

Effect of Mechanical Cleaning of Endotracheal Tubes With Sterile Urethral Catheters to Reduce Biofilm Formation in Ventilator Patients

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Objectives: To investigate the effectiveness of mechanical cleaning with sterile urethral catheters to prevent formation of biofilms on endotracheal tubes.

Methods: Forty-five children were randomized in equal numbers to endotracheal tube cleaning group for three times a day (group A), twice daily (group B), or to a control group with no endotracheal tube cleaning (group C). Bacterial studies and confocal laser scanning microscopy were performed to assess bacterial colonization and biofilm thickness on the internal surface of the endotracheal tube.

Results: In group B, the quantities of viable bacteria adhering to the endotracheal tube after 1 day of ventilation were similar to the control group but were significantly less by 3 days ($p < 0.05$). The quantities of viable bacteria adhering to the endotracheal tube in group A were significantly lower than group C from day 1 to day 7 ($p < 0.05$). The numbers of culture-positive endotracheal tube and lower respiratory tract secretions were both reduced in groups A and B compared with group C. Confocal laser scanning microscopy showed progressive development of mature biofilms in group C. Scattered bacteria were seen in group A with no biofilm formation. In group B, a small amount of extracellular polymeric substance was seen, with more bacterial cells than in group A. The biofilms in group B were significantly thinner than those in group C ($p < 0.05$). The occurrence of ventilator-associated pneumonia was significantly reduced by endotracheal tube cleaning.

Conclusion: Mechanical cleaning with sterile urethral catheters reduced bacterial colonization, prevented formation of endotracheal tube biofilm, and reduced the occurrence of ventilator-associated pneumonia. (*Pediatr Crit Care Med* 2013; 14:e338–e343)

Key Words: biofilm; confocal laser scanning microscopy; endotracheal tube; urethral catheter; ventilator-associated pneumonia

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Ventilator-associated pneumonia (VAP) is a common complication in patients receiving mechanical ventilation (MV) and is an important cause of increased mortality, length of hospital stay, and cost. Endotracheal intubation is an independent risk factor for VAP (1, 2). After 3 days of intubation and MV, the lumen of the endotracheal tube (ETT) becomes heavily colonized by bacteria, which often organizes into a thick, mature biofilm. The formation of ETT biofilms and subsequent, recurrent transfer of bacteria to the lower respiratory tract may lead to development of lower respiratory tract infection and VAP (3). A device, the Mucus Shaver, was developed by Berra et al (4) to keep the internal surface of the ETT free of mucus secretions and to prevent the formation of BF, but the design of the Mucus Shaver is complicated, and it is only applicable to adults. Therefore, increased availability of interventions that reduce bacterial adhesion to ETTs and avoid biofilm formation is needed for prevention of VAP. This randomized study evaluated a mechanical cleaning method that used sterile urethral catheters, which are widely available in hospital units caring for mechanically ventilated patients. We measured the effect of removing residual secretions within the ETT on bacterial adhesion and the formation of biofilms.

PATIENTS AND METHODS

Patients

This study enrolled 45 children receiving MV in the PICU of Children's Hospital, Chongqing Medical University, Chongqing, China, following cardiac surgery. Eligible children were greater than 1 month of age, received MV for the first time, and expected to need ventilation for more than 6 hours. The parents of all enrolled children gave prior written informed consent.

Study Design

Patients were randomized to three study groups of 15 children each. The ETTs were mechanically cleaned with an 8F sterile urethral catheter after routine suctioning: three times a day in group A and twice daily in group B. Mechanical ETT cleaning

was not performed in the control patient group. Routine tracheal suctioning was performed as needed. Based on previous studies of biofilm formation (5), each of the three study groups were divided into three subgroups of 1 day, 3 days, and 7 days duration of ventilation.

Cleaning Method

The volume of air needed to inflate the catheter balloon was determined from in vitro measurements. After routine suctioning, a sterile urethral catheter was inserted from the connector of the ETT to the tip. The balloon was then inflated with a 2-mL syringe so that it was pressed firmly against the wall of the ETT. The urethral catheter was then gently pulled out of the ETT over a period of 3–5 seconds to remove accumulated mucus from the lumen of the ETT. An assistant held the ETT while it was being cleaned.

