Anesthesiology 2004; 100:1446–56

Endotracheal Tubes Coated with Antiseptics Decrease Bacterial Colonization of the Ventilator Circuits, Lungs, and Endotracheal Tube

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Background: **Formation of a bacterial biofilm within the endotracheal tube (ETT) after tracheal intubation is rapid and represents a ready source of lung bacterial colonization. The authors investigated bacterial colonization of the ventilator circuit, the ETT, and the lungs when the ETT was coated with silver-sulfadiazine and chlorhexidine in polyurethane, using no bacterial/viral filter attached to the ETT.**

Methods: **Sixteen sheep were randomized into two groups. Eight sheep were intubated with a standard ETT (control group), and eight were intubated with a coated ETT (study group). Animals were mechanically ventilated for 24 h. At autopsy, the authors sampled the trachea, bronchi, lobar parenchyma, and ETT for quantitative bacterial cultures. Qualitative bacterial cultures were obtained from the filter, humidifier, inspiratory and expiratory lines, and water trap. ETTs were analyzed with light microscopy, scanning electron microscopy, and laser scanning confocal microscopy.**

Results: **In the control group, all eight ETTs were heavily colonized (10^5–10^8 colony-forming units [cfu]/g), forming a thick biofilm. The ventilator circuit was always colonized. Pathogenic bacteria colonized the trachea and the lungs in five of eight sheep (up to 10^9 cfu/g). In the study group, seven of eight ETTs and their ventilator circuits showed no growth, with absence of a biofilm; one ETT and the respective ventilator circuit showed low bacterial growth (10^3–10^4 cfu/g). The trachea was colonized in three sheep, although lungs and bronchi showed no bacterial growth, except for one bronchus in one sheep.**

Conclusions: **Coated ETTs induced a nonsignificant reduction of the tracheal colonization, eliminated (seven of eight) or reduced (one of eight) bacterial colonization of the ETT and ventilator circuits, and prevented lung bacterial colonization.**

VENTILATOR-ASSOCIATED pneumonia is the leading cause of morbidity and mortality in mechanically ventilated patients,[1–3] and it is also associated with significant morbidity, mortality, and cost of care in surgical patients.[4–6] After tracheal intubation, a bacterial biofilm rapidly forms on the polyvinyl-chloride endotracheal tube (ETT)[7] and represents a significant source of bacterial inoculation of the lungs.[8,9] Fragments of the ETT biofilm can detach spontaneously or become dislodged by the suction catheter and enter the lungs with the inspiratory gas flow.[10] In animal studies, such biofilm aggregates were considerably more pathogenic than aerosolized bacteria[11] and were a persistent source of infection.[12,13] *In vitro* and *in vivo* studies have shown that bacterial biofilm is already well organized in a sessile antibiotic-resistant structure within 24 h.[14,15] In clinical studies and in an animal study, Craven *et al.*[7,16] and Christopher *et al.*[17] showed that after a few hours of mechanical ventilation (MV), the ventilator tubing can also become colonized with high bacterial counts and can become an important source of lower respiratory tract colonization. Because of to the universal colonization of the ventilator circuit and our inability to prevent it, Craven *et al.*[7] concluded by saying that "trying to maintain a sterile ventilator circuit for 24 h is a difficult and perhaps impossible task."

Despite recent previous publications investigating the efficacy of coated ETTs,[18,19] there are no published *in vivo* studies showing absence of bacterial biofilm, and bacterial colonization, in the ETT, the ventilator circuit, and the water condensate in the ventilator tubing after 24 h of MV or longer. We report here the use of ETTs coated with silver-sulfadiazine and chlorhexidine in polyurethane[20] to prevent bacterial growth on the ETT, on the ventilator circuit, and in the lungs. Chlorhexidine gluconate is known to reduce the incidence of nosocomial pneumonia when used as an oral rinse in patients undergoing open heart surgery.[21] Also, sulfadiazine acts synergistically with chlorhexidine,[22] and both are used in burn treatment[23] and to coat central venous catheters.[24]

We performed a controlled, randomized study in sheep on MV for 24 h, intubated with either a standard uncoated ETT or an ETT coated with silver-sulfadiazine and chlorhexidine in polyurethane, to asses reduction/elimination of bacterial colonization of the ETT and of the ventilator circuit when such tubes are used.

Materials and Methods

Study Design and Animal Monitoring

This study was conducted at the National Institutes of Health animal research laboratory and was approved by

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Received from the National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland. Submitted for publication September 10, 2003. Accepted for publication December 30, 2003. Supported by the National Heart, Lung and Blood Institute, Division of Intramural Research, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland. Presented at the Annual Meeting of the American Thoracic Society, Seattle, Washington, May 18–21, 2003, and Simposio e Mostra Anestesia Rianimazione e Terapia Intensiva, Milan, Italy, May 28–30, 2003.

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the Animal Care and Use Committee at the National Institutes of Health, Bethesda, Maryland.[25]

This 24-h controlled study involved 16 young female Dorset sheep in two groups. All microbiologic and microscopic studies were performed by observers blinded to the groups.

Control Group. The control group consisted of eight sheep intubated with standard uncoated ETTs and mechanically ventilated for 24 h.

Study Group. The study group consisted of eight sheep intubated with ETTs internally coated with silversulfadiazine and chlorhexidine in polyurethane and mechanically ventilated for 24 h.

Sheep were anesthetized (induction: 7 mg/kg ketamine; maintenance: 2 mg · kg^{-1} · h^{-1} sodium pentobarbital), paralyzed (0.1 mg · kg^{-1} · h^{-1} pancuronium bromide), and mechanically ventilated (volume-controlled ventilation: tidal volume, 8–10 ml/kg; respiratory rate, 14–25 breaths/min, positive end-expiratory pressure, 5 cm H_2O, fraction of inspired oxygen [F_{IO_2}], 0.4) using a Servo 900C (Siemens Elema, Solna, Sweden). Arterial carbon dioxide tension (Pa_{CO_2}) was kept between 35 and 45 mmHg, and F_{IO_2} was adjusted to maintain arterial oxygen tension (Pa_{O_2}) above 80 mmHg, with peak airway pressure under 20 cm H_2O. A heated (37.5°C) respiratory humidifier (MR850 JHU; Fisher & Paykel, Auckland, New Zealand) was connected to a sterile heated infant ventilator circuit (Isothermal respiratory circuit, infant respiratory circuit MR850; Allegiance Healthcare Corporation, McGaw Park, IL). Hemodynamic and respiratory parameters, body temperature, blood gas analysis, blood cell counts, urinary output, chest radiographs, fluid replacement, and parenteral nutrition were monitored/administered as previously described.[26] Sheep were placed prone with the neck, the ETT, and the trachea oriented 30° above horizontal to simulate the orientation of the ETT in the human in the semirecumbent position. No systemic or topical antibiotics were used at any time. After 24 h of MV, sheep were euthanized with an overdose of sodium pentobarbital.

