Effectiveness of Pediatric Tracheostomy Tube Cleaning

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Objective: To determine the effectiveness of typical reprocessing of pediatric tracheostomy tubes (TTs) with detergent-soaked gauze pads.

Design: In vitro microbiologic study.

Setting: Department of Otolaryngology, University of Florida, Gainesville.

Participants: Polyvinyl chloride and silicone TTs of 3 different manufacturers.

Intervention: Thirty TTs were cultured with Pseudomonas aeruginosa or Staphylococcus aureus and reprocessed after 4 days. Eighteen additional TTs were exposed to plasma and then cultured with P aeruginosa or S aureus for 7 days.

Main Outcome Measures: The presence of biofilms was assessed before and after cleaning by quantitative bacterial counts and scanning electron microscopy.

Results: Bacterial counts that were obtained before cleaning of the tubes did not differ among brands. Reprocessing reduced P aeruginosa and S aureus bacterial counts in the 4-day group (P=.003 and P=.004, respectively), but clean TTs had a mean count of 10^5 colony-forming units/mL. Reprocessing did not significantly reduce S aureus or P aeruginosa bacterial counts in TTs pretreated with plasma and exposed to 7-day culture.

Conclusions: P aeruginosa and S aureus biofilms in pediatric TTs are not eradicated by standard cleaning methods. Further research is needed to determine the clinical significance of these findings and whether strategies to prevent biofilm formation or more effective disinfection methods would result in improved clinical outcomes.


Limited data support the current practices of tracheostomy tube (TT) care in children, including reuse of tubes, frequency of tube changes, and disinfection methods, and there is a wide variation of practices based on physician preferences and availability of resources. To our knowledge, the efficacy of household disinfection methods commonly used to reprocess pediatric TTs has never been evaluated. Adequate cleaning of pediatric TTs is likely more challenging than adult ones because of the smaller diameter and absence of a removable inner cannula. It is possible that persistent bacterial colonization in the form of adherent biofilms persists after cleaning.

Biofilms are recognized as a significant source of morbidity related to various indwelling medical devices. Bacterial colonization of endotracheal tubes is implicated in the development of ventilation-associated pneumonia, the main source of morbidity and mortality in patients receiving mechanical ventilatory support. The presence of biofilms has been identified in neonatal endotracheal tubes, laryngeal stents, and adult and pediatric TTs, and may induce granulation tissue formation, chondritis, and lower respiratory tract infections.

This study aimed to test the efficacy of the manufacturer-recommended cleaning methods to eradicate biofilms from pediatric TTs. Furthermore, we compared the propensity of bacterial adhesion and biofilm formation between polyvinyl chloride (PVC) and silicone TTs in vitro.

Methods

Two separate in vitro experiments were performed. In the first experiment, 15 TTs were exposed to Staphylococcus aureus–rich cul-
ture medium for 96 hours and 15 tubes to *Pseudomonas aeruginosa* for the same period. Samples for bacterial counts were obtained before and after cleaning of the tubes. In the second experiment, we used a total of 18 tubes, which were first exposed to human plasma and then cultured with *P aeruginosa* or *S aureus* for 7 days. Tube sections were obtained before and after cleaning for bacterial counts and scanning electron microscopy (SEM) analysis.

**BACTERIAL PREPARATION**

Frozen aliquots of *P aeruginosa*, strain Rochester, and *S aureus* ATCC 29213 were quad-streaked on tryptic soy agar plates. A single colony was picked and grown overnight at 37°C in a tryptic soy broth growth medium. The bacteria were transferred to fresh media and grown to early log phase (optical density of 0.2 to 0.4 at 640 nm). This yielded approximately 1×10^9 colony-forming units (CFU)/mL of both *P aeruginosa* and *S aureus*, as determined by measuring optical density at 640 nm and interpolating the CFU count from a predetermined linear optical density–CFU regression.