Safety Evaluation

All the patients were maintained in a 30° supine Fowler position. Continuous measurements of heart rate, respiration, blood pressure, and oxygen saturation were made before and after mechanical cleaning with the urethral catheter. Patients were auscultated for symmetrical lung volumes and to monitor the insertion depth of the ETT. In addition, the duration of intubation, ICU stay, and occurrence of VAP were recorded.

Diagnostic Criteria of VAP

The diagnostic criteria for VAP included MV for more than 48 hours and new or progressive infiltration on lung X-ray plus any two of the following signs: fever, that is, body temperature $\geq 37.5^{\circ}\text{C}$ or $\geq 1^{\circ}\text{C}$ higher than basal body temperature; WBC $\geq 10 \times 10^6/\text{iL}$, or $\leq 4.0 \times 10^6/\text{iL}$; appearance of purulent airway secretions or an increase in secretion volume; confirmed etiology of a new infection (6).

Microbiological Sampling

Specimens of lower respiratory tract secretions were obtained through the ETT after intubation and before extubation. After extubation, each ETT was sealed in a sterile bottle and rinsed gently inside and outside with sterile phosphate-buffered

saline in order to remove nonadherent bacteria and nonbiofilm complexes. A 1-cm segment was cut from the bottom of ETT and divided longitudinally into two parts: one for a bacterial colony count and the other for confocal laser scanning microscopy. The former was placed in L-Broth culture medium, and after ultrasonic oscillation, the solution was used for bacteriological monitoring. Sputum specimens and the L-broth medium were sent to the hospital's clinical laboratory for bacteriological isolation, quantitative culture, and bacterial identification using the MicroScan WalkAway-40 Automatic Identification System (Dade-MicroScan International, West Sacramento, CA).

Bacterial Colony Count of ETT Biofilm

Ten-fold serial dilutions of 200 μL volumes of solution obtained from ETT biofilms by ultrasonic oscillation were streaked onto fresh blood agar plates (20 $\mu\text{L}/\text{plate}$). Visible colonies were counted after incubation for 24 hours at 37°C . Colony forming units (CFU) per square centimeter of ETT surface area were calculated as follows: $\text{cm}^2 = \text{inner diameter of the ETT} \times 0.1\pi$; and $\text{CFU}/\text{cm}^2 = \frac{\text{visible colonies} \times \text{dilution} \times 2 \text{ mL}}{20 \text{ .L} \times \text{area}}$. The standard colony count was expressed as lg (CFU/cm² + 100).

Confocal Laser Scanning Microscopy

Segments of ETT were fixed in 4% paraformaldehyde, cut into smaller strips with a sterile scalpel, and stained with *Canavalia ensiformis* A (concanavalin A, con A), labeled fluorescein isothiocyanate (FITC-Con A), and propidium iodide (PI) (Sigma, UK) solution at room temperature in the dark. Each strip was then rinsed three times. The fixed, stained specimens were viewed using a confocal laser scanning microscope. The bacterial cell population and extracellular polysaccharide (EPS) were observed, and the biofilm thickness was measured.

Statistical Analysis

One-way analysis of variance was used for significance testing of all measurement data. The Student-Newman-Keuls test was used for postanalysis of variance group comparisons. Fisher exact test was used for analysis of colony count data. A *p* value of less than 0.05 was considered statistically significant. Statistical analysis was done using SSPS 13.0 software.

TABLE 1. Patient Characteristics and Clinical Data

Baseline Data	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	<i>p</i>
Age (yr) mean \pm SD	10.13 \pm 17.94	15.36 \pm 19.37	16.66 \pm 19.24	0.609
Weight (kg) mean \pm SD	5.92 \pm 2.81	8.27 \pm 4.7	7.57 \pm 3.41	0.219
Male/female (n)	9/6	8/7	11/4	0.516
Mechanical ventilation duration (d) mean \pm SD	4.25 \pm 2.91	4.53 \pm 2.89	4.81 \pm 3.65	0.888
ICU stay (d) mean \pm SD	6.80 \pm 2.98	8.24 \pm 7.46	10.73 \pm 12.06	0.289
Ventilator-associated pneumonia prevalence, <i>n</i> (%)	1.75 ^a	1.64 ^a	10.48	<0.05

^aSignificant compared with Group C.