Microbiologic Sampling

Blood Cultures. Using sterile techniques, we took blood samples through direct puncture of the tibial vein every 8 h (four samples). Three milliliters blood was introduced into an aerobic soy broth bottle (Bactec Peds Plus/F; BD Diagnostic Systems, Franklin Lakes, NJ) and sent to the microbiologic laboratory for culture.

ETT Swab. The internal lumen of the ETT was sampled 10–12 cm from the ETT connector piece every 8 h (four samples) for bacterial growth using a cotton culture swab (Bacti-swab; Remel, Lenexa, KS).

Just before the end of the study, using the same techniques as used above, we sampled the air filter between the ventilator and the humidifier, the water from the humidifier, the inspiratory and expiratory lines of the mechanical ventilator approximately 10–12 cm from the ETT connector piece, and the expiratory line condensate water trap (fig. 1).

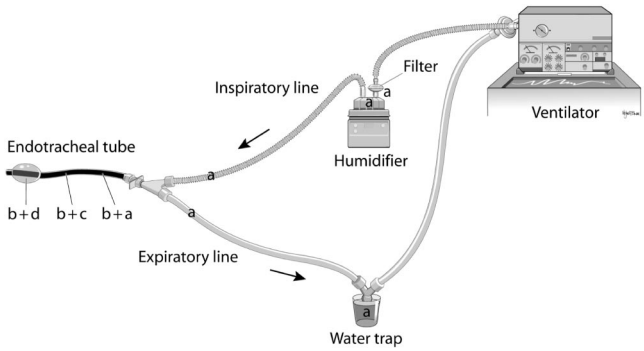

Fig. 1. Ventilator circuit and endotracheal tube (ETT). Sample sites: (*a*) Swabs from the air filter, humidifier, inspiratory lines, ETT, expiratory lines, and water trap; (*b*) ETT biofilm scraped for light microscopy studies; (*c*) secretions from inside the ETT for bacterial culture (colony-forming units [cfu]/g); (*d*) two rings of the ETT were cut, one for scanning confocal laser microscopy studies and one for scanning electron microscopy.

Quantitative Studies. At autopsy, the thorax was opened using strict aseptic techniques, and the lungs were exposed, excised, and weighed. Tissue samples were collected for quantitative culture from each lobe and the lobar bronchi. The trachea and the larynx were opened through an anterior–midline–longitudinal incision up to the carina for visual inspection of the mucosa and of the ETT (fig. 1). The trachea was excised, fixed in 10% buffered formalin, and sent to the pathologist for microscopic study.

As previously described,[26] we took a total of 12 tissue samples weighing approximately 50 mg each: 5 samples from the five lobes of the lungs, 5 samples from the five corresponding lobar bronchi, 1 sample from the trachea 2 cm above the carina, and 1 sample from the middle part of the ETT. The oral cavity was sampled at the beginning of the study.

All tissue/mucus and fluids were placed in sterile vials containing 1 ml sterile physiologic buffered saline and were immediately sent to the microbiology laboratories for quantitative and qualitative aerobic cultures using standard bacteriologic techniques. All indwelling devices (intravenous catheters, arterial catheter, and urinary catheter) were cultured.

Light Microscopy

Mucus and secretions from the inner surface of the ETTs were retrieved with a sterile No. 10 blade, spread on a glass slide, and stained with Gram stain for light microscopic studies (Optiphot-2; Nikon, Tokyo, Japan) (fig. 1). We sampled three locations: (1) 6 cm from the ETT connector piece, (2) 16 cm from the connector piece, and (3) 26 cm from the ETT connector piece.