**BIOFILM FORMATION AND REPROCESSING**

**Experiment 1**

Shiley (Tyco Healthcare Group LP), Tracoe Mini (Bryan Medical Inc), and Bivona (Portex Inc) uncuffed pediatric TTs of the same inner diameter (3.5 mm) were used. Shiley and Tracoe Mini tubes are primarily composed of PVC, whereas Bivona tubes are made of silicone. Five tubes of each brand were immersed in 15-mL conical tubes (Thermo Fisher Scientific) containing *P aeruginosa*–rich culture medium (tryptic soy broth) for 96 hours. Five additional TTs of each brand were immersed in *S aureus*–rich culture medium for the same period. The broth was changed every 24 hours to prevent nutrition depletion. The tubes were then removed from the culture media and flushed with 50 mL of sterile water. One 2-mm section was cut from the tip and used as controls for bacterial counts. The TTs were then thoroughly rinsed with sterile water. Two contiguous 2-mm sections were cut from the tip and used as controls for bacterial counts and SEM. The TTs were then immersed in 15-mL conical tubes (Thermo Fisher Scientific) containing *P aeruginosa*–rich culture medium (9 tubes) or *S aureus*–rich culture medium (9 tubes) for 7 days. The culture media were changed every 24 hours to prevent nutrition depletion. The TTs were then removed and flushed with 50 mL of sterile water. Two contiguous 2-mm sections were cut from the tip and used as controls for bacterial counts and SEM. The TTs were then sonicated 5 times for 11⁄2 minutes with a 1-minute rest in between. The samples were serially diluted and plated on tryptic soy agar plates. These plates were then incubated for 18 to 24 hours, and the colonies were counted to determine the colony count of the diluting solution. The bacterial counts were normalized for the weight of each section.

**QUANTITATIVE CULTURES**

Each 2-mm section was placed in a 15-mL microtube and covered with 4 mL of sterile phosphate-buffered saline. The samples were then sonicated 5 times for 11⁄2 minutes with a 1-minute rest in between. The samples were serially diluted and plated on tryptic soy agar plates. These plates were then incubated for 18 to 24 hours, and the colonies were counted to determine the colony count of the diluting solution. The bacterial counts were normalized for the weight of each section.

**SCANNING ELECTRON MICROSCOPY**

The tube sections were transferred to Trump solution for fixation. The samples were dehydrated in a graded ethanol series of 25%, 50%, 75%, 95%, and 100% at 10-minute intervals and dried using a critical point dryer. Each tube section was then mounted on an SEM stub and stored in a desiccator until sputter coated. They were coated with gold and palladium using an argon gas sputter coating unit (Desk II sputter coater; Denton Vacuum USA) for 45 seconds and stored under vacuum until imaged (PhenomVR SEM; FEI Company). The images were analyzed by one of us (C.P.O.-D.), who is a microbiologist certified in electron microscopy.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using JMP 7.0 statistical software (SAS Institute, Inc). Total bacterial counts among all study groups in experiment 1 were compared using analysis of variance, followed by the Tukey studentized range test if the overall test result was significant. Paired t test with a Welch correction was used to compare the bacterial counts before and after reprocessing. The confidence level was set to 95% for all tests.

**RESULTS**

**EXPERIMENT 1**

After 4-day exposure to *S aureus* and *P aeruginosa*, bacterial counts that were obtained before cleaning of the tubes did not differ among brands. Mean *P aeruginosa* counts of Shiley tubes had significant reduction (*P* = .002) as opposed to Tracoe and Bivona tubes (Figure 1). Reprocessing significantly reduced mean *P aeruginosa* counts when all tubes (*n* = 30) were combined: 4.5 × 10^6 CFU/mL before cleaning compared with 2.3 × 10^5 CFU/mL after cleaning (*P* = .004, Figure 2). Reprocessing significantly reduced mean *S aureus* counts overall (*P* = .005), although the cleaned tubes had a mean count of 10^5 CFU/mL (Figure 3). Mean *S aureus* counts were significantly reduced in Shiley (*P* = .01) and Tracoe (*P* = .01) but not Bivona tubes (Figure 4). Cleaning led to a 3-log reduction in *S aureus* counts compared with approximately 1-log reduction observed with *P aeruginosa*.

