Biofilm formation on three different endotracheal tubes: a prospective clinical trial

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Abstract

**Background:** Biofilm formation on endotracheal tubes (ETTs) is an early and frequent event in mechanically ventilated patients. The biofilm is believed to act as a reservoir for infecting microorganisms and thereby, contribute to development and relapses of ventilator-associated pneumonia (VAP). Once a biofilm has formed on an ETT surface, it is difficult to eradicate. This clinical study aimed to compare biofilm formation on three widely used ETTs with different surface properties and to explore factors potentially predictive of biofilm formation.

**Methods:** We compared the grade of biofilm formation on ETTs made of uncoated polyvinyl chloride (PVC), silicone-coated PVC, and PVC coated with noble metals after > 24 hours of mechanical ventilation in critically ill patients. The comparison was based on scanning electron microscopy of ETT surfaces, biofilm grading, surveillance and biofilm cultures, and occurrence of VAP.

**Results:** High-grade (score ≥ 7) biofilm formation on the ETTs was associated with development of VAP (OR 4.17 [95% CI 1.14–15.3], p = 0.031). Compared to uncoated PVC ETTs, the silicone-coated and noble-metal-coated PVC ETTs were independently associated with reduced high-grade biofilm formation (OR 0.18 [95% CI 0.06–0.59], p = 0.005 and OR 0.34 [95% CI 0.13–0.93], p = 0.036, respectively). No significant difference was observed between silicon-coated ETTs and noble-metal-coated ETTs (OR 0.54 [95% CI 0.17–1.65] p = 0.278). The microbes found in the ETT biofilm were frequently found in surveillance cultures at intubation and often remained in the biofilm despite appropriate antibiotic therapy. High-grade biofilm formation on ETTs was not predicted by either colonization with common VAP pathogens in surveillance cultures or duration of invasive ventilation.

**Conclusion:** High-grade biofilm formation on ETTs was associated with development of VAP. Compared to the uncoated PVC ETTs, the silicone-coated and noble-metal coated PVC ETTs were independently associated with reduced high-grade biofilm formation. Methods aimed at the continuous monitoring of biofilm formation are warranted. Routines for biofilm removal need further study.

**Trial registration:** ClinicalTrials.gov, NCT02284438. Retrospectively registered on 21 October 2014, URL: https://clinicaltrials.gov/ct2/show/NCT02284438.

Background

Hospital-acquired pneumonia (HAP) is a heavy burden on modern healthcare [1,2]. With intubation and mechanical ventilation, the risk of pneumonia increases ten-fold [3]. Ventilator-associated pneumonia (VAP) results in prolonged length of hospitalization, higher rates of morbidity and mortality, and a significant increase in treatment costs [4–7]. Despite the introduction of preventive strategies and modifications of endotracheal tubes (ETTs) in recent years, the rate of VAPs remains substantial [8–10].

The pathogenesis of VAP is assumed to be a process requiring colonization or overgrowth of the upper gastrointestinal tract with potentially causative pathogens [11], followed by micro-aspiration of contaminated secretions that overcome lower respiratory tract defense mechanisms, resulting in an infection [11,12]. The ETT has a central role in the pathogenesis of VAP [13], which is not surprising since it involves opening the body’s natural barrier (the vocal cords), thereby providing pathogens access to and changing the microbiota of the lower respiratory tract [14]. In recent years, increasing evidence has emerged indicating that biofilm formation on the surfaces of ETTs is an important link in the VAP pathogenesis [15–21]. Furthermore, these biofilms act as reservoirs for pathogens that are believed to contribute to VAP relapses [19].

Biofilms are structured communities of microbial cells enclosed in a self-produced polymeric matrix attached to a surface [22–24]. As biofilm formation and fragments of it entering the lower respiratory tract is considered a major source for VAP [17,19], a number of different ETT surfaces or materials that have an action against microbial adhesion or viability have been developed. Different biocide coatings (e.g. silver, chlorhexidine,
sulfadiazine, gendine) have been tested in this context, although only silver-coated ETTs have been subjected to multiple clinical trials that have shown some beneficial effects [25]. However, there are some impediments to widespread use, including concerns over antibiotic resistance and the relatively high costs.

Today, several different ETT materials are commercially available and they differ markedly in price. Two materials that are used extensively for this purpose are polyvinyl chloride (PVC) and silicone-coated (SC) PVC. To the best of our knowledge, these two materials have not been compared regarding biofilm formation in a clinical setting. Another ETT coated with a thin layer of a noble metal alloy (NbMC) containing silver, gold, and palladium (Bactiguard® AB, Sweden), has been on the market since 2013, and the manufacturer claims that this coating does not release any silver ions into the environment. Urinary catheters with this coating have been successful in reducing urinary tract infections[26], but the effectiveness of the coating in preventing biofilm formation on ETTs has not been evaluated in intensive care settings.

The primary aim of the present clinical prospective observational study was to compare the two most widely used ETT materials on the market (uncoated PVC and silicone-coated PVC) and an NbMC PVC ETT, evaluating the grade of biofilm formation on the three different ETT surfaces. The secondary objective was to explore possible associations between patient characteristics, the development of VAP, and increased biofilm formation on the ETT surfaces. We hypothesized the following: that the three different ETT materials would differ with regard to the grade of biofilm formation; and that the biofilm grade, along with colonization with common VAP pathogens in the oropharynx and lower airways, would be correlated with the development of VAP.