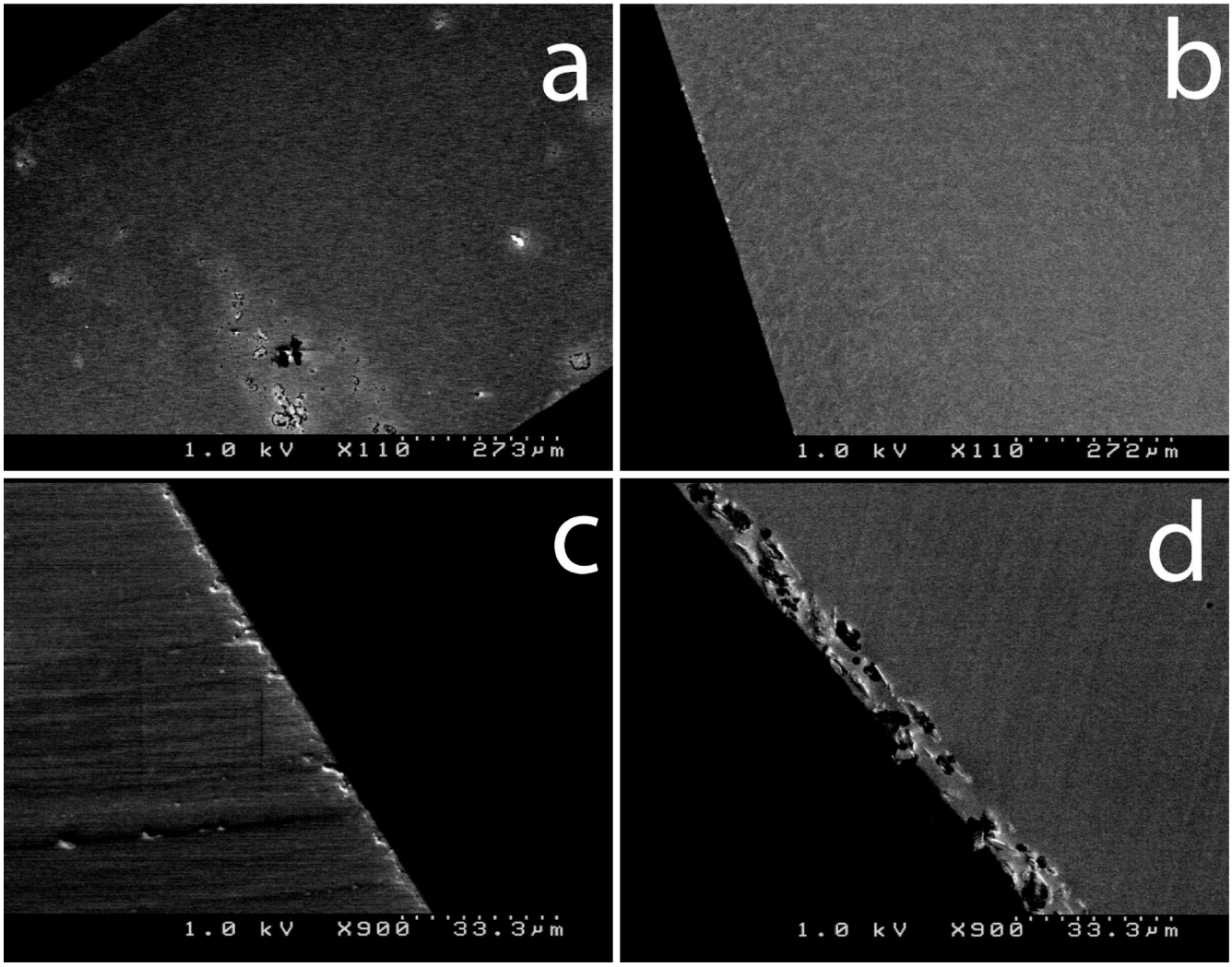

Fig. 2. Micrographs of appearance of a new standard endotracheal tube (*a*, *c*) and coated endotracheal tube (*b*, *d*) using scanning electron microscopy. Note multiple irregularities in the surface of the new endotracheal tube (*a*) and on transverse cut (*c*). When coated with silver-sulfadiazine and chlorhexidine in polyurethane, the coating seems uniform (*b*). Note thickness of the coating (approximately 20 μm) on transverse cut.

Laser Scanning Confocal Microscopy and Scanning Electron Microscopy

One centimeter-long section of the distal ETT (27–28 cm from the ETT connector piece) was cut and immersed in 2.5% glutaraldehyde. A small sample from the cut ETT, approximately 2 mm^2, was stained with 0.0025% acridine orange for 15 min and then mounted with the cut surface firmly against a cover glass. The cross section and the internal coating of the specimen were viewed with a laser scanning confocal microscope (TCS-4D; Leica, Heidelberg, Germany) with excitation and emission wavelengths of 568 and 590 nm, respectively.

Another section of the ETT was cut (28–29 cm from the ETT connector piece), immersed and fixed in 2.5% glutaraldehyde solution for 60 min, and dehydrated in graded alcohol. Then, we used 50%–50% hexamethyldisilazane (EM Sciences, Fort Washington, PA) and in the end 100% hexamethyldisilazane and air dried. The samples were sputter coated (MED 010, Balzers Union, Manchester, NH) with 15 nm of gold and examined using a Hitachi S-4500 scanning electron microscope (Hitachi Ltd., Tokyo, Japan) equipped with a cold cathode field emission gun at an accelerating voltage of 1.0 kV. All images were recording using the secondary electron detector. Micrographs were recorded on a personal computer using PCI quartz cards (Quartz Imaging Corporation, Vancouver, Canada).

ETTs Coated with Silver-sulfadiazine and Chlorhexidine in Polyurethane

We prepared a dispersion of 4 g silver-sulfadiazine, 2 g chlorhexidine, and 6 g polyurethane in 200 ml *N*,*N*-dimethylacetamide. We inserted a standard 8-mm-ID ETT (Lo-Contour; Mallinckrodt Inc., St. Louis, MO) into a hollow transparent acrylic tube to keep the ETT straight.

With the plastic tube positioned vertical, we immersed the tip of the ETT into the dispersion and rapidly aspirated the dispersion up to the level of the connector piece. Immediately thereafter, we let the filled ETT drain for 2–4 s and then placed the transparent plastic tube with the ETT horizontal in a rotational device, through which a stream of air was gently passed to dry the dispersion (fig. 2). After 12 h, the coated ETT was removed from the plastic tube and sterilized with ethylene oxide gas.

Statistical Analysis

We used the Wilcoxon (Mann–Whitney) rank sum test for group comparisons of continuous variables.[27] The Fisher exact test was used for the analysis of categorical variables.[28] We report individual data for each sheep on bacterial colonization of the ETT, saliva, and lower respiratory airways; bacteria isolated from blood cultures and retrieved through swabbing from within the ETT and ventilatory circuit; thickness of the silver-sulfadiazine and chlorhexidine in polyurethane coating; the protein deposit; and biofilm formation. In addition, sheep weight; bacterial colonization of the ETT, saliva, and lower respiratory airways; biofilm; and thickness of ETT coating are summarized as median and range. A P value less than 0.05 was considered statistically significant. All tests were two sided. We performed all analyses using the Stata statistical package (Stata Corporation, College Station, TX; release 7.0).

Results

Study Population, Clinical, and Autopsy Findings

We studied 16 young female Dorset sheep with a median body weight of 30.5 kg (range, 25–37 kg). All animals were healthy based on clinical findings, laboratory data, and chest radiograph 3 days before the study. Sheep were excluded from the protocol if they received antibiotics at any time during the 4 weeks before the study. Intubation was achieved without difficulty on first attempt in all 16 sheep. Both groups maintained a Pao_2:F_{IO_2} ratio greater than 400 at all times. During the study, there was no fever, abnormal leukocyte counts, or change in chest radiographs. No purulent secretions were detected in ETTs of any sheep. On opening the chest after 24 h of MV, we found no gross abnormalities in any sheep of either group, and the tracheal mucosa appeared normal in all.

Bacteriologic Findings

Oropharyngeal Contents and ETT. Samples from the oropharynx of sheep in both groups before intubation showed heavy multibacterial colonization (control group: median, 1.0×10^6; range, 2.5×10^4–9.8×10^6 colony-forming units [cfu]/g; study group: median, 2.8×10^6; range, 2.8×10^5–7.0×10^7 cfu/g), with no significant statistical difference between the two groups ($P = 0.21$) (table 1).