RESULTS

Patient Characteristics and Clinical Data

There were no significant differences among the three groups in age ($p = 0.609$), gender ($p = 0.516$), or weight ($p = 0.219$; **Table 1**). There were significant differences in the prevalence of VAP in both groups A and B versus group C ($p < 0.005$). There were no significant differences in length of ICU stay in groups A and B versus group C ($p = 0.289$). Between-group differences in the duration of MV were not significant (**Table 1**).

Regarding safety, there were no significant differences in the changes in heart rate, respiration, blood pressure, or oxygen saturation before and after mechanical cleaning in groups A and B versus the control group ($p > 0.05$). Respiratory auscultation was symmetrical in all groups. No adverse effects were observed during cleaning of the ETT with the urethral catheter.

Microbiological Culture

Microbiological culture findings are summarized in **Tables 2–4**. In group A, eight of 15 ETTs were colonized, including one mixed population (Hemolytic staphylococci/*Streptococcus viridans*). Seven of 15 samples of lower respiratory tract secretions were colonized with bacteria, five with normal flora. In group B, 10 of 15 ETTs were culture positive, with one mixed population (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*). Nine samples of lower respiratory tract secretions were culture positive, six with normal flora. In group C, 13 ETTs were

colonized including three mixed populations (*Staphylococcus epidermidis* and *Staphylococcus faecalis*, *Enterobacter cloacae* and *Candida albicans*, *P. aeruginosa*, and *A. baumannii*). Twelve samples of lower respiratory tract secretions were colonized, three with normal flora.

CFUs per square centimeter calculated from colony counts in cultures of viable bacteria adhered to the ETT were significantly lower in group A than in group C ($p < 0.05$). The difference in number of colonies isolated from the ETTs in group B versus group C were not statistically different at 1 day ($p > 0.05$), but after 3 days of intubation, the differences were statistically significant ($p < 0.05$) (**Table 4**).

Confocal Laser Scanning Microscopy

The FITC-Con A and PI labels of extracellular polysaccharide (green fluorescence) and bacterial cell DNA (red fluorescence) clearly show reduced bacterial cell adherence and biofilm formation on ETTs that had been mechanically cleaned compared with controls (**Fig. 1**).

Microscopy revealed gradual thickening of the biofilms that formed in the control group with prolongation of MV. Biofilm thickness increased by about 11 μm from day 1 to day 3 and then continued to develop somewhat slowly, increasing by an additional 6 μm through day 7. Biofilms were significantly thinner ($p < 0.05$) in both groups A and B than in group C (**Table 5**).

TABLE 2. Distribution of Bacteria on the Surface of Endotracheal Tube

Microorganisms	No. of Cultures		
	Group A	Group B	Group C
Gram-negative bacteria			
<i>Klebsiella pneumoniae</i>	—	—	1
<i>Bacillus coli</i>	1	2	1
<i>Aerobacter cloacae</i>	—	1	1
<i>Pseudomonas aeruginosa</i>	—	2	1
<i>Acinetobacter baumannii</i>	2	1	1
<i>Stenotrophomonas maltophilia</i>	—	—	2
<i>Enterobacter aerogenes</i>	1	—	—
Gram-positive bacteria			
<i>Staphylococcus aureus</i>	—	1	2
Hemolytic staphylococci	1	—	—
<i>Staphylococcus epidermidis</i>	—	—	2
Fecal Enterococcus	1	—	1
<i>Streptococcus viridians</i>	1	1	1
Normal flora	2	1	—
Fungi			
<i>Candida albicans</i>	2	3	2

TABLE 3. Distribution of Bacteria in Lower Respiratory Tract Secretions

Microorganisms	Group A	Group B	Group C
Gram-negative bacteria			
<i>Bacillus coli</i>	—	—	1
<i>Aerobacter cloacae</i>	—	—	2
<i>Pseudomonas aeruginosa</i>	1	—	1
<i>Stenotrophomonas maltophilia</i>	—	—	3
Gram-positive bacteria			
<i>Staphylococcus aureus</i>	—	—	1
Normal flora	6	5	3
Fungi			
<i>Candida albicans</i>	1	2	1
<i>Candida tropicalis</i>	—	1	1

