



BRIEF REPORT

Low-cost antiseptic-impregnated tracheostomy tube for the prevention of ventilator-associated pneumonia caused by multidrug-resistant bacteria: *In vitro* and pilot study in humans

Juliano Gasparetto^{a,b}, Leandro Bressianini Jurkonis^{a,b}, Leticia Ramos Dantas^a, Paula Hansen Suss^a, Felipe Francisco Tuon^{a,*}

^a Laboratory of Emerging Infectious Diseases, School of Medicine, Pontificia Universidade Católica do Paraná, Curitiba, Brazil

^b Intensive Care Unit, Hospital Universitario Cajuru, Pontificia Universidade Católica do Paraná, Curitiba, Brazil

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Abstract Ventilator-associated pneumonia (VAP) is one of the most common causes of nosocomial infections. The aim of this study was to evaluate the antimicrobial and anti-biofilm activity of an in-house low-cost tracheostomy tube impregnated with chlorhexidine and violet crystal. The impregnated tracheostomy tubes demonstrated antimicrobial activity, including for multidrug-resistant bacteria. Fourteen patients were evaluated. During ventilation, VAP occurred in one patient in the coated group and in three patients in the control group ($p = 0.28$). A reduction of biofilm cells was observed. This study provides preliminary evidence to support that the antiseptic impregnation of a tracheostomy tube provides significant antimicrobial activity.

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PALABRAS CLAVE

Antimicrobiano;
Tubos endotraqueales;
Clorhexidina;
Cristal violeta;
Neumonía asociada a la ventilación

Impregnación económica de tubo traqueostómico con antiséptico para la prevención de la neumonía asociada a la ventilación mecánica causada por bacterias multirresistentes: estudio *in vitro* y piloto en humanos

Resumen La neumonía asociada a la ventilación mecánica (NAV) es una de las causas más comunes de infecciones nosocomiales. El objetivo de este estudio fue evaluar la actividad antimicrobiana y antibiofilm de un tubo traqueostómico impregnado en el propio establecimiento con clorhexidina y cristal violeta. Los tubos traqueostómicos impregnados demostraron

* Corresponding author.

E-mail address: felipe.tuon@pucpr.br (F. Francisco Tuon).

actividad antimicrobiana, incluso contra bacterias multirresistentes. Se evaluaron catorce pacientes. Durante la ventilación, se produjo NAV en un paciente del grupo con recubrimiento antiséptico y en tres pacientes del grupo control ($p=0,28$). Se observó una reducción de células de biofilm, pero sin diferencia significativa. Este estudio proporciona una evidencia preliminar de que la impregnación antiséptica del tubo traqueostómico proporciona una actividad antimicrobiana significativa.

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Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections affecting patients in the intensive care unit (ICU)¹⁰. Antiseptic-coated endotracheal tubes (ETTs) can reduce bacterial adhesion to the devices, thereby decreasing biofilm formation, lung colonization, and risk of VAP^{4,7,11}. Chlorhexidine and gentian violet may be viable options for ETT coating. Chlorhexidine-coated ETTs showed no bacterial growth when compared to uncoated tubes, and were associated with less bacterial colonization in bronchial samples and the lung parenchyma². Thus, antiseptic-coated ETTs can be a valuable intervention to prevent VAP. All previous studies have evaluated the efficacy of antiseptic-coated ETTs, but not of coated tracheostomy tubes. Tracheostomy is commonly performed in patients that are not extubated within a few days of intubation. VAP in patients with tracheostomy tubes is called late-VAP, which is commonly associated with multidrug-resistant bacteria. This study aimed to characterize and evaluate the antimicrobial and anti-biofilm activity of an in-house low-cost tracheostomy tube impregnated with chlorhexidine and violet crystal.