Table 1. Bacterial Colonization of the Oropharynx at the Beginning of the Study and of the ETT after 24 Hours of Mechanical Ventilation

Group/Sheep	Bacterial Species	Oropharynx (cfu/g)*	ETT (cfu/g)†
Control group			
1	Mo	2.7×10^4	
	Ps	3.2×10^4	1.2×10^5
	αS	1.7×10^6	
	Ph	3.5×10^6	2.0×10^5
	Sa		2.0×10^4
2	Ph	1.2×10^6	1.1×10^7
	αS	1.5×10^5	
	Ps	1.8×10^5	
3	Ps	2.8×10^6	1.3×10^5
	αS	7.0×10^6	3.5×10^4
4	Ps	1.5×10^3	1.0×10^7
	αS	6.5×10^4	3.5×10^6
5	Ph	6.5×10^5	3.1×10^6
	αS	7.4×10^5	
6	Ph	2.3×10^3	2.0×10^7
	Kp	8.9×10^4	3.0×10^2
7	Ph	1.9×10^4	1.0×10^8
	Mo	6.6×10^3	2.0×10^8
8	Ph	2.2×10^4	3.0×10^5
	Mo		4.0×10^5
	αS	6.0×10^5	
Study group			
1	Ps	8.6×10^4	
	αS	3.5×10^7	
	Pm	4.0×10^5	
2	Ps	7.0×10^7	
3	Ps	2.7×10^5	
	Mo	8.0×10^3	
4	αS	1.0×10^5	
	Ps	4.0×10^5	
	Mo	8.0×10^3	
5	Pa	4.6×10^5	
6	Ps	1.2×10^6	1.2×10^3
	Mo	6.8×10^5	2.0×10^2
7	Pm	2.3×10^6	
	Mo	2.0×10^5	
	αS	1.3×10^6	
8	Ps	4.0×10^5	
	Pa	8.0×10^6	

* Oropharyngeal secretions were heavily colonized in both groups (control group: median, 1.0×10^6; range, 2.5×10^4–9.8×10^6 colony-forming units (cfu)/g; study group: median, 2.8×10^6; range, 2.8×10^5–7.0×10^7 cfu/g) with no difference in total colonization ($P = 0.21$). † Endotracheal tube (ETT) bacterial colonization was lower in the study group (control group: median, 7.1×10^6 cfu/g; range, 1.7×10^5–3.0×10^8 cfu/g; study group: median, 0; range, 0–3.2×10^3 cfu/g; $P < 0.001$).

αS = α hemolytic *Streptococcus* species (not *Streptococcus pneumoniae*); Kp = *Klebsiella pneumoniae*; Mo = *Moraxella* species; Ph = *Pasteurella haemolytica*; Pm = *Pasteurella multocida*; Ps = *Pasteurella* species; Pa = *Pseudomonas aeruginosa*; Sa = *Staphylococcus aureus.*

After 8 h of MV, the lumen of the standard ETTs in the control group were all pervasively colonized by multiple bacterial species. None of the coated ETTs from the study group were colonized ($P < 0.001$) (table 2). Sim-

Table 2. Bacterial Species Retrieved through Swabbing from Within the ETT and Ventilator Circuit

	ETT				Ventilator Tubing (after 24 h of MV)				
Group/Sheep	0 h*	8 h†	16 h†	24 h‡	Filter*	Humidifier*	Inspiratory Tubing†	Expiratory Tubing‡	Water Trap‡
Control group									
1		Ps	Ps	Ps		Ps	Ps	Ps	Ps
				Mo			Mo	Mo	
		αS	αS	αS			αS	αS	αS
2		Ph	Ph	Ph			Ph	Ph	Ph
			αS	αS				αS	αS
3		Ph	Ph	Ph		Ph	Ph	Ph	Ph
		αS	αS				αS	αS	αS
4			Ph	Ph			Ph	Ph	Ph
		αS	αS	αS				αS	αS
5		Ph	Ph	Ph			Ph	Ph	Ph
			αS	αS			αS	αS	αS
6			Kp	Kp		Kp	Kp	Kp	Kp
		Ph	Ph	Ph		Ph	Ph	Ph	Ph
7			Ph	Ph			Ph	Ph	Ph
		Mo	Mo	Mo			Mo	Mo	Mo
8		Ph	Ph	Ph			Ph	Ph	Ph
		Mo		Mo			Mo	Mo	Mo
Study group									
1									
2									
3									
4									
5									
6				Ph				Ph	Ph
				Mo				Mo	Mo
				αS				αS	αS
7									
8									

Bacterial colonization of the endotracheal tube (ETT) (immediately after intubation and after 8, 16, and 24 h of mechanical ventilation [MV]) and of the ventilator tubing (after 24 h of MV).

* No statistical difference between the two groups in the number of sheep showing colonization. † $P < 0.001$ for difference in the number of sheep showing colonization. ‡ $P = 0.001$ for difference in the number of sheep showing colonization.

αS = α hemolytic *Streptococcus* species (not *Streptococcus pneumoniae*); Kp = *Klebsiella pneumoniae*; Mo = *Moraxella* species; Ph = *Pasteurella haemolytica*; Ps = *Pasteurella* species.

ilarly, after 16 h of MV, there was no bacterial growth in the lumen of coated ETTs ($P < 0.001$). At the end of the 24-h study, the median bacterial growth in the lumen of the ETTs of the control group was 7.1×10^6 cfu/g (range, 1.7×10^5–3.0×10^8 cfu/g) (table 1). The same species of bacteria were found in the ETT as in the oral cavity. There was no bacterial growth in seven of eight coated ETTs; a low level growth of multiple bacteria (1.4×10^3 cfu/g) was found in one sheep ($P = 0.001$, test for difference in cfu/g between the two groups).

