Antiseptic-impregnated tracheostomy tube for the prevention of ventilator-associated pneumonia caused by multidrug-resistant bacteria: in-vitro and pilot study in humans

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Abstract

Background: Ventilator-associated pneumonia (VAP) is one of the most common causes of nosocomial infections and is associated with prolonged hospitalization, increased health care costs, and high mortality of critically ill patients during hospitalization in intensive care units (ICUs). Objective: To characterize and evaluate in vitro and in vivo antimicrobial and anti-biofilm activity of an in-house tracheostomy tube impregnated with chlorhexidine and violet crystal. Methods: The tracheostomy tubes were tested in vitro for their ability to prevent biofilm formation by standard strains of S. aureus, P. aeruginosa, and E. coli, and multidrug-resistant bacteria obtained from clinical cultures: Meticillin-resistant S. aureus (MRSA), and carbapenem-resistant Acinetobacter baumannii, Pseudomonas aeruginosa and Klebsiella pneumoniae. Results: The impregnated tracheostomy tubes demonstrated antimicrobial activity, including for multidrug-resistant bacteria. In this pilot study, 14 patients were evaluated, seven in the chlorhexidine and violet crystal-coated group and seven in the control group. During ventilation, VAP occurred in one patient in the coated group and in three patients in the control group (p=0.28). The biomass in the impregnated tubes did not differ from the control group and no difference was found in the production of sessile cells by the quantitative method, with a median of 15.50 cfu/mL (IQR25-75% 12.00-196.50) and 168.00 cfu/mL ((IQR25-75% 78.50-250.00), respectively. Conclusion: This study provides preliminary evidence to support that antiseptic impregnation of tracheostomy tube provides significant antimicrobial activity.

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RUNNING TITLE:
Tracheostomy tube impregnation with antiseptics

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**Background**: Ventilator-associated pneumonia (VAP) is one of the most common causes of nosocomial infections and is associated with prolonged hospitalization, increased health care costs, and high mortality of critically ill patients during hospitalization in intensive care units (ICUs). **Objective**: To characterize and evaluate *in vitro* and *in vivo* antimicrobial and anti-biofilm activity of an in-house tracheostomy tube impregnated with chlorhexidine and violet crystal. **Methods**: The tracheostomy tubes were tested *in vitro* for their ability to prevent biofilm formation by standard strains of *S. aureus*, *P. aeruginosa*, and *E. coli*, and multidrug-resistant bacteria obtained from clinical cultures: Meticillin-resistant *S. aureus* (MRSA), and carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. **Results**: The impregnated tracheostomy tubes demonstrated antimicrobial activity, including for multidrug-resistant bacteria. In this pilot study, 14 patients were evaluated, seven in the chlorhexidine and violet crystal-coated group and seven in the control group. During ventilation, VAP occurred in one patient in the coated group and in three patients in the control group (p=0.28). The biomass in the impregnated tubes did not differ from the control group and no difference was found in the production of sessile cells by the quantitative method, with a median of 15.50 cfu/mL (IQR25-75% 12.00-196.50) and 168.00 cfu/mL (IQR25-75% 78.50-250.00), respectively. **Conclusion**: This study provides preliminary evidence to support that antiseptic impregnation of tracheostomy tube provides significant antimicrobial activity.

**KEYWORDS**

Antimicrobial; endotracheal tubes; chlorhexidine; violet crystal; ventilator-associated pneumonia

**INTRODUCTION**

Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections affecting patients in the intensive care unit (ICU) \(^1\). VAP occurs in 9–27% of all intubated ICU patients \(^2\). The etiology of VAP seems to be related to colonization of the aerodigestive tract by the pathogenic bacteria. Endotracheal tubes (ETTs) appear to be an independent risk factor for development of VAP \(^3\). ETTs are disposable tubes used for invasive mechanical ventilation; it is inserted into the trachea to establish and maintain a patent airway for sufficient exchange of oxygen and carbon dioxide. The median duration of mechanical ventilation with ETTs is estimated to be < 10 days; up to 75% of patients are extubated within 10 days of intubation \(^4\). The risk of developing VAP is reportedly highest in the first 10 days of mechanical intubation \(^5\).

Most ETTs used today are made from polyvinyl chloride (PVC), which can be colonized by bacteria within a few hours of mechanical ventilation. The bacteria organize into a thick biofilm, representing a large reservoir of microorganisms that can spread to the lungs and cause pneumonia \(^6\). Antiseptic-coated ETTs can reduce bacterial adhesion to the devices, thereby decreasing biofilm formation \(^7\). Recent studies have shown that the use of antiseptic-coated ETT to reduce lower lung colonization also reduces the risk of VAP \(^8\)-\(^10\). 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 parenchyma \(^7\). Thus, antiseptic-coated ETT 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 who 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 tracheostomy tube impregnated with chlorhexidine and violet crystal.

**METHODS**

**Design**

This study was carried out in two stages. The first stage was the development of the impregnated tracheostomy tubes and microbiological tests. The second stage was the pilot study in which patients intubated with impregnated and non-impregnated tubes were assessed. This study was approved by local ethics committee.