This study was conducted in two stages. The first stage involved the development of the impregnation for the tracheostomy tubes and subsequent microbiological testing. The second stage was the pilot study in which patients intubated with impregnated and non-impregnated tubes were assessed. This study was approved by the local ethics committee (PUCPR - 44535521.0.0000.0020). For impregnation, the PVC tracheostomy tubes (Smiths Medical ASD, Minneapolis, MN, USA) were completely immersed in an 8:2:1:1 solution of methanol, acetone, 2% crystal violet solution, and 20% chlorhexidine digluconate for 1 h. Subsequently, the tubes were removed from the solution and placed in a 50 °C oven for 1 h to dry. Thereafter, they were washed three times with ultrapure water and dried at room temperature. The tubes were sterilized in ethylene oxide; seven of them were used for clinical studies and five for microbiological studies.

Staphylococcus aureus ATCC 25923TM, *Pseudomonas aeruginosa* ATCC 27853TM and *Escherichia coli* ATCC 25922TM strains were used for the microbiological tests. We have also included multidrug-resistant bacteria, obtained from clinical cultures: methicillin-resistant *S. aureus* (MRSA) and carbapenem-resistant *Acinetobacter baumannii*, *P. aeruginosa* and *Klebsiella pneumoniae*. The microorganisms from a solution equivalent to 0.5 McFarland standard were plated

on Muller Hinton agar. Axial sections of the impregnated and non-impregnated tubes were also placed onto the plate. The plates were placed in an oven at 35 °C for 24 h, after which halo formation was analyzed.

The impregnated tubes were used in seven consecutive patients. Seven non-impregnated tracheostomy tubes were used as controls. The study was performed in a University Hospital with 207 beds, serving as reference center in trauma and surgery, between January/23 and March/23. The mean rate of VAP in the center was 22 VAP/1000-patient-days during the period, and the most important bacteria were *S. aureus* (45% oxacillin-resistant), *A. baumannii* (95% carbapenem-resistant), *P. aeruginosa* (18% carbapenem-resistant), *klebsiella pneumoniae* (3% carbapenem-resistant), and *E. coli* (0% carbapenem-resistant). There was no randomization or blinding, and the tubes were used sequentially. Inclusion criteria were: (1) age >18 years; (2) ICU admission; (3) need for mechanical ventilation; (4) clinical indication for tracheostomy at the discretion of the attending team. Patients with a previous history of tracheostomy or use of a tube other than an orotracheal tube were excluded. Patients did not undergo any intervention of the researcher regarding other procedures. The decision to retain or remove the tracheostomy tube was at the discretion of the assistant team. Epidemiological data, such as sex, age, comorbidities, severity indices at admission (APACHE and SOFA), reason for ICU admission, need for mechanical ventilation, and clinical outcomes, were evaluated. VAP was defined according to the guidelines provided by the Center for Disease Control and Prevention (CDC), and includes the presence of compatible bacteria in a tracheal aspirate or bronchial wash, clinical signs of systemic and pulmonary infection, and absence of another focus³.

All the extracted tracheostomy tubes were immediately taken to the laboratory and stored in a refrigerator until microbiological studies could be performed. The biofilm was quantitatively analyzed by weighing the dry biofilm and counting colonies as previously described^{5,12}.

A 0.5 cm height ring of all the used tubes was analyzed by electron microscopy for residual biofilm as previously described⁵. The PVC was also characterized using Fourier-transform infrared (FTIR) spectroscopy (Spotlight 200i FTIR Microscope System; Perkin Elmer, Akron, OH, USA). One specimen of each group was analyzed and the antiseptic-coated tubes were compared. The samples were scanned