**EXPERIMENT 2**

Because of the smaller sample size, differences among brands could not be adequately assessed. *P aeruginosa* and *S aureus* formed mature biofilms on PVC and silicone after 7 days in culture. Postcleaning images revealed persistent bacteria adhered to the tube surfaces (Figure 5). Reprocessing did not reduce *P aeruginosa* counts when
all tubes \((n=18)\) were combined: 4.5 \(\times \) 10^6 CFU/mL before cleaning vs 6.5 \(\times \) 10^6 CFU/mL after cleaning (Figure 6). When the 3 brands were considered separately, the \(P \) aeruginosa bacterial counts of Shiley and Traco were significantly reduced after cleaning \((P=0.04 \text{ and } P=0.049)\) but not the Bivona tubes (Figure 7). Reprocessing did not significantly reduce \(S\) aureus counts overall \((P=0.13)\) (Figure 8). The cleaned tubes had a mean count of 5.9 \(\times\) 10^5 CFU/mL. Reprocessing did not reduce \(S\) aureus counts in any of the 3 brands (Figure 8). Cleaning led to a 3-log reduction in \(P\) aeruginosa counts compared with an approximately 1-log reduction observed with \(S\) aureus.

**COMMENT**

This is the first study, to our knowledge, to investigate the effectiveness of a decontamination procedure of pediatric TTs. We identified persistent \(P\) aeruginosa and \(S\) aureus biofilms on PVC and silicone tubes after in vitro biofilm formation and typical cleaning with water and detergent. Cleaning of the tubes was not effective when biofilms developed 7 days after exposure to human plasma. Blood proteins have long been recognized as key binding sites for bacterial colonization of biomedical implants.\(^1\) Also, 3 additional days of culture allowed further maturation of the biofilms.

Growing evidence has demonstrated the presence of bacterial biofilms in a number of medical devices and various chronic wounds, including pressure sores, diabetic foot ulcers, and venous stasis ulcers.\(^1\) Although the deleterious effect of biofilms on healing of chronic wounds has been increasingly recognized, the significance of these bacterial communities on TTs and tracheostomas remains unclear. Bacterial biofilms are prevalent in chronic wound beds and may interfere with wound healing by induction of a chronic nonhealing inflammation and evasion of the immune system with the protection of an extracellular polysaccharide matrix.\(^1\) Schierle et al\(^3\) observed that the presence of staphylococcal biofilms significantly delayed reepithelialization of cutaneous wounds in a murine model. Although the reasons for that delay are not completely elucidated, it is possible that biofilms act as a physical barrier to reepithelialization, cause
chemical-environmental change, and promote chronic inflammation.\textsuperscript{17}

In the airway, there is evidence of an association between \textit{S aureus} and \textit{P aeruginosa} colonization of Montgomery TTs at specific sites and the formation of granulation tissue.\textsuperscript{18} The bacteria on the granulation tissue itself had been previously described as variable combinations of gram-positive, gram-negative, and anaerobic organisms.\textsuperscript{19} Similarly, colonization of Silastic stents with \textit{S aureus} and \textit{P aeruginosa} is associated with the occurrence of airway granulation tissue after laryngotracheal reconstruction.\textsuperscript{10} We speculate that colonization of TTs with bacterial biofilms may be implicated in the local wound complications frequently observed in patients with tracheostomies. The repeated use of persistently colonized pediatric tubes might be involved in recurring stoma infections and airway granulomas. This notion may support the use of disposable tubes until practical and effective decontamination techniques are identified or surface modifications make tubes more resistant to fouling, bacterial attachment, and biofilm formation. Alternatively, although further clinical studies outside the scope of this study are necessary, it seems reasonable to advise more frequent tube changes and methodic mechanical cleansing in patients with recurrent granulomas, infections, or plugging. Although most otolaryngologists currently recommend pediatric TT changes every 7 days, patients who develop complications may benefit from changes within 4 days to prevent the formation of more mature, adherent, and difficult-to-clear biofilms. Also, the safety and efficacy of added chemical disinfectants, such as chlorhexidine or sodium hypochlorite, to the cleaning solution should be investigated.