**Impregnation**

For impregnation, a PVC polymer swelling technique was employed using two solvents, methanol and acetone. The swelling of the polymer allowed deposition of the antiseptic onto the polymer webs; after volatilization of the solvent, the polymer structure returned to normal, retaining the antiseptic by physical adsorption.

The PCV 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 one hour. Subsequently, the tubes were removed from the solution and placed in a 50 degC oven for one hour 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.

**Microbiological tests**

*S. aureus ATCC 25923™, P. aeruginosa ATCC 27853™ and E. coli ATCC 25922™ strains were used. We have also included multidrug-resistant bacteria, obtained from clinical cultures: Methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas 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 degC for 24 hours, after which the halo formation was analyzed.

**Patient Study**

The impregnated tubes were used in seven consecutive patients. Seven non-impregnated tracheostomy tubes were used as controls. There was no randomization or blinding, and the tubes were used sequentially. The 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. The patients did not undergo any intervention of the researcher about 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 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 focus.

**Post-use study**

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. The tracheostomy tubes were sectioned at the base and washed three times with 0.9% NaCl to remove planktonic cells. Thereafter, they the sections were submerged in Falcon...
tubes and 0.9% NaCl was added until the 50 mL mark. Subsequently, the tubes were closed and sonicated at 40 kHz for 30 minutes. The 100 mcL of aliquot was plated at $6\log_{10}$, $4\log_{10}$, $2\log_{10}$, and neat dilutions to estimate colony counts. For dry biofilm analysis, the remaining liquid was filtered through a 0.22 mm membrane. After complete drainage, the membrane was placed in a 50 °C oven for 24 hours, and thereafter, weighed. An unfiltered membrane was used as a control at each weighing.

Scanning electron microscopy

After sonication, a 0.5 cm height ring of all the used tubes was analyzed by electron microscopy for residual biofilm. For qualitative scanning electron microscopy (SEM), the samples were transferred into sterile glass Petri dishes filled with the primary fixative agent (0.68 g sucrose, 0.42 g sodium cacodylate, 0.6 mL 30% glutaraldehyde) (Merck & Co., Inc., Darmstadt, Germany) and 19.4 mL of deionized water in sufficient quantity to submerge the specimen for 45 min. Thereafter, the specimen was transferred to a buffer solution (composed of sucrose and sodium cacodylate at the above concentrations) for 10 min. Subsequently, the samples were dehydrated in alcohol in a series of increasing concentrations for 10 min each: 35% ethanol, 50% ethanol, 70% ethanol, 100% ethanol, and 100% HMDS (hexamethyldisilazane) (Merck & Co., Inc., Darmstadt, Germany). The samples were coated with gold particles using the Q150R ES rotary pump (Quorum Technologies, Lewes, UK), and later fixed in a metal base for observation under SEM (PentaFET Precision; Oxford Instruments, Abingdon, UK).

Fourier-transform infrared spectroscopy

The PVC was 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 between 650 and 4,000 cm$^{-1}$, and the average spectra of five scans was obtained.

Statistical Analysis

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 $P<0.05$.

RESULTS

After impregnation, the material turned violet, which was compatible with the dye coloration (Figure 1). 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 the carbapenem-resistant $A. baumannii$, $P. aeruginosa$ and $K. pneumoniae$ (Figure 2).

In the 14 patients who were evaluated, the clinical data and VAP development 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 3. 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 production 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 for possible structural alterations that may have been caused. No fissures or other alterations that could compromise the tube structure were found (Figure 4).
It was possible to confirm the formation of organic compounds peaks compatible with PVC on FTIR spectroscopy. However, owing to the overlapping of peaks, it was not possible to identify the impregnation components either before or after use on the patients.

**DISCUSSION**

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 VAP risk

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. The novel, styrene-based, antimicrobial agent (BCP3) has shown significant growth inhibition against *S. aureus* and *P. aeruginosa*, with a more substantial effect on *S. aureus*; inhibition of bacterial growth greater than 80%.

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 group using coated-ETT. VAP was confirmed in 11 (6.5%) and 18 (11.6%) patients in the NMA-coated and control groups, respectively.

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 VAP. 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 formation.

As this was a pilot and in vitro study, it is too early to assume that the device effectively reduces VAP risk. 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 we 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.

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.

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None.

**CONFLICT OF INTEREST/DISCLOSURES**

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

**FUNDING**

None.
REFERENCES


**FIGURE LEGENDS**

Figure 1. Segment of tracheostomy tube impregnated with chlorhexidine and violet crystal.

Figure 2. Segment of tracheostomy tube impregnated with chlorhexidine and violet crystals (orange circles) and control tube were tested on a plate with multidrug-resistant bacteria: A) carbapenem-resistant *Klebsiella pneumoniae*; B) MRSA; C) carbapenem-resistant *Acinetobacter baumannii*, and D) carbapenem-resistant *Pseudomonas aeruginosa*.

Figure 3. 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).

Figure 4. SEM of the impregnated tube before tracheostomy tube implantation (A), and after extubation (B), with the arrow showing the biofilm (26x magnification). In Figures C and D the biofilm has been demonstrated at 500x and 5000x magnification, respectively.