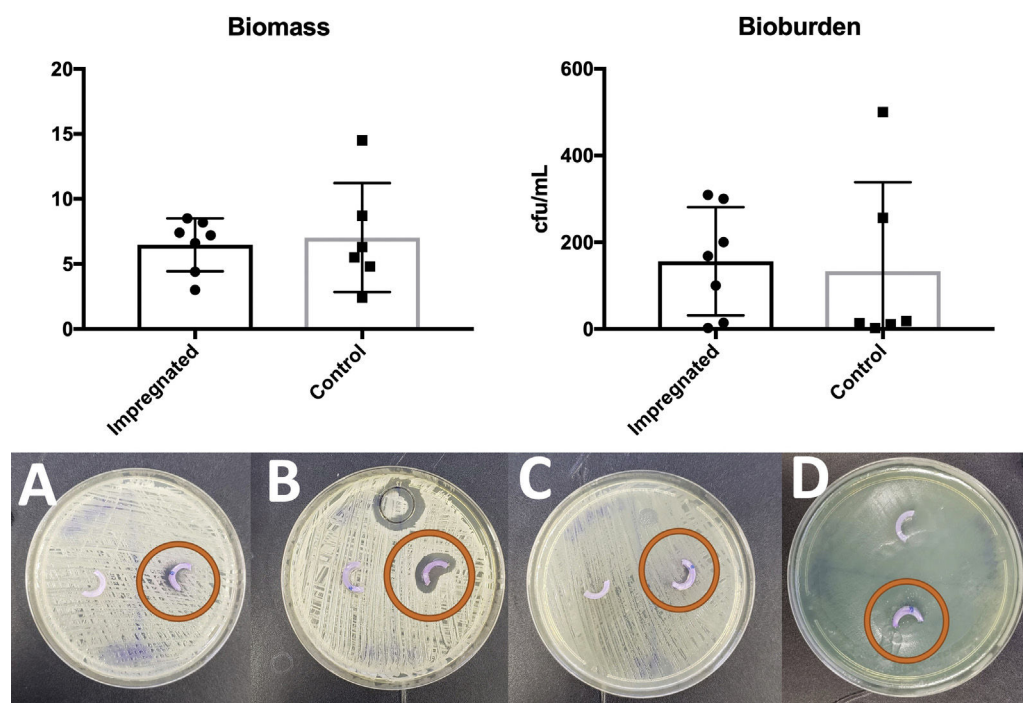


Figure 1 Above: biomass and bioburden was evaluated in seven impregnated tracheostomy tubes and seven control tubes after being used in a patient. Biomass was quantified in mg and bioburden as colony forming units per ml (CFU/ml). Below: a segment of a tracheostomy tube impregnated with chlorhexidine and violet crystals (orange circles) and a control tube were tested on a plate containing multidrug-resistant bacteria: (A) carbapenem-resistant *Klebsiella pneumoniae*; (B) MRSA; (C) carbapenem-resistant *Acinetobacter baumannii*, and (D) carbapenem-resistant *Pseudomonas aeruginosa*.

between 650 and 4000 cm^{-1} , and the average spectra of five scans was obtained⁹.

Qualitative data are described as percentages, and quantitative data as arithmetic mean or median value according to the distribution pattern (normality). Standard deviation (SD) and 25% and 75% interquartile ranges (IQR) were the distribution variables for mean and median, respectively. Mann-Whitney test was used for statistical analysis. Statistical significance was set at $p < 0.05$.

After impregnation, the material turned violet, which was compatible with the dye coloration. Microbiological plaque tests showed an inhibition distance of >5 mm (measured from the device) for the ATCC bacteria. For the multidrug-resistant bacteria, inhibition was significant for MRSA, but weak for carbapenem-resistant *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* (Fig. 1).

The clinical characteristics of patients are described in Table 1. Of the seven patients in whom impregnated tubes were used, one developed VAP; three patients in the control group developed VAP ($p = 0.28$). The microorganisms identified in these VAP cases were as follows: two cases of *P. aeruginosa*; one case of extended-spectrum beta-lactamase-producing *E. coli*; and one case of negative culture.

