

Endotracheal Tube Biofilm and its Impact on the Pathogenesis of Ventilator-Associated Pneumonia

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ABSTRACT

Ventilator-associated pneumonia (VAP) is a common and serious nosocomial infection in mechanically ventilated patients and results in high mortality, prolonged intensive care unit- (ICU) and hospital-length of stay and increased costs. In order to reduce its incidence, it is imperative to better understand the involved mechanisms and to identify the source of infection. The role of the endotracheal tube (ET) in VAP pathogenesis became more prominent over the last decades, along with extensive research dedicated to medical device-related infections and biofilms. ET biofilm formation is an early and constant process in intubated patients. New data regarding its temporal dynamics, composition, germ identification and consequences enhance knowledge about VAP occurrence, microbiology, treatment response and recurrence.

This paper presents a structured analysis of the medical literature to date, in order to outline the role of ET biofilm in VAP pathogenesis and to review recommended methods to identify ET biofilm microorganisms and to prevent or decrease VAP incidence.

Keywords: biofilm, endotracheal tube, ventilator-associated pneumonia, culture dependent and independent pathogen identification, colonization, oral flora, antibiotic resistance, ventilator-associated pneumonia prevention

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INTRODUCTION

Nosocomial infections represent a major health care problem associated with high mortality and increased costs. Medical device-related infections represent almost one fourth of nosocomial infections [1]. Researchers devote extensive work in order to discover efficient prevention and treatment strategies. The most common type is nosocomial pneumonia with a reported incidence of 6.8-27% [1, 2].

Nosocomial pneumonia is a hospital-acquired life-threatening infection. Critically ill patients requiring mechanical ventilation in intensive care unit (ICU) is the group at the highest risk to develop the most severe form, ventilator-associated pneumonia (VAP).

VAP is defined as a nosocomial pneumonia occurring in a patient after 48 hours of mechanical ventilation via an endotracheal tube (ET) or tracheostomy tube [3] and it is the most common infectious complication in critically ill patients [4]. VAP prevalence varies between 9-65% [5, 6], mortality rates are high (15-76%), ICU- and hospital-length of stay are increased (by 5-7 ICU days, respectively by 2-3 folds), with significantly increased costs per patient [6, 7].

ENDOTRACHEAL TUBE AND RISK OF VENTILATOR-ASSOCIATED PNEUMONIA

Although mechanical ventilation is a life-saving procedure, the use of endotracheal intubation has its risks.

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The ET provides an ideal opportunity for bacterial and fungal adhesion and biofilm formation on both its inner luminal and outer surface [8]. Being recognized as an independent risk factor for pulmonary infection in intubated patients [9], ET increases this risk by 6-10 times [10].

While necessary to facilitate mechanical ventilation, the ET results in several host-device interactions described to be involved in VAP pathogenesis. ET has direct effects, which impair host's local defense mechanisms: it keeps the epiglottis open altering the cough reflex and muco-ciliary clearance; it modifies the phenotype of trachea-bronchial cells promoting the bacterial binding; low airway tract inoculation with the endogenous oropharyngeal flora and airway injury during intubation can also create bacterial binding sites; the ET surface is a nest for bacterial biofilm formation [7, 11, 12]. In addition, it was proven that the accumulation of contaminated secretions from the oropharynx or gastro-intestinal tract in the subglottic space above the inflated ET cuff is a source of microaspiration leading to ET and airway colonization and VAP [3].

It is also presumed that apart from interfering with respiratory tract defense, the ET presence causes an imbalance in the lung microbiome [13]. The use of culture-independent techniques proves that bacteria exist in normal individuals even in the lower airway tract and this microbial population is termed lung microbiota [14]. In never-smokers these bacterial communities are very few and composed of different types of bacteria [15]. The lung microbiota is a biological defense barrier of the respiratory tract. Changes of its composition promote pathogen invasion and occurrence and progression of lung infections and acute exacerbation of chronic diseases [16]. Such changes are enhanced in intubated patients [13].

■ BIOFILMS – DEFINITION AND FORMATION

80% of human bacterial infections are biofilm-related [17]. It is known that ET acts as a reservoir for infecting microorganisms. Soon after intubation a mixed biofilm harboring microbial pathogens is formed on the ET [18], especially lining in the interior of the tube distal third [3]. In 1967 Redman and Lockey were the first to demonstrate ET bacterial colonization by culturing the distal end of the ET and in 1986 Sottile *et al.* were the first to use scanning microscopy to demon-

strate biofilm presence on the inner surface of polyvinylchloride ETs [19, 20].

Microorganisms can exist as planktonic organism – individual cells freely suspended in a liquid medium, or as a sessile community into biofilms [biofilm]. A microbial biofilm is a three-dimensional structured aggregate of microbial cells surrounded by a self-produced polymer matrix, which protects it from the hostile environment [21]. The matrix-enclosed bacterial populations are adherent to each other and to inert or living surfaces [11]. Consequently, the hosted microorganisms can survive in a dormant state in these protected communities [22]. These metabolically inactive persister cells are a small part of the biofilm, which survive in this state because of a slowed down metabolism, where they are less sensitive to the effects of antimicrobials [23]. Biofilm formation is a dynamic process. There are several described stages of biofilm formation: adhesion stage, aggregation stage, maturation stage, mature biofilm stage and dispersion stage [23]. After attachment bacteria have to mature into a differentiated biofilm and they secrete signaling molecules to determine if there are enough bacteria to initiate the expression of a particular phenotype (“quorum sensing”). Then the sessile forms of the bacteria coating the biofilm can give rise to planktonic bacteria, which may also generate biofilm and disperse into the environment [11].