DISCUSSION

The narrowing of the intraluminal diameter of ETTs resulting from accumulation of secretions increases with the duration of intubation. Such narrowing may significantly increase airway resistance, which can contribute to failure to be weaned off MV (7, 8). When the duration of intubation exceeds 1 day, large numbers of bacteria become established in the secretions that accumulate on the ETT. At 3 days, both bacterial aggregations embedded in an amorphous matrix and mature biofilms have been demonstrated using STY09 and PI fluorescence microscopy (9). The ETT provides an environment suitable for the development of biofilms by bacteria present in respiratory secretions. As the secretions accumulate on the ETT, the surface and the available nutrients favor bacterial adhesion and biofilm formation. Bacterial colonization and ETT biofilm formation increase the risk of VAP (10). Therefore, one key to preventing VAP is elimination of accumulated secretions on

the ETT, which prevents bacterial adhesion and formation of ETT biofilm.

In this study, extensive, thick secretions accumulated on the walls of the ETTs in control patients. They were seen in gross appearance and were obvious at the bottom of ETT when extubated. Bacteria, mainly common pathogens, were cultured from the ETT of 13 control patients (86.7%) and from the lower respiratory tract secretions of nine control patients. Our results are, thus, in line with the above studies and others (11), confirming that traditional suctioning methods do not eliminate the secretions within the ETT and the risk that they represent.

In this study, after cleaning with a sterile urethral catheter, the ETT was free of visible secretions like a new ETT. The effect was most obvious in group A. The results were affected by the frequency of cleaning, as bacterial colonization decreased the most in group A, that is, by 3.42×10^2 CFU/cm². The difference

TABLE 4. Comparison of Bacterial Colony Count in Endotracheal Tube Biofilm

	Duration of ventilation		
	1 d	3 d	7 d
	CFU/cm ²	CFU/cm ²	CFU/cm ²
	lg (CFU/cm ² + 100) ^a	lg (CFU/cm ² + 100) ^a	lg (CFU/cm ² + 100) ^a
Group A	(1.46 ± 1.22) × 10 ²	(1.74 ± 2.04) × 10 ²	(7.06 ± 6.93) × 10 ²
	2.34 ± 0.24 ^a	2.33 ± 0.35 ^a	2.65 ± 0.60 ^a
Group B	(6.53 ± 6.30) × 10 ²	(5.19 ± 6.0) × 10 ²	(9.70 ± 9.56) × 10 ²
	2.72 ± 0.46	2.62 ± 0.46 ^a	2.73 ± 0.68 ^a
Group C	(5.36 ± 4.43) × 10 ³	(5.10 ± 10.46) × 10 ⁴	(2.94 ± 2.55) × 10 ⁵
	3.38 ± 0.85	3.72 ± 1.20	5.10 ± 0.80
<i>p</i>	0.041	0.034	0.000

CFU = colony forming units.

p value represents in the same time; the *p* value of analysis of variance of the lg (CFU/cm² + 100) of three groups.

^aRepresents in the same time compared with the C group; the difference was statistically significant (*p* < 0.05).