The microbiological and biofilm data of all the extubated tracheostomy tubes are depicted in Figure 1. The biomass in the impregnated tubes did not differ from that in the control group; the median was 5.90 (IQR 5.15–8.1) in the impregnated group and 7.40 (IQR 7.05–8.35) in the control group. Similarly, no difference was found in the produc-

tion of sessile cells by the quantitative method; the median was 15.50 CFU/ml (IQR 12.00–196.50) in the control group and 168.00 CFU/ml (IQR 78.50–250.00) in the impregnated group.

SEM was performed to evaluate the tube microstructure before and after use, to assess possible structural alterations that may have been caused. No fissures or other alterations that could compromise the tube structure were found (Fig. 2). The formation of organic compound peaks compatible with PVC on FTIR spectroscopy could be confirmed. However, owing to the overlapping of peaks, the impregnation components either before or after use in the patients could not be identified.

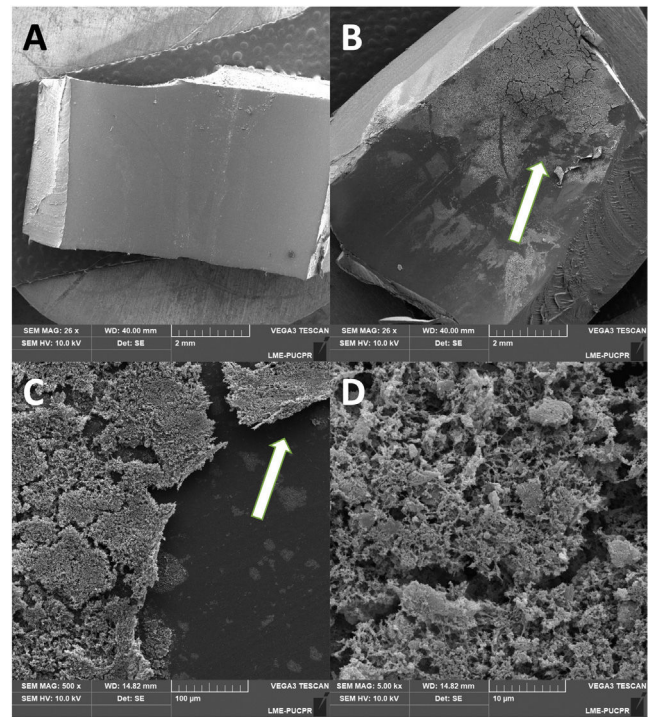
This is the first study to evaluate a tracheostomy tube impregnation protocol for the prevention of late-VAP. Microbiological analyses showed that impregnation of the tracheostomy tube was effective, and demonstrated antimicrobial activity, including against multidrug-resistant bacteria. The study also demonstrated that impregnation was safe, as evidenced by the lack of adverse events in this pilot human study.

The impregnated tube showed activity against the ATCC bacterial strains, but its activity against the carbapenem-resistant gram negative bacilli was weaker, suggesting that impregnation may be unsuccessful in these infections. Furthermore, the impregnation did not inhibit biofilm formation. Biomass is not necessarily associated with microorganism viability, and there was a clear tendency of reduction in the viable cells (bioburden). The bioburden is an important factor in reducing the risk of VAP¹. The

Table 1 Clinical data of patients included in the pilot study of impregnated tube of tracheostomy.