**Methods**

This clinical observational study was carried out at Skåne University Hospital in Lund, Sweden. The study protocol was reviewed and approved by the Regional Ethical Review Board, Lund, Sweden (protocol 2013/583). Informed consent, including permission to collect and publish anonymous data, was obtained from all patients or their relatives. The manuscript was prepared according to the STROBE guidelines for observational studies [27].

**Patients and materials**

We included patients 18 years and older who were admitted to our intensive care unit (ICU) and were expected to require invasive mechanical ventilation for at least 24 hours. Patients were allowed to participate only once and were included during six separate time periods from February 2014 to April 2017. Depending on the period, patients were intubated on clinical indications with one of the three different types of ETTs tested in our study. Each type of ETT was used in two of the six periods. The use of the different ETTs according to study period rather than by randomization was done for logistic reasons. Patients in study periods one and four received an uncoated PVC ETT (Oral/Nasal Endotracheal Tube, Mallinckrodt™, Medtronic, Dublin, Ireland ), which is standard in our hospital; patients in periods two and six received an SC ETT (Siliconized PVC, Oral/Nasal Soft Seal® Cuffed Tracheal Tube, Portex™, Smith’s Medical, Kista, Sweden); and patients in periods three and five received an NbMC PVC ETT, coated with a thin noble metal alloy coating consisting of gold, silver, and palladium (Bactiguard Infection Protection Endotracheal Tube, Bactiguard®, Tullinge, Sweden). Each of the six study periods was planned to last for about three months and the goal was to include a minimum of 15 patients in each period. Bundles of maneuvers (e.g. placing patients in a semi-recumbent position) to prevent VAP are standard at our facility. With the exception of use of different ETT materials during the study periods, all patients received standard intensive care according to diagnosis and the clinical decisions of the responsible physicians.

**Microbiological procedures**

Surveillance cultures (i.e., oropharyngeal swabs and endotracheal aspirates), were collected on days 1, 2, 3, 5, 7, 14 and 21, and thereafter once a week for all included patients. On the day of extubation, surveillance cultures were also collected if not previously scheduled.

All oropharyngeal swabs and endotracheal aspirates were processed in the same manner at the Department of Clinical Microbiology by use of standardized, extended microbiological procedures [28]. Samples were inoculated
on three different selective plates, one differentiating and one non-selective agar plate as follows: (1) agar plate with 5% horse blood (LabM, Heywood, Lancashire, UK) supplemented with 10 mg/L colistin and 15 mg/L nalidixic acid; (2) agar plate with 5% horse blood supplemented with 2 mg/L gentamicin and 25 mg/L nalidixic acid for Gram-positive cocci including S. pneumoniae; (3) Hematin agar plate (OxoidTM, Thermo Science, Basingstoke, UK) supplemented with 300 mg/L bacitracin for fastidious Gram-negative rods including Haemophilus influenzae (selective); (4) Uriselect 4 agar (Bio-Rad Laboratories, Copenhagen, Denmark) supplemented with 10 mg/L vancomycin for non-fastidious Gram-negative rods (differentiating); and (5) Hematin agar with a colistin disk (non-selective). All plates were manufactured in-house, and they were inspected for growth after 16 and 40 hours of aerobic, anaerobic, or CO2 incubation at 35 - 37 °C. Identification of bacterial species was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MALDI Biotyper Microbial Identification system, Bruker, Boston, MA, USA). Differentiation of Candida spp was based on colony appearance on CHROM Candida agar (CHROMagar, Hägersten, Sweden) after 48 hours of incubation at 35 °C.

Patient data

Age, sex, body-mass-index (BMI), days with invasive ventilation, antibiotic and/or proton pump inhibitor (PPI) use during mechanical ventilation, Simplified Acute Physiology Score 3 (SAPS 3), and main diagnosis on ICU admission were documented for all patients. Data on the occurrence of VAP, together with data on microorganisms isolated in surveillance cultures and ETT biofilm were collected for all patients.

Processing of the ETT

Patients were extubated at the discretion of the treating physician or in the case of a patient’s death. After extubation, the ETTs were collected, avoiding contamination from other than oropharyngeal flora, and rinsed (inside and outside) with 1 L of sterile saline to eliminate excess mucus. Thereafter, the distal tip of the ETT was divided into four pieces for both scanning electron microscopy (SEM) (2 pieces) and microbial cultures (2 pieces). Finally, the ETT tip was cut in a cross-sectional manner 1.5 cm above the distal tip. Pieces of the ETT for microbial cultures were sonicated in phosphate-buffered saline (PBS) with 47 kHz for 1.5 minutes to dislodge biofilm microbes. The solution was then homogenized by vortex mixing and subsequently cultured using the same procedures as applied for the oropharyngeal swabs and endotracheal aspirates. A pilot study (not published) comparing different methods for processing of the ETTs indicated that the method outlined above was optimal for removing the biofilm and for dislodging the biofilms’ microbes before culturing. For SEM, pieces of an ETT were fixed in a solution of 4% formaldehyde diluted with PBS at room temperature for 30 minutes, dehydrated with crescent ethanol concentrations, air-dried overnight, and sputter-coated with 15 nm of Au/Pd thin film (Gatan PECS Mod 682, Gatan, Inc., Pleasanton, CA, USA) to prevent charging during SEM analysis.

