pneumonia associated with bacteremia.

In conclusion, nebulized colistin provides rapid and efficient bacterial killing in ventilated piglets with inoculation pneumonia caused by *P. aeruginosa*. Further randomized and comparative clinical studies are required to assess the efficiency of nebulized colistin for treating VAP caused by multidrug-resistant Gram-negative pathogens.

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