Patient	Group	Age	Gender	Comorbidities	Admission	Admission SOFA	Total MV duration (days)	MV before tracheostomy (days)	Outcome	Length of stay
1	Control	77	F	SAH/DW	Hydrocephalus	4	16	7	Death	38
2	Control	68	M	None	Traumatic brain injury	10	8	6	Death	88
3	Control	74	M	SAH	Myocardial infarction	7	9	7	Death	17
4	Control	50	F	SAH	Subarachnoid hemorrhage	8	27	13	Survived	205
5	Control	63	M	None	Traumatic brain injury	9	20	12	Death	19
6	Control	82	M	SAH/DW/HF	Traumatic brain injury	5	21	9	Death	56
7	Control	47	F	None	Hemorrhagic stroke	8	23	7	Survived	74
8	Impregnated	75	M	SAH/stroke	Intraparenchymal hematoma	5	8	7	Death	18
9	Impregnated	70	M	SAH/DW/HF	Severe acute pancreatitis	7	14	11	Survived	77
10	Impregnated	55	M	None	Perforated gastric ulcer	9	18	15	Death	52
11	Impregnated	34	F	None	Atrial fibrillation	7	7	5	Survived	58
12	Impregnated	39	M	None	Hydrocephalus	6	26	17	Death	45
13	Impregnated	61	F	SAH	Ischemic stroke	6	12	8	Survived	80
14	Impregnated	22	M	None	Pulmonary sepsis	10	19	14	Survived	34

SAH: systemic arterial hypertension; DM: diabetes mellitus; HF: heart failure.

**Figure 2** SEM of the impregnated tube before tracheostomy tube implantation (A), and after extubation (B), with the arrow showing the biofilm (26× magnification). In (C) and (D), the biofilm is shown at 500× and 5000× magnification, respectively.

most prevalent etiologic agents in late-VAP are the ones for which the applied technique did not show good results, and this should be improved given the increasing resistant rates observed in medical centers. On the other hand, the VAP observed between 4 and 7 days of hospitalization are usually caused by non-resistant microorganisms, in which the method seems to work for early VAP.

Simple antimicrobial coatings may be prone to delamination during use, thus compromising the antimicrobial effect. The development of new, active, antimicrobial coatings has received extensive attention. Damas et al. conducted a multicenter clinical study using an ETT coated with a sub-micron layer of noble metal alloy (NMA) of gold, silver, and palladium. They found a delayed onset of VAP and a trend toward decreased antibiotic use in the group using coated ETT. VAP was confirmed in 11 (6.5%) and 18 (11.6%) patients in the NMA-coated and control groups, respectively⁵.

In another study on S-nitrous-N-acetylpenicillamine-coated ETTs, Homeyer et al. showed promising data with greater effectiveness against *S. aureus*; this study showed a reduction of 92% in *P. aeruginosa*-associated VAP⁸. An *in vitro* experiment by Zangirolami et al. evaluated the biofilm's kinetics on curcumin-coated ETT. There was a significant decrease in bacterial colonies in all conditions; microbial reduction of approximately 95% for *S. aureus*, 72% for *E. coli*, and 73% for *P. aeruginosa*, when compared with the control. In this study, the presence of curcumin photosensitizer on the ETT may have produced an alteration in the mechanical cell forces, consequently modifying and reducing the biofilm formation¹³.

As this was a pilot and *in vitro* study, it is too early to assume that the device effectively reduces the risk of VAP. Though the sample size was small, the aim of this study was to identify adverse events and mechanical complications related to antiseptic-coated tracheostomy tubes, which could be accomplished. Biomechanical tests will need to be performed to determine polymer compromise. The findings described in this study support the initiation of a randomized clinical trial, to confirm the efficacy of the impregnated tracheostomy tube.

In conclusion, the impregnated tracheostomy tube demonstrated a significant antimicrobial activity against standard bacteria, and to a lesser extent against multidrug-resistant bacteria. The impregnation produced a non-significant bioburden reduction in bacterial cells. A randomized clinical trial is currently under consideration to evaluate the efficacy of impregnated tracheostomy tubes in reducing VAP incidence. The cost of impregnation is extremely cheap and could be applied in public health settings for the prevention of infections.

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None declared.

Authors' contributions

JG – manuscript draft and idealization; LBJ – manuscript review and clinical study; LRD – microbiological study and analysis; PHS – biofilm analysis; FFT – idealization, microbiological study and final review.

Conflict of interest

Felipe Tuon is a CNPq researcher. All authors declare no conflicts of interests.

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