Scanning electron microscopy and grading of the biofilm

The inner and outer surfaces of the ETTs were examined by SEM (Zeiss Supra 40VP, Carl Zeiss Microscopy GmbH, Jena, Germany). The analysis was performed using a secondary electron detector set at a working distance of 8–10 mm and electron acceleration of 3.68–4.05 kV. Low magnification (100x to 1,000x) was used to rank biofilm coverage as follows: 0, no biofilm; 1, scarce coverage of < 10%; 2, clusters with 10–70% coverage; 3, confluent film with > 70% coverage. High magnification (10,000x to 50,000x) was used to evaluate biofilm density (0, no biofilm; 1, low/very porous; 2 medium; 3, high/compact) and level of thickness (0, no biofilm; 1, thin 0.1-1.0 µm; 2, medium 1.1-7 µm; 3, thick > 7 µm). Film thickness was estimated as the difference in focus distance between the outer surface of the biofilm and the ETT surface. When measuring the thickness, the mean was calculated based on multiple representative points in the sample. The final grade of the biofilm was then calculated by adding the scores from coverage, density, and thickness together to give a score of 0 to 9. A high/advanced biofilm grade was defined as a score of ≥ 7. The grading system is summarized in Table 1. Grading of the biofilm was performed by a researcher at RISE who was blinded to all patient information including type of ETT analyzed.

Surveillance cultures and ETT tip cultures with growth of at least two species of bacteria usually found in the oral cavity were classified as having normal flora (negative cultures). Species not normally found in the oral cavity (e.g., pathogens, gut flora, or overgrowth of normal oral flora) were classified as abnormal flora (positive cultures).
cultures). All culture results were reviewed by a microbiologist to ensure correct classification. The diagnosis of VAP was made by two independent physicians evaluating the cases based on clinical and radiological examinations. VAP was defined by the following: 1) new or progressive lobar infiltrate > 48 hours after intubation; 2) two or more of the minor criteria fever, leukocytosis/leukopenia, and/or purulent respiratory secretions; 3) microbiological confirmation in endotracheal aspirate [29]. Colonization with common VAP pathogens included cultures with Enterococcus faecium, E. faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Streptococcus pneumoniae, Haemophilus influenzae, and other Enterobacteriaceae species.

VAP relapse was defined as previously reported[19]. Microbial persistence was defined as persistence of the causative agent of VAP in at least two surveillance cultures despite 48 hours of appropriate antibiotic therapy [30].

Statistical analysis

Results were expressed as median (interquartile range [q1-q3]) for continuous variables and numbers (percentages) for dichotomous variables. A p value of < 0.05 was considered significant, and all statistical tests were two-tailed. Groupwise comparisons were conducted using Fisher’s exact test for categorical variables and the Mann-Whitney U-test for continuous variables. Multivariable logistic regression analysis was applied to identify independent factors associated with the formation of high-grade biofilm on the ETT (score ≥ 7 in the scoring system described above). This cut-off was chosen based on data from an earlier study [18]. The Hosmer-Lemeshow test was used to determine goodness of fit. Univariable logistic regression analyses were conducted to evaluate patients’ characteristics that could be associated with the development of VAP. Multivariable regression analysis of VAP was not performed due to too few cases of the condition (n = 12). Clinical studies comparing biofilm formation on ETTs using the scoring system applied in this study are lacking. Based on previous laboratory models using other scoring systems to grade biofilm formation [31,32] and given a power of 0.8 and an alfa level of 0.05, a sample size of 27 was needed in each group. We carried out all analyses with SPSS 26 (SPSS Inc, Chicago, IL, USA).

Results

Inclusion of patients is shown in Fig. 1. During the six study periods 505 patients were orally intubated. Among the 293 patients receiving mechanical ventilation for >24 hours, 129 patients were included in our study (Fig. 1). One-hundred and sixty-four patients were not included for logistical reasons and during weekends and holidays. Twenty-three of the included patients (n=129) were excluded because the ETT was lost after extubation, mechanical ventilation lasted < 24 hours and/or informed consent was not obtained from the patient or a relative. The remaining 106 patients were included in the final analysis as follows: 34 were intubated with an uncoated PVC tube; 30 were intubated with an SC PVC tube; and 42 were intubated with the NbMC PVC tube (Fig. 1). Patient characteristics and main diagnoses on ICU admission were similar in the three study groups (Table 1).

Biofilm formation

Biofilm was present on the ETTs of 97% (n = 103) of the patients and could be seen on the surface of the tubes after 24 hours of intubation. The grade of biofilm formation varied from low, porous (Figure 2a) to confluent, abundant biofilm matrices (Figure 2b). Colonies of different microorganisms embedded in the biofilm matrix were often recognized by SEM (Fig. 2c).

When analyzing possible predictors of high-grade biofilm formation (score ≥ 7 ) on the ETT using multivariable logistic regression analysis, the SC ETTs and the NbMC ETTs were independently associated with reduced high-grade biofilm formation compared to the uncoated PVC tubes (odds ratio [OR] 0.18, 95% confidence interval [CI] 0.06–0.59, p = 0.005 for the former, and OR 0.34, 95% CI 0.13–0.93, p = 0.036 for the latter). There was no significant difference between SC and NbMC ETTs (OR, 0.54; 95% CI 0.17–1.65; p = 0.278). Age, sex, days with invasive ventilation, and colonization with common VAP pathogens in surveillance cultures did not predict higher
biofilm formation in univariable or multivariable analyses (Table 3).