Ventilator Circuit. In all sheep of the control group, there was colonization of the inspiratory and expiratory lines of the ventilator circuit and the water trap, with multiple bacterial species, found also in the ETT and in the oropharynx. In the study group, none of the ventilator inspiratory tubes was colonized ($P < 0.001$ *vs.* control group); one expiratory tube and the water trap were colonized by same bacteria found in the ETT ($P = 0.001$ *vs.* control group). Three humidifiers in the control group and none in the study group were colonized ($P = 0.20$). None of the air filters were colonized in either group (table 2)

Trachea-bronchial Tree and Lungs. The trachea was colonized by multiple bacterial species in seven of eight sheep in the control group and in three of eight sheep in the study group ($P = 0.119$, test for difference between the two groups in the number of sheep showing bacterial colonization). The level of colonization (cfu/g), even though it did not reach statistical difference, suggests a lower level of colonization in the study group (overall median of tracheal bacterial colonization in the control group, 7.5×10^5; range, 0–6.6×10^8 cfu/g; overall median of tracheal bacterial colonization in the study group, 0; range, 0–0.7×10^7 cfu/g; $P = 0.052$, test for difference in cfu/g between the two groups).

In the control group, at least one lobe of the lungs was colonized in six of eight sheep with potentially pathogenic bacteria (overall median of lung parenchyma bacterial colonization in the control group, 7.9×10^6; range, 0–4.1×10^9 cfu/g; overall median of bronchial bacterial

Table 3. Bacterial Colonization of the Lower Respiratory Airways (cfu/g)

Group/Sheep	Species	Trachea*	RUB†	RMB†	RLB†	LUB†	LLB†	RUL‡	RML‡	RLL‡	LUL‡	LLL‡
Control group												
1	Mo	5.2×10^4	2.8×10^6			7.1×10^4		4.1×10^6			7.1×10^4	
	Ps	3.6×10^4	1.6×10^6	1.1×10^4		2.1×10^6		8.2×10^6	2.0×10^6		1.2×10^6	
	αS		2.2×10^7									
2	Ph	3.7×10^8	9.2×10^7		3.6×10^7		2.1×10^6	1.6×10^9		2.0×10^9		1.1×10^5
	αS	1.2×10^7			1.1×10^7					5.3×10^8		
	Ps	2.8×10^8										
3												
4	Ps	2.2×10^6			6.9×10^9					1.4×10^5		
	αS	9.3×10^6			1.5×10^6					7.8×10^4		
5	Ph	2.5×10^5	1.6×10^7	2.8×10^7		1.7×10^7	7.6×10^3	1.5×10^9	1.5×10^9	5.6×10^4	1.2×10^5	1.5×10^4
	αS	6.5×10^5						8.1×10^8			1.2×10^5	
6	Ph	2.8×10^7	3.2×10^9		9.0×10^8	8.1×10^4	4.5×10^8	2.1×10^7		8.4×10^8	1.6×10^4	2.2×10^5
	Kp	2.3×10^7			1.6×10^4		4.0×10^3			2.4×10^4		
7	Ph	8.9×10^4										
8	αS	6.0×10^5		1.8×10^4		1.3×10^4			1.3×10^5	3.6×10^4	1.6×10^4	3.0×10^4
Study group												
1	Ps	1.6×10^7										
	αS	9.9×10^5										
2												
3												
4												
5												
6	Ps			1.7×10^6								
	Mo	9.0×10^5										
7												
8	Pa	8.0×10^3										

* Trachea: Total colony-forming units (cfu)/g bacterial colonization of the trachea in the two groups was not statistically different (control group: median, 7.5×10^5; range, 0–6.6×10^8 cfu/g; study group: median, 0; range, 0–1.7×10^7 cfu/g; $P = 0.052$). † Bronchi: Total cfu/g bacterial colonization of bronchi was lower in the study group (control group: median, 4.5×10^7; range, 0–6.9×10^9 cfu/g; study group: median, 0; range, 0–1.7×10^6 cfu/g; $P = 0.01$). ‡ Pulmonary parenchyma: Total cfu/g bacterial colonization of lungs was lower in the study group (control group: median, 7.9×10^6; range, 0–4.1×10^9 cfu/g; study group: median, 0; range, 0–0 cfu/g; $P = 0.004$).

αS = α hemolytic *Streptococcus* species (not *Streptococcus pneumoniae*); Kp = *Klebsiella pneumoniae*; LLB = left lower bronchus; LLL = left lower lobe; LUB = left upper bronchus; LUL = left upper lobe; Mo = *Moraxella* species; Pa = *Pseudomonas aeruginosa*; Ph = *Pasteurella haemolytica*; Ps = *Pasteurella* species; RLB = right lower bronchus; RLL = right lower lobe; RMB = right middle bronchus; RML = right middle lobe; RUB = right upper bronchus; RUL = right upper lobe; T = trachea.

colonization in the control group, 4.5×10^7; range, 0–6.9×10^9 cfu/g). When present, the bacterial species found in bronchi and the lungs reflected those in the ETT and oropharynx. In the study group, with the exception of the right middle lobe bronchus of one sheep, bronchial samples showed no growth (overall median of bronchial bacterial colonization in the study group, 0; range, 0–1.7×10^6 cfu/g; $P = 0.004$ *vs.* control group), and none of the lungs were colonized (overall median of lung parenchyma bacterial colonization in the study group, 0; range, 0–0 cfu/g; $P = 0.01$ *vs.* control group) (table 3).

Blood Cultures.

Control Group. Of 32 blood cultures, 11 were positive, 10 of which included potentially pathogenic bacteria (α-hemolytic *Streptococcus*, *Klebsiella*, *Pasteurella*). Blood cultures remained negative throughout the study in three of eight sheep. Five sheep had positive blood cultures, with more than one positive blood culture in four sheep. In those sheep, the same bacterial species were also found in the lungs at autopsy.

Study Group. Of 32 blood cultures, 4 were positive for bacterial growth, only 1 of which showed pathogenic bacteria. No sheep had more than one positive blood culture. Two blood cultures were positive (nonpathogenic bacteria) at the beginning of the study, and two immediately before autopsy ($P = 0.07$ *vs.* control group) (table 4).

Light Microscopy

Control Group. In the control group, the ETT contained mucus with bacterial aggregates or singly at all three sites in all sheep. Polymorphonuclear cells and epithelial cells were common in all sites (fig. 3 and table 5).