\textbf{Figure 5.} Scanning electron micrographs of a polyvinyl chloride tracheostomy tube after exposure to human plasma and culture with \textit{Pseudomonas aeruginosa} for 7 days. A, Before cleaning; B, after cleaning.

\textbf{Figure 6.} Mean \textit{Pseudomonas aeruginosa} colony counts in colony-forming units (CFU) per milliliter on all 7-day tubes combined before and after cleaning ($P=.13$). Error bars indicate SD.

\textbf{Figure 7.} Mean \textit{Pseudomonas aeruginosa} colony counts in colony-forming units (CFU) per millimeter on 7-day tracheostomy tubes before and after cleaning. Asterisks indicate $P<.05$. Error bars indicate SD.
In this study, we also observed decreased cleaning efficacy with silicone tubes compared with PVC. Processing with detergent did not significantly reduce bacterial counts of silicone tubes exposed to either P. aeruginosa or S. aureus with or without human plasma. However, other studies\(^6\)\(^7\) have not demonstrated an increased prevalence of biofilms on silicone compared with PVC tubes. Furthermore, standard silicone tubes were not associated with an increased incidence of stomal complications.\(^2\) It is possible that this lack of clinical correlation derives from the fact that silicone may undergo additional sanitization by exposure to boiling water, which would further reduce the number of viable bacteria. Yet it remains unknown whether repeated exposure to high temperatures would accelerate material degradation and require more frequent disposal of tubes.

The limitations of this in vitro preliminary study are evident. It is inherently difficult to reproduce the tracheal environment in an in vitro setting. A colonized trachea contains multiple bacteria and fungi interacting with the tube surface, which in turn is coated with a slime composed of variable amounts of respiratory mucus, blood, and saliva. The mechanical forces produced by the constant airflow inside the TT may also influence bacterial attachment and biofilm formation. We induced the formation of single-species biofilms by immersion of TTs in culture media. Although this method does not entirely replicate the clinical scenario in terms of biofilm formation, it is a valid tool to assess the effectiveness of a decontamination technique. Our findings contrast with those of Björling et al.,\(^3\) who found minimal bacterial growth after 2 decontamination procedures of adult inner cannulae. Although these authors flushed the reprocessed tubes with 10 mL of saline and cultured the flushed solution, we obtained tube sections that were subsequently sonicated. Sonication is necessary to disrupt biofilms and liberate firmly attached bacteria that would not be recovered otherwise.\(^6\)\(^7\) Finally, the considerably smaller inner diameter of the pediatric tubes may reduce cleaning effectiveness and partly account for the differences.

This study provided evidence that bacterial biofilms in pediatric TTs are not cleared by standard cleaning methods. Further research is needed to determine the clinical significance of these findings and whether strategies to prevent biofilm formation or more effective disinfection methods would result in improved clinical outcomes.

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**Author Contributions:** Dr Silva had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Silva and Ojano-Dirain. Acquisition of data: Silva and Ojano-Dirain. Analysis and interpretation of data: Silva, Ojano-Dirain, and Antonelli. Drafting of the manuscript: Silva. Critical revision of the manuscript for important intellectual content: Silva, Ojano-Dirain, and Antonelli. Statistical analysis: Ojano-Dirain. Administrative, technical, and material support: Ojano-Dirain and Antonelli. Study supervision: Ojano-Dirain.

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**REFERENCES**