**Surveillance cultures**

Surveillance cultures were obtained for all patients but missing cultures, according to the predefined culture scheme, were 5% (n=41) of all planned cultures. For all oropharyngeal (n = 376) and endotracheal (n = 381) cultures, 66% (n = 248) and 64% (n = 242), respectively, turned positive with about 22% noted as polymicrobial. A majority of the patients developed abnormal oropharyngeal flora (82%, n = 87) and became colonized in the lower airways during invasive ventilation (endotracheal aspirate [ETA], 79%, n = 84).

Colonization with common VAP pathogens was found in oropharyngeal cultures from 33% (n = 35) of the patients and in endotracheal cultures from 27% (n = 29). In colonized patients, the median time from intubation to culture positivity was 1 day (1–2 days) for both oropharyngeal and endotracheal cultures. In patients colonized in oropharyngeal or in endotracheal cultures (all positive cultures), the same microbe was found on the ETT in 48% and 49% of the cases, respectively. In patients colonized with common VAP pathogens, the same microbe was found on the ETT in 29% (10 out of 35) of the oropharyngeal cultures and 38% (11 out of 29) of the endotracheal cultures.

Microbes isolated from surveillance cultures in the three study groups are listed in Table 4. The surveillance cultures turned positive for several different microbes, most often *Candida albicans* and other candida species. Among bacteria, *Enterococcus faecalis*, *E. faecium*, *Staphylococcus aureus*, *Klebsiella* species, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* occurred most frequently (Table 4).

**Microbial isolation from endotracheal tubes**

At extubation, 99% (n = 105) of the ETTs were cultured and 47% (n = 49) turned positive. No significant difference in positive ETT cultures was observed between the groups (uncoated PVC, 41% [n = 14], SC PVC 45% [n = 13]; NbMC PVC 52% [n = 22]; p = 0.61). Colonization with common VAP pathogens in oropharyngeal and endotracheal cultures predicted a positive ETT culture with common VAP pathogens at extubation (OR, 17.6; 95% CI 3.8–81.6; p < 0.001 and OR, 10.3; 95% CI 3.1–34.2; p < 0.001, respectively). If the ETT tip turned positive, the microbe was found in oropharyngeal cultures in 86% (n = 42) of the patients and in endotracheal cultures in 88% (n = 43). The microbes found on the ETTs at points of extubation could be detected in the first oropharyngeal culture in 60% (n = 29) of the patients and in the first endotracheal cultures in 58% (n = 28). *Candida albicans* (39%) and other candida species (11.5%) were the microbes that occurred most frequently on the ETTs, followed by *Staphylococcus aureus* (8.2%), *Enterococcus faecalis* (8.2%), *Pseudomonas aeruginosa* (6.6%), and *Stenotrophomonas maltophilia* (6.6%). Microbial isolations from the different ETTs are presented in Table 5.

**VAP**

Twelve patients developed 15 episodes of VAP during their stay in the ICU, and three of those episodes were VAP relapses. The most common pathogens involved in VAP were *Enterococcus faecium*, *E. faecalis*, *Staphylococcus aureus*, *Klebsiella* spp., and *Acinetobacter* spp. High-grade biofilm formation (score ≥ 7) on the ETTs, days of invasive ventilation, and age were significantly associated with the development of VAP (OR, 4.17; 95% CI 1.14–15.3; p = 0.031 and OR 1.11; 95% CI 1.01–1.22; p = 0.026 and OR 0.96; 95% CI 0.92–0.99; p = 0.046, respectively). ETT material, sex, and colonization with common VAP pathogens in surveillance cultures were not associated with development of VAP (Table 6).

Microbial persistence in surveillance cultures could be evaluated in seven of 12 patients who developed VAP, and it occurred in five patients (71%) after appropriate antibiotic treatment for VAP. At extubation, the microbes previously causing VAP could be found in the ETT biofilm in 56% of the cases (5 out of 9 patients) despite appropriate antibiotic therapy. The microbes most often involved in microbial persistence were *Klebsiella* spp., *Candida parapilosis*, *Enterococcus faecium*, and *E. faecalis*. In about half of the VAP cases, an evaluation of microbial persistence in surveillance cultures (n = 5) or in the ETT biofilm (n = 3) could not be done, because the course of antimicrobial treatment (mostly antifungal treatment < 7days) was too short, the pathogens were resistant to initial treatment (inappropriate antibiotic therapy), or the patient received a tracheostomy or died.
Discussion

In this prospective clinical observational study, we have demonstrated that biofilm formation on ETTs is an early and frequent event in intubated patients. Furthermore, we found that high-grade biofilm formation on ETTs was associated with the development of VAP. Among the studied ETT materials, both the SC ETT and the NbMC ETT were associated with reduced high-grade biofilm formation as compared to the standard uncoated PVC tube. The microbes detected in the ETT biofilm were frequently found in surveillance cultures at intubation. Microbial persistence despite appropriate antibiotic treatment is common in ICU patients with VAP, and the causative microbe can often be found in the ETT biofilm at extubation.

Our results confirm earlier studies showing that biofilm formation on the ETT surface is an early and frequent event in intubated patients [15–19]. In that context, our investigation adds important knowledge illustrating that formation of a high-grade biofilm on the ETT surface (not just the presence of biofilm at any stage) is associated with development of VAP. Given that 60% of the first oropharyngeal cultures and 58% of the first endotracheal cultures contained the microbes in the ETT biofilm, our data also demonstrate a microbial link between early surveillance cultures and microbial content of biofilms. In addition, microbial persistence is common, despite appropriate antibiotic treatment, when an ETT is left in place after an episode of VAP. These observations indicate that the choice of ETT material can influence the grade of biofilm formation on the ETT surface. Sottile et al. [16] were among the first to use SEM to describe biofilm formation on ETTs, but only in recent years has the role of biofilm formation in the pathogenesis of VAP become clearer. There is increasing evidence [19], including the present results, suggesting that the ETT biofilm is a significant and persistent source of infection that should be taken into account when treating critically ill patients.