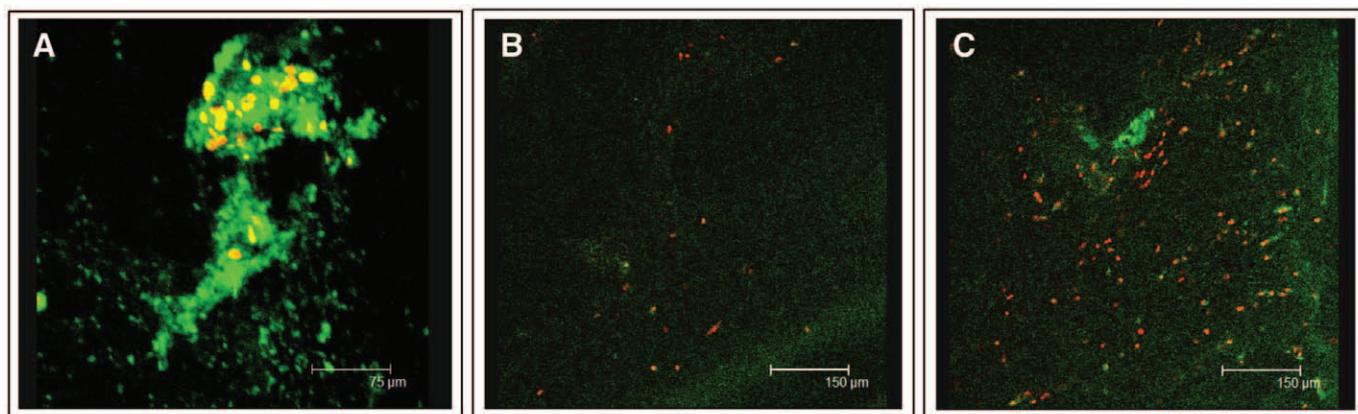


Figure 1. Confocal scanning laser microscopy. **A**, Group C: Bacteria (red fluorescence) encased in mass of extracellular polysaccharide (green fluorescence), and forming a mature biofilm. **B**, Group A: Scattered red bacteria with no biofilm formation. **C**, Group B: A small amount of extracellular polysaccharide (green fluorescence), and a greater number of bacteria than in Group A.

increased with increasing duration of MV, where bacterial growth decreased from 10^5 CFU/cm² to 10^2 CFU/cm². The number of bacterial colonies observed in groups B and C were not different over 2 days of MV, but with the prolongation of MV over 7 days, the difference of 7.45×10^2 CFU/cm² was significant.

Both the lower respiratory tract secretions and ETTs became culture positive, with the same species isolated from both in more than 60% of patients. This shows that the ETT biofilms include a high density of bacteria that colonized the inner surface of the ETT. Other studies have shown biofilms were easily detached by the ventilator air flow, suction catheters, and changes in body position, and could then travel from within the ETT into the lower respiratory tract (12), potentially causing lower respiratory tract infections or VAP.

Fewer ETT and fewer lower respiratory tract secretions became colonized in groups A and B than in group C. The observation of culture-negative lower respiratory tract secretions in patients with colonized ETTs suggests that mechanical cleaning with a urethral catheter can remove adherent bacteria and ETT biofilms before detectable bacteria can be transferred to secretions in the lower respiratory tract.

Staining with PI and FITC-Con A allowed observation of the distribution of the bacteria and extracellular polymeric substance (EPS) on the ETT inner surface and measurement of the thickness of biofilms. Mature biofilms developed in

group C but were not observed in either group A or group B. The number of ETT-adherent bacteria and the amount of EPS were significantly reduced in groups A and B compared with the ETTs in group C, which had not been cleaned. The formation of biofilms is a dynamic process, with several stages including attachment of cells to a substrate, bacterial population growth, aggregation of bacterial cells into micro-colonies, and finally maturation and maintenance of the biofilm structure (13). Our results showed bacteria adhered to the surface of the ETTs within 12 hours after intubation. Cleaning the inner surface of ETT every 8 hours or 12 hours to clear the adherent bacteria interrupted the subsequent bacterial growth, aggregation, and EPS formation required for biofilm formation.

In addition, there was a statistically significant difference of VAP prevalence in groups A and B versus group C. The prospective, randomized controlled design of this trial supports the reliability of the data obtained. Sterile urethral catheters are easily obtained, widely available, inexpensive, safe, and requirements of use are a familiar clinical nursing practice. The study results showed that mechanical clearing of secretions within the ETT at 8- or 12-hour intervals interfered with the adhesion of bacteria on the surface of ETT and the formation of biofilms. We conclude that this mechanical cleaning method using sterile urethral catheters is safe, effective, prevented the formation of BF, and reduced the prevalence of VAP.

TABLE 5. Biofilm Thickness Observed in the Study Groups and Subgroups

	Duration of Ventilation			p1
	1 d	3 d	7 d	
Group A	14.09 ± 5.18 ^a	14.94 ± 4.31 ^a	15.31 ± 4.23 ^a	0.912
Group B	19.11 ± 3.46 ^a	20.49 ± 3.55 ^a	21.37 ± 3.35 ^a	0.595
Group C	26.73 ± 3.52	37.43 ± 4.86 ^b	43.51 ± 2.30 ^b	0.000
p	0.001	0.000	0.000	

^aSignificant difference compared with Group C ($p < 0.05$).

^bSignificant difference compared with day 1 ($p1 < 0.05$).

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