Study Group. Epithelial cells, polymorphonuclear cells, and individual bacteria were observed only at the tip of three of eight ETTs ($P = 0.026$ *vs.* control group). Bacterial aggregates were seen in one ETT ($P = 0.01$ *vs.* control group). In the middle sections of ETTs, and adjacent to the ETT connector piece, we observed only mucus without bacteria or epithelial cells, except in one sheep, where we found some bacteria and rare polymor-

Table 4. Blood Cultures

Group/Sheep	0 h	8 h	16 h	24 h
Control group				
1				
2		Ps		Ps
3				
4	*Mi*			
5		Ph	Ph	Ph
6			Kp	Kp
7				
8		αS	αS	αS
Study group				
1				Pm
2				*Ba*
3				
4				
5				
6	*Mi*			
7	*Se*			
8				

No statistical difference was found between the two groups ($P = 0.07$). *Bacillus* species, *Micrococcus* species, and *Staphylococcus epidermidis* are considered commensal (nonpathogenic bacteria) in sheep. In italics: nonpathogenic bacteria in sheep. In bold: pathogenic bacteria in sheep.

Blood cultures were performed every 8 h.

αS = α hemolytic *Streptococcus* species (not *Streptococcus pneumoniae*); Ba = *Bacillus* species; Kp = *Klebsiella pneumoniae*; Mi = *Micrococcus* species; Ph = *Pasteurella haemolytica*; Pm = *Pasteurella multocida*; Ps = *Pasteurella* species; Se = *Staphylococcus epidermidis.*

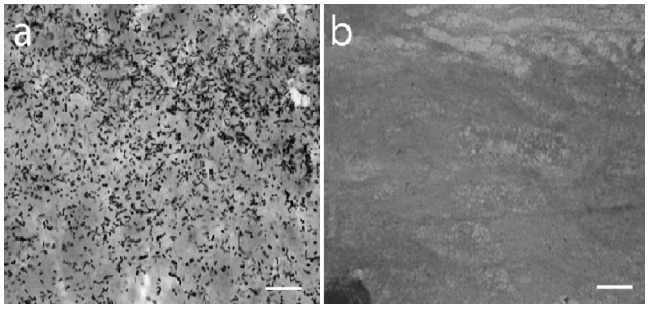

Fig. 3. Gram stain of secretions from the endotracheal tube. (*a*) Individual bacteria or aggregates in all samples in standard endotracheal tubes (control group). (*b*) No bacteria were seen in secretions using a silver sulfadiazine-chlorhexidine coated endotracheal tube (study group). *Bar*, 15 μm.

Table 6. Laser Scanning Confocal Microscopy: Thickness of the Silver-sulfadiazine and Chlorhexidine in Polyurethane Coating, Protein Deposit, and Biofilm

Sheep	Control Group: Biofilm Thickness, μm	Study Group: Protein Deposit Thickness, μm*	Study Group: Silver-sulfadiazine and Chlorhexidine in Polyurethane Coating Thickness, μm
1	253.5	53.9	32
2	204.5	49.2	31
3	224.0	25.8	26
4	223.8	21.7	26
5	248.4	32.8	27
6	193.3	51.3	11
7	405.6	24.3	31
8	317.2	31.2	29
Median (range)	236.2 (193.3–405.6)	32.0 (21.7–53.9)	28 (11–32)

* Thickness of protein deposits in the coated tracheal tube (ETT) was lower than the biofilm in the noncoated endotracheal tube ($P < 0.001$).

phonuclear cells ($P = 0.01$ *vs.* control group) (fig. 3 and table 5).

Laser Scanning Confocal Microscopy

The thickness of silver-sulfadiazine and chlorhexidine in polyurethane ETT coating in the study group was similar in all the sheep (range, 26–32 μm), with the exception of sheep number 6 (11 μm) (table 6). By using scanning confocal microscopy, it was possible to examine the biofilm without significant previous dehydration (fig. 4). The thickness of the bacterial biofilm in the uncoated ETTs (control group) was 236.2 μm (range, 193.3–405.6 μm) (table 6); eukaryotic cells and bacteria were observed in abundant amorphous material at higher magnification (×1,000). Commonly, bacteria were aggregated and densely packed, rather than isolated. In the silver-sulfadiazine and chlorhexidine coated ETTs, we found only a few random eukaryotic cells attached to the surface, which formed a continuous amorphous layer 32.0 μm thick (range, 22–54 μm; $P < 0.001$ *vs.* control group) (table 6).

Table 5. Analysis of the Internal Surface of the ETT through Light Microscopy

	Control Group (n = 8)			Study Group (n = 8)		
	ETT at Mouth	ETT Middle	ETT at Tip	ETT at Mouth	ETT Middle	ETT at Tip
Bacteria						
Random scatter or individual bacteria	8	8	8	1†	1†	3‡
Clumps or aggregates	8	8	8	0*	0*	1†
Leukocytes						
Polymorphonuclear cells	8	8	8	1†	1†	3‡

Light microscopy: Secretions of the internal surface of the endotracheal tube (ETT) were scraped at 6, 16, and 26 cm from the connector piece of the ETT and analyzed with light microscopy for presence of bacteria and leukocytes.

* $P < 0.001$ compared with control group. † $P = 0.001$ compared with control group. ‡ $P = 0.026$ compared with control group.

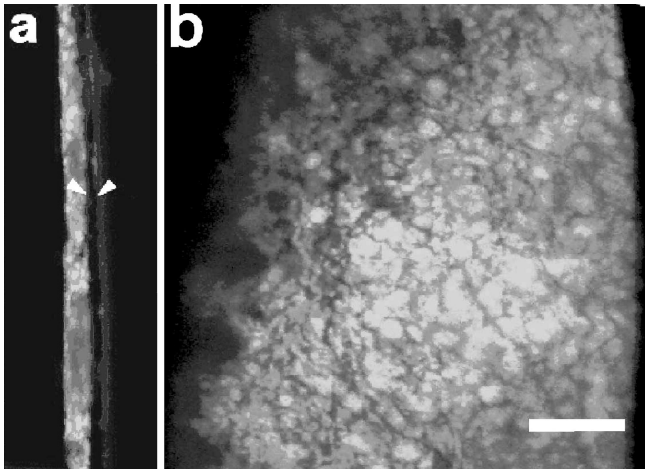

Fig. 4. Confocal scanning micrograph of the internal surface of a standard endotracheal tube and coated endotracheal tube after 24 h of mechanical ventilation. Confocal scanning micrograph of coated endotracheal tube (*a*) and uncoated endotracheal tube (*b*) showing layering of interfaces. Note great increase in biofilm thickness in *b* compared with thin proteinaceous layer in *a*. Between *arrows*, silver sulfadiazine-chlorhexidine in polyurethane. *Bar* (*bottom right*), 40 μm.