The determinants of high-grade biofilm formation on ETTs in critically ill patients are not clear and are probably multifactorial. In this study, we found that, high-grade biofilm formation on ETTs was associated with the development of VAP; as has also been observed in one prior study of thirty-two patients[18]. Our study confirms these finding but in a much larger population (n=106). Unfortunately, there is no gold standard for biofilm grading when using SEM, which makes it difficult to compare findings from different studies. The majority of studies that have applied SEM to analyze biofilms have only used the coverage or the existence of a biofilm to grade biofilm formation[19,33–35]. The disadvantage of that approach is that two biofilms exhibiting the same coverage can differ substantially in thickness and density and most likely also with regard to their degree of maturity and how prone they are to dispersal [36]. This may be one reason why the authors of some earlier studies have not found an association between biofilm coverage/existence and VAP development[19,37].

Although biofilm formation is a rapid process, we were unable to predict biofilm grade solely by determining the duration of invasive ventilation, as has been noted in earlier studies [15,18,19]. This indicates that the grade of biofilm formation is dependent on additional factors. It is not clear what causes high-grade biofilm to rapidly develop on the ETT surface in one patient, whereas it happens much more slowly or not at all in another. Similarly, not all intubated patients ultimately develop VAP [18]. Being able to monitor or predict the grade of biofilm formation is of clinical importance, as our findings indicate that high-grade biofilm formation (not just biofilm formation at any stage) is associated with the development of VAP. Methods for continuous monitoring of biofilm formation on ETTs are described in laboratory models utilizing optical fiber sensors, incorporated into the lumen of the ETT[38]. This could be an interesting tool for clinical use, but needs clinical evaluation.

Despite the knowledge that VAP is the most common hospital acquired infection in the critically ill, and that ETT biofilms act as a significant and persistent source of infection in the intubated patient, routines for biofilm removal including ETT exchange are not well studied, and in many cases, not considered safe. As biofilms are hard to eradicate once they have formed, it would be interesting to study if changing the ETT in selected VAP patients could reduce microbial persistence and the risk of VAP relapse. The risk-to-benefit ratio must be evaluated for each patient, but the benefits of an ETT change may be greater than the risks in some cases. It has been pointed out that reintubation is associated with VAP, although some of the data underlying that conclusion have not been obtained in evaluations of ETT exchange, but rather in extubation trials[39]. Nevertheless, well controlled studies in this field are warranted before any recommendations can be made. Early
performance of a tracheostomy has been considered to be a measure that can reduce VAP. However, a systematic review found no such reduction [40], possibly because the tracheostomy tube is also a foreign material that is prone to biofilm formation in the same manner as the conventional ETT. With the emergence of multiresistant bacteria and fewer choices for antimicrobial treatment of VAP, ETT routines may change [41]. Methods aimed at the continuous monitoring of biofilm formation[38] are warranted, because few predictive factors are known at the moment. Biofilm removal without ETT removal by use of tools such as the mucus shaver or photodynamic inactivation are promising but must be further evaluated [42,43].

Considering the lifecycle of a biofilm, it is likely that high-grade biofilm formation (grade ≥ 7 in our study) reflects a more mature biofilm containing several pillar- and mushroom-shaped masses that are susceptible to breakage caused by manipulation such as suction with a catheter (as shown in Fig. 2d) or due to turbulent airflow. Detachment and dispersal are natural developments in a mature biofilm that lead to the spread of highly contagious biofilm fragments into the lower airways [44]. On the other hand, a low-grade biofilm is thinner and more firmly anchored to the surface when host- and tissue-specific adhesins on pili and fimbria are attached [45].

Colonization with common VAP pathogens in surveillance cultures was not associated with high-grade biofilm formation or VAP in our study. This finding was somewhat unexpected, considering that colonization with pathogenic bacteria is assumed to precede the development of VAP [11,46]. Previous research has shown that there is a microbial link between oropharyngeal, tracheal, and biofilm cultures [19], but to the best of our knowledge, the correlation between positive cultures and biofilm grade has not yet been elucidated. Vandecandelaere et al. used both culture and culture-independent methods and found no significant difference in biofilm flora between patients who developed VAP and those who did not [47]. Furthermore, those authors observed no difference in biofilm flora between patients with longer ( > 5 days) and shorter ( < 5 days) intubation periods. Other scientists have shown that culture-dependent methods (as used in our study) detect only a small portion of microorganisms in ETT biofilms. Biofilms are multi-microbial, and culture-independent methods have revealed that they contain approximately 70% oral flora [47]. It has been suggested that many microbes in the oral flora initiate biofilm formation that may facilitate colonization of more pathogenic bacteria, although those floral microbes may not be responsible for development of VAP per se [48]. One such interaction has been observed between Candida albicans and Pseudomonas aeruginosa [49], in which an antifungal treatment was found to be associated with a reduced risk of P. aeruginosa VAP [50]. The impact of each microbe or combination of microbes is not clear and must be elucidated in larger trials.

This is the first study of critically ill patients to compare biofilm formation on widely used ETT materials. We found that, compared to the standard uncoated PVC ETT, the SC ETT and the NbMC ETT were associated with reduced high-grade biofilm formation. Although silicone is applied extensively in health care, there are no previous evaluations of use of SC ETTs in the critical care setting. Silicone is also used for industrial purposes: in fluids (free chains) for producing cosmetics; in gels (crosslinked chains) for fabricating soft tissue implants; in resins (heavily crosslinked chains) for producing optically clear silicone; and in elastomers (crosslinked and reinforced chains) for manufacture of medical catheters including ETTs and various kinds of prostheses (e.g., breast, hand, foot, ear, nose, eye, and voice prostheses). It should be mentioned that there are a number of subtypes of silicone elastomers, and the exact composition of the particular subtype used in the present SC ETTs is the manufacturer’s trade secret and hence was not available to us. Silicone elastomers have gained widespread use mainly due to their durability, flexibility, and biocompatibility when in contact with skin or human tissue [51]. On the other hand, some studies indicate that silicone elastomers have reduced blood compatibility [52,53], and assessments in rabbit models have shown that, compared to central venous catheters made of PVC, such catheters made of silicone increase the risk of infection [54]. These differences indicate that there may not be one successful surface or material in particular that fits all environments of the body.

Bacterial adhesion and biofilm formation on different catheter materials have been studied in vitro. Although results are conflicting, some of the authors have reported significantly reduced bacterial adhesion and biofilm formation on silicone surfaces compared to PVC surfaces[31,55]. Also, studies have demonstrated that the properties of PVC and silicone catheters from different manufacturers vary, indicating differences in the composition of the materials [32]. Hence, our findings may not apply to all silicone or PVC ETTs. It has also been observed that biofilm formation is facilitated on surfaces with greater roughness [56]. Even though evaluation of
The NbMC ETTs showed reduced high-grade biofilm formation compared to uncoated PVC tubes, but although both those tube types were made of PVC, they were not from the same manufacturer. As for silicone, the composition of PVC can differ between manufacturers. Thus far, NbMC ETTs have not been evaluated in the intensive care settings, with the exception of a short-term intubation trial during elective surgery [57]. During our study no adverse reactions were registered that could be related to the NbMC ETT coating. According to some reports, the noble metal coating has been shown to reduce catheter-related urinary tract infections with long-term use [58–60]. However, it should be noted that the NbMC urinary catheter is made of silicone or latex, not PVC like the NbMC ETT in our study. PVC ETTs have been shown to be prone to bacterial adhesion in vitro [61], which indicates that silicone coated ETTs with NbMC may be more efficient in preventing microbial adhesion and biofilm formation. This possibility must be further assessed before any conclusions can be drawn.

A majority of the current patients received PPIs (n = 99) or antibiotic therapy (n = 103) during intubation, which made it impossible to evaluate the impact of these variables on VAP or biofilm formation. Previous studies considering the effect of systemic antibiotics on bacterial persistence in the respiratory tract have shown that the persistence of colonization after antibiotics differs significantly among pathogens [30]. Thus, it is likely that the effectiveness of antibiotics in reducing the bacterial load on ETTs varies depending on the microbe’s present. Moreover, it is well established that a biofilm that has formed on the surface of a medical device is highly resistant to antibiotics [48,62]. The association between use of PPIs, colonization with more pathogenic bacteria in the oropharynx, and development of VAP has been described in previous reports [28,63,64]. The impact of PPI and/or antibiotic therapy on biofilm formation and VAP could not be evaluated in our study and should be further assessed in future trials.

We recognize the limitations of the present study. First, our ambition was to include all patients that were expected to receive mechanical ventilation for >24 hours. For logistical reasons and during weekends and holidays, a large number of patients were not included (n = 164). The authors have speculated if this could have led to any kind of selection bias. As patient characteristics are similar among the three groups, and the same method of inclusion was applied to all six inclusion periods, we find that unlikely. The six study periods were also equally spread through the year, containing some holiday periods and summer months. Second, one would expect to see the same significant association between ETT material and VAP as is seen between ETT material and high-grade biofilm formation. Not all patients who had high grade biofilm formation on the ETT surface are though expected to develop VAP, the odds are only increased according to the analyses. One probable explanation is lack of power as a much larger sample size is needed to observe a difference in VAP than in grade of biofilm formation. In the same way, the impact of each microbe or combination of microbes is not clear and could not be evaluated due to the lack of statistical power. Finally, microbial persistence could not be assessed in many cases due to the death of a patient or because a patient underwent a tracheostomy a few days after the VAP episode.

In this study, formation of biofilm on ETTs was an early and frequent event in critically ill patients. Moreover, high-grade biofilm formation on the ETT was associated with the development of VAP. Compared to the uncoated PVC ETTs, the silicone-coated and noble-metal coated PVC ETTs were independently associated with reduced high-grade biofilm formation. The microbes found in the ETT biofilms were frequently found in surveillance cultures at intubation and often remained in the biofilms after appropriate antibiotic therapy. Methods aimed at the continuous monitoring of biofilm formation are warranted. Routines for biofilm removal need further study.
The study protocol was reviewed and approved by the Regional Ethical Review Board, Lund, Sweden (protocol 2013/583). Informed consent, including permission to collect and publish anonymous data, was obtained from all patients or their relatives.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

**Competing interests**

This study was performed as part of a national project entitled Innovation against Infection (in Swedish: *Innovation Mot Infektion* [IMI]), and was conducted in collaboration with Skane University hospital, Lund, and RISE Research Institutes of Sweden, Borås, Sweden. The authors declare that they have no competing interests.