Scanning Electron Microscopy

The internal surface of the ETT at low-power scanning electron microscopy showed effects of critical point drying: Deep fissures and cracks were observed in all samples. A very thick multilayer biofilm (range, 40–65 μm) was seen in the control group; this thickness measured a few microns in the study group (fig. 5).

At higher magnification, amorphous material was observed on the inner surface of all ETT in both groups. In the control group, globular formations (1–2 μm) were easily recognized, structured in lines suggesting presence of bacteria partly submerged in the matrix, with only the outline discernible (fig. 6). No such structures were observed in the study group.

Discussion

Pneumonia is the most frequent nosocomial infection in the intensive care unit[1–3] and is of major concern in both postoperative patients[4] and surgical intensive care units,[5,6] likely related to prolonged intubation and MV.[29] Langer *et al.*[30] were among the first to report high incidence of pulmonary infection during the first days of MV. They raised the possibility that early pneumonia might be ascribed to contaminated ventilator circuits, but frequent replacement of the ventilator circuit does not reduce ventilator-associated pneumonia.[31] However, it is now well documented that bacterial colonization of the ventilator circuit (ETT, inspiratory, expiratory lines, filters) is an early process after only a few hours of MV.[7–9,16] Ventilator-associated pneumonia is associated with thick, bacteria-laden biofilm within the ETT that can rapidly colonize the ventilator circuit.[8] This biofilm within the ETT is greatly resistant to antibacterial agents[32–34] because (1) bacteria are physically isolated from blood flow; (2) sessile forms of dense multibacterial plaques offer potent protection as antimicrobial agents fail to penetrate the full depth of the biofilm[35,36]; and (3) bacteria in a biofilm frequently exist in a quiescent state,[37] with characteristic gene expression,[38] morphology,[39] and phenotype,[40] and readily adapt to change in pH and oxygen deprivation.[41] The use of bacterial/viral filters during anesthesia has been shown to protect ventilator circuit from viral/bacterial colonization[42] but does not prevent bacterial colonization and biofilm formation of the ETT. Previous studies have shown an association between bacterial biofilm and ventilator-associated pneumonia.[8,9] This was believed to be related to (1) aerosolization of the biofilm through the inspiratory gas flow, (2) dislodgement of biofilm into the lungs through spontaneous detachment of bacterial colonies, or (3)

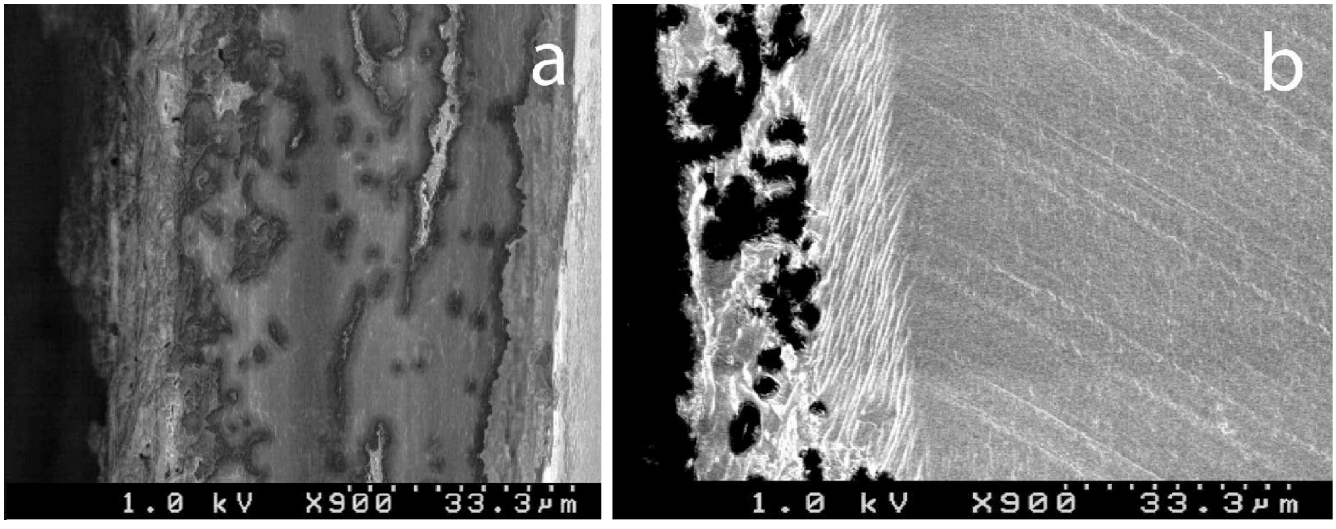

Fig. 5. Scanning electron micrograph of the internal surface of standard endotracheal tube and coated endotracheal tube after 24 h of mechanical ventilation. (*a*) Note thick (41–52 μm) multilayering on lateral view. (*b*) Study group: no biofilm visible. Note nonhomogeneous coating of silver-sulfadiazine and chlorhexidine (28–34 μm).