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**Authors’ contributions**

BK designed the study. BK and HRT coordinated the inclusion of patients. BK, HRT, and research nurses handled the ETTs after extubation and prepared pieces of the tubes for the SEM analysis. HRT collected the data, built the database, and wrote the first version of the manuscript. TK performed the first revision of the manuscript. HRT and TK performed the statistical analyses. HRT, AH, SP, and BK created the scoring system for biofilm grading used in the study. SP examined and ranked all ETTs by SEM. All authors contributed to the interpretation of the data, revised the manuscript critically, and gave final approval of the version to be published.

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**Abbreviations**

ETT: endotracheal tube; SEM: scanning electron microscopy; HAP: hospital-acquired pneumonia; ICU: intensive care unit; MALDI-TOF: matrix-assisted laser; desorption/ionization time-of-flight; NbMC: noble-metal-coated; PBS: phosphate-buffered saline

PPI: proton pump inhibitor; PVC: polyvinyl chloride; SAPS 3: Simplified Acute Physiology Score 3; SC: silicone-coated; SEM: scanning electron microscopy; VAP: ventilator-associated pneumonia

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Tables

Table 1

| Biofilm coverage | Biofilm density | Biofilm thickness scale |
|------------------|-----------------|------------------------|
| Determined at 100x to 1000x mag | Determined at 10000x to 30000x mag | Determined at 10000x to 50000x mag |
| 0 no biofilm | 1 | 0 no biofilm |
| scarce ( < 10% coverage) | 2 | 1 low/very porous |
| clusters (10% to 70% coverage) | 2 | 2 intermediate |
| 3 confluent ( > 70% coverage) | 3 | 3 high/compact |
| thin biofilm (0.1 to 1.0 mm) | 1 | medium biofilm (1.1 to 7 mm) |
| thick biofilm ( > 7 mm) | 3 | |

The biofilm grade was calculated by adding together the scores for biofilm coverage, density, and thickness. Mag: magnification.
## Table 2

### Baseline characteristics of patients and diagnosis on admission to the ICU (n = 106)

| Variable                                | PVC tube (n = 34) | SC tube (n = 30) |
|-----------------------------------------|-------------------|------------------|
| **Patient characteristics**             |                   |                  |
| Age, median (range)                     | 67 (57–74)        | 69 (54–76)       |
| Sex, male                               | 16 (47)           | 17 (57)          |
| BMI                                     | 26 (23–29)        | 28 (24–32)       |
| SAPS III score                          | 67 (53–75)        | 63 (57–79)       |
| PPI use ≥ 24 h before intubation        | 19 (56)           | 13 (43)          |
| PPI use while intubated                 | 32 (94)           | 29 (97)          |
| Antibiotic use ≥ 24 h before intubation | 28 (82)           | 22 (73)          |
| Antibiotic use while intubated          | 33 (97)           | 28 (93)          |
| Days with invasive ventilation          | 3.3 (2.0–5.5)     | 3.0 (1.8–6.8)    |
| **Main diagnosis on admission to the ICU** |                   |                  |
| Sepsis                                  | 8 (23)            | 12 (40)          |
| Cardiovascular                          | 5 (15)            | 1 (3.3)          |
| Respiratory insufficiency               | 11 (32)           | 13 (43)          |
| Trauma                                  | 4 (12)            | 1 (3.3)          |
| Coma GCS < 7                            | 4 (12)            | 1 (3.3)          |
| Other                                   | 2 (5.8)           | 2 (6.7)          |

Data are presented as median (q1–q3) or n (%). Abbreviations: ICU intensive care unit, PVC polyvinyl chloride, SC silicone-coated, NbMC noble-metal-coated, BMI body mass index, SAPS III Simplified Acute Physiology Score III, PPI proton pump inhibitor, GCS Glasgow Coma Scale.
### Table 3

**Possible predictors of high-grade (score ≥ 7) biofilm formation on the endotracheal tube**

| Factor          | Univariable analysis | Mul |
|-----------------|----------------------|-----|
|                 | No n = 65            | Yes n = 41 |
| ETT type        |                      | OR  | 95% CI | p- value | OR  |
| PVC (reference) | 15 (23)              | 19 (46) | NA | NA | NA | NA | 0.18 |
| SC              | 23 (35)              | 7 (17)   | 0.24 | 0.08 - 0.71 | 0.010 | 0.18 |
| NbMC            | 27 (42)              | 15 (37)  | 0.44 | 0.17 - 1.11 | 0.081 | 0.34 |
| Age             | 69 (58 - 76)         | 66 (53 - 75) | 0.98 | 0.95 - 1.00 | 0.088 | 0.98 |
| Sex, male       | 34 (52)              | 26 (63)  | 1.58 | 0.71 - 3.52 | 0.262 | 1.78 |
| Days with invasive ventilation | 3.1 (2.0 - 5.8) | 3.8 (2.0 - 9.2) | 1.06 | 0.98 - 1.14 | 0.157 | 1.06 |
| Colonized       | 33 (51)              | 18 (44)  | 0.76 | 0.35 - 1.67 | 0.491 | 0.59 |

Data are presented as median (range) or number (percentage). Abbreviations: OR odds ratio, CI confidence interval, ETT endotracheal tube, SC silicone-coated, NbMC noble-metal-coated, VAP ventilator-associated pneumonia, NA not applicable. “Colonized” with common VAP pathogens in surveillance (oropharyngeal or endotracheal) cultures.

### Table 4

**Microbial Isolation from Surveillance Cultures in the Three Study Groups**

-
The total number of different microbes isolated

| Microorganism                                      | OPC  | OPC - ETT match | E  |
|---------------------------------------------------|------|-----------------|----|
|                                                   | 150 (100) | 51 (100) | 133 |

**Microorganism**

**Gram-positive bacteria:**

- *Enterococcus faecalis*: 15 (10) 4 (7.8) 10
- *Enterococcus faecium*: 13 (8.6) 2 (3.9) 5
- *Staphylococcus aureus*: 9 (6.0) 3 (5.9) 15
- *Staphylococcus epidermitis*: 2 (1.3) 1 (2.0) 2
- *Beta hemolytic streptococcus group A*:
- *Streptococcus pneumoniae*:

**Gram-negative bacteria:**

- *Klebsiella species*: 9 (6.0) 1 (2.0) 4
- *Stenotrophomonas maltophilia*:
- *Pseudomonas aeruginosa*:
- *Haemophilus influenzae*:
- *Entrobacter cloacae*:
- *Serratia marcescens*:
- *Escherichia coli*:
- *Citrobacter freundii*:
- *Chryseobacterium indolgenes*:
- *Acinetobacter sp.*:

**Other bacterial species:**

**Yeast:**

- *Candida species*:
- *Candida albicans*:
- *Candida dubliniensis*:
- *Candida parapsilosis*:
- *Candida tropicalis*:
- *Other candida spp.*:
- *Other yeasts, not candida*:

Note. Data are presented as numbers (percentages). The percentage of positive cultures is related to how many patients were microbe. *Includes Escherichia coli producing extended spectrum beta-lactamase (ESBL). Approximately 25% of oropharyngeal endotracheal cultures were polymicrobial. OPC: oropharyngeal culture; ETA: endotracheal culture; ETT: endotracheal tube.

Table 5
Microbial isolation from positive endotracheal tube tip cultures

| Organism                           | PVC tube  
|                                   | \( n = 14 \) | SC tube  
|                                   | \( n = 13 \) | NbMC tube  
|                                   | \( n = \) |
|-----------------------------------|-----------|-----------|
| Gram-positive bacteria:           |           |           |
| Staphylococcus aureus             | 2 (11)    | 2 (13)    | 1 (3. |
| Enterococcus faecalis             | 3 (16)    |           | 2 (7. |
| Enterococcus faecium              | 1 (5.6)   | 1 (6.2)   |       |
| Staphylococcus epidermidis        |           |           | 1 (6.3)|
| Gram-negative bacteria:           |           |           |
| Pseudomonas aeruginosa            | 1 (5.6)   |           | 3 (1  |
| Stenotrophomonas maltophilia      |           | 4 (1)     |       |
| Klebsiella species                |           | 1 (6.2)   | 1 (3. |
| Chryseobacterium indologenes      |           |           | 2 (7. |
| Escherichia coli                  |           | 1 (6.2)   |       |
| Serratia marcescens               | 1 (5.6)   |           |       |
| Acinetobacter species             |           | 1 (6.2)   |       |
| Citrobacter freundii              |           |           | 1 (3. |
| Yeast:                            |           |           |
| Candida spp.                      | 9 (50)    | 9 (56)    | 13 (4 |
| Candida albicans                  | 8 (44)    | 7 (44)    | 9 (3  |
| Other yeasts                      | 1 (5.6)   |           |       |
| **The total number of microbes cultured from ETTs:** | **18 (100)** | **16 (100)** | **27 (100)** |

Data are presented as number (percentage). Approximately 20% of all endotracheal tube tips were polymicrobial. Abbreviations: PVC polyvinyl-chloride, SC silicone-coated, NbMC noble-metal-coated.
Table 6
Analyzing possible predictors of ventilator associated pneumonia (n = 83)

| Factor                          | OR   | 95% CI         |
|---------------------------------|------|----------------|
| ETT type                        |      |                |
| PVC (reference)                 | NA   | NA             |
| SC                              | 0.66 | 0.14–3.16      |
| NbMC                            | 0.54 | 0.13–2.26      |
| Age                             | 0.96 | 0.92–0.99      |
| Sex, male                       | 0.68 | 0.19–2.48      |
| Days with invasive ventilation  | 1.11 | 1.01–1.22      |
| High-grade biofilm formation on ETT | 4.17 | 1.14–15.3      |
| Colonized                       | 1.52 | 0.44–5.26      |

Abbreviations: OR odds ratio, CI confidence interval, ETT endotracheal tube, PVC polyvinyl chloride, SC silicone-coated, NbMC noble-metal-coated, NA not applicable. “Colonized” refers to patients colonized with common VAP pathogens in surveillance (oropharyngeal or endotracheal) cultures.
Figure 1. Flow chart showing inclusion of patients in the study. PVC: polyvinyl-chloride; SC: silicone-coated; NbMC: Noble
Figure 1

Flow chart showing inclusion of patients in the study. No consent, refers to cases were the patient did not give his consent to participate after the ICU stay. Endotracheal tube materials: uncoated PVC; Poly-vinyl-chloride, SC; Silicon-coated PVC, NbMC; nobel-metal-coated PVC.
Figure 2

Scanning electron microscopy of biofilm formation on the surface of endotracheal tubes. a Typical low-grade (score < 4) and b typical high-grade (score ≥ 7) biofilm formation (low magnification). c Colonies of microorganisms embedded in biofilm matrix (high magnification). d Scrape marks on the surface of an endotracheal tube probably caused by use of a suction catheter.