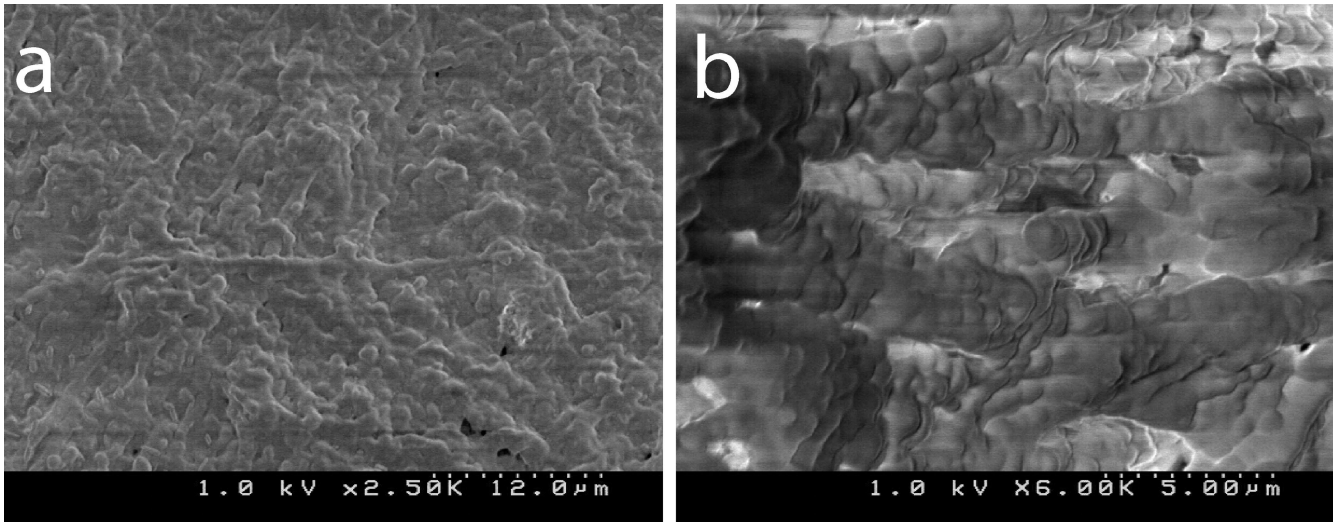

Fig. 6. Scanning electron micrograph of the internal surface of the standard endotracheal tube and coated endotracheal tube after 24 h of mechanical ventilation. (*a*) Amorphous matrix covers the surface of the standard endotracheal tube. Cocci in chains and rods are visible in the dense matrix. (*b*) At higher magnification, the cocci are organized in clumps and chains.

dislodgement of biofilm into the lungs through the use of a suction catheter.

In this prospective, randomized, controlled trial in sheep, we have demonstrated that bacterial growth within the ETT and the entire ventilator circuit during 24 h of MV was prevented in seven of eight sheep and was greatly reduced in the remaining one sheep when the internal surface of the ETT was coated with silver-sulfadiazine and chlorhexidine in polyurethane and while the orientation of the trachea and ETT was elevated 30° degrees above horizontal, simulating the semirecumbent position in the human. Absence of bacterial colonization of the ETT and of the ventilator circuit correlated with absence of bacterial colonization of the bronchi and of the lungs after 24 h of MV in seven of eight sheep, whereas with uncoated ETTs, we found rapid bacterial colonization of the ETT and the entire ventilator circuit and frequent widespread, heavy bacterial colonization of the lower respiratory tract. However, in the study group, the trachea was colonized in three sheep, with no statistical difference compared with the control group ($P = 0.119$, test for difference between the two groups on the number of sheep showing bacterial colonization, and $P = 0.052$, test for difference in cfu/g between the two groups).

In a previous 72-h study in sheep,[26] we showed that both the ETT and the lungs were always heavily colonized when the ETT and trachea were elevated 30° above horizontal, as in this study; however, the trachea, bronchi, and lungs were free from bacterial colonization when the ETT was kept horizontal, even though the ETT was always heavily colonized. In the current study, we showed that the use of ETTs internally coated with bactericidal agents in polyurethane prevents bacterial colonization of the ETT, with the bronchi and lungs also free from bacterial growth, even when the trachea and ETT were elevated 30° above horizontal. These two studies in an animal model lead us believe that an important mechanism in the pathogenesis of the lung bacterial colonization during the first days of MV, along with leakage of heavily colonized oropharyngeal contents across the inflated ETT cuff,[3,43,44] is to be ascribed to bacteria-laden mucus, or microdroplets, and bacteria-laden water condensate that enter the lungs through the colonized ETT.[8–10]

In a recent study, Olson *et al.*[18] showed reduced bacterial growth in lungs of dogs intubated with ETTs coated with a novel antimicrobial silver-hydrogel. However, the concentration of aerobic bacteria from the sampled inner lumen segments of the ETTs at the time of necropsy was only reduced (study group, 6.1 ± 1.3 *vs.* control group, 4.1 ± 2.1 log cfu/cm; $P = 0.009$), never eradicated. In addition, the ventilator circuit was not humidified and heated to 37°C, possibly reducing bacterial growth.

Jones *et al.*[19] showed prevention of bacterial biofilm using novel surfactant bacterial antiadherent coatings in an *in vitro* setting. However, we believe that *in vivo* studies are required to assess whether antiadhesive bacterial coatings alone are efficacious in the prevention of lung bacterial colonization.

In our study, we coated the ETTs internally with silver-sulfadiazine and chlorhexidine in polyurethane because silver ions have long been known to be bactericidal and fungicidal[45] and because relatively nontoxic silver-sulfadiazine and chlorhexidine[46] have been used to coat intravenous[24] and urinary catheters[47] and in burn patients.[48,49] Moreover, chlorhexidine and sulfadiazine in combination are known to exhibit synergistic antifungal and antimicrobial properties, in particular against strains of *Pseudomonas*, *Proteus*, and *Staphylococcus*.[20] Signif-

icantly, bacteria are not known to develop resistance to silver-sulfadiazine and chlorhexidine.[20]

Our studies are now being extended beyond 24 h to establish optimal loading of silver-sulfadiazine and chlorhexidine in polyurethane and to learn whether chlorhexidine or silver-sulfadiazine alone in polyurethane is equally effective. In addition, toxicology studies should test the safety of prolonged use of these coated ETTs.

Although animals of both groups did not present any clinical finding suggesting pneumonia during the 24 h of MV (chest radiograph, laboratory finding, fever) and we did not perform histologic analysis of lung tissue, it is clear that coated ETTs, as in our current studies, may already potentially be highly beneficial in the prevention of ventilator-associated pneumonia during surgical procedures during general anesthesia requiring intubation and MV and particularly during expected prolonged postoperative MV.

The authors thank Martha Vaughan, M.D. (Deputy Chief, Pulmonary and Cardiac Assist Devices, Pulmonary Critical Care Medicine Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland), and Joel Moss, M.D. (Chief, Pulmonary Critical Care Medicine Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Department of Health and Human Services), for critical review of the manuscript, and the staff of the Laboratory of Animal Medicine and Surgery, National Heart, Lung and Blood Institute, National Institutes of Health, and of the Veterinary Resources Program, National Institutes of Health, for assistance throughout the animal studies and the bacteriological analysis.

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