■ ENDOTRACHEAL TUBE SHELTERED BIOFILM

The ET is rapidly, within hours after insertion, colonized by microorganisms that form a biofilm on its surface [24]. The sequence of colonization with pathogenic bacteria in mechanically ventilated patients was firstly reported by Feldman *et al.*: the oropharynx (within 36 hours), the stomach (within 36-60 hours), the lower respiratory tract (within 60-84 hours) and thereafter the ET (within 60-96 hours) [25]. Yan *et al.* assessed by scanning electron microscopy patchy biofilms on the ET surface after 2-7 days of ventilation initiation, 87.5% of ETs being covered by biofilms after 7-10 days. Day 10 was the breakpoint when all the ETs housed biofilms on their surfaces [26].

In fact, ET colonization occurs much earlier and the capacity to demonstrate this fact depends on the assessment methods. Perkins *et al.* showed ET colonization by quantitative polymerase chain reaction (PCR) even after 24 hours of intubation [27]. Gil-Perotin *et al.* showed the same by electron microscopy and culture [12].

New data show that ET biofilm contains multiple bacterial populations. Usually the composition of ET biofilms is often misrepresented due to the fact that the gold standard assessment method is the traditional culture analysis. 99% of bacteria in the natural environment cannot be cultured [8]. Taking into account that ET biofilm flora may originate in the oral cavity it is important to know that 50% of the oral microflora is considered to be unculturable – difficult to culture or have not yet been cultured [28]. Culture identification results are at best available 48 hours after sampling and the interpretation is often difficult. Also, some loss of microbial viability between the time of patient's ET removal and it's processing for culture is likely to be responsible for the incomplete biofilm characterization [7]. Vandecandelaere *et al.* suggest that using combined surveillance cultures (throat swab, nose swab and sputum samples) may increase the sensitivity but decrease specificity to identify ET biofilm flora [18]. Ferreira *et al.* compare tracheal aspiration culture versus ET originated biofilm germ identification through sonication technique in 27 pediatric patients, proving the benefit of the later technique [10].

In time numerous culture-independent approaches became available in order to analyze these microbial communities [7], which usually consist of a wide variety of bacteria [9]. Meligy *et al.* identifies biofilms in 20 ET mechanically ventilated patients by biofilm electronic microscopy and culture analysis [29]. Pan *et al.* uses 16S ribosomal RNA gene polymerase chain reaction, denaturing gradient gel electrophoresis (DGGE), cloning and sequencing in 15 pediatric VAP to compare throat swabs and tracheal aspirates versus ET biofilm samples [30]. Cairns *et al.* also uses DGGE and PCR in order to characterize microbial biofilms on the inner lumen of 24 extubated ETs from ICU patients [7]. All of them conclude that a combination of culture and different molecular methods should be used to obtain a complete picture of the bacterial diversity of ET biofilms.

■ ENDOTRACHEAL TUBE BIOFILMS AND VENTILATOR-ASSOCIATED PNEUMONIA

Several mechanisms are responsible for ET biofilm involvement in VAP pathogenesis: biofilm pieces dispersed and passively moved towards the lung, biofilm cells aerosolized and aspirated into the lungs due to gas flow during artificial ventilation and individual cells

can be dislodged by liquids and transferred deep into the lungs [24, 30].

VAP diagnosis has its limits due to definition and currently available diagnostic methods. Its etiology is poly-microbial with a considerable inter-patient and intra-patient diversity [7, 31], and unfortunately, often the causative agent is not known at the time of VAP suspicion [18]. The differentiation colonization versus infection is very important [32], but also very difficult. It is the clinician's responsibility to decide if the ET biofilm flora is either the primary source of infection or a concomitant colonization site [10].

The multidrug resistant ESKAPE pathogens (*Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*) play a dominant role in VAP etiology, and these organisms were frequently identified in ET biofilms. Members of the normal oral flora were also identified but considered to initiate ET biofilm formation and not to be directly involved in VAP development [33]. It is supposed that an indicator of VAP is the enrichment of pathogen strains in the biofilm [27] and a change in the prevalence of detectable organisms identified by molecular methods [8].

Adair *et al.* reported that 70% of VAP patients have identical pathogens present in the ET biofilm and in the lung, suggesting that the biofilm represents a significant and persistent source of pathogenic bacteria [7]. Bardes *et al.* found using 16S ribosomal RNA gene analysis a maximum 87 different bacterial species on 20 ET biofilms with a mean number of 16±9 identified species [9]. This huge variety is greater in smokers, but similar in patients with or without pneumonia [9].

Despite the early presence of ET biofilm and despite its bacterial diversity, VAP occurs later. Perkins *et al.* demonstrated that longer intubation period increases the opportunity for the potentially pathogenic bacteria to proliferate [27] and De Souza *et al.* showed that an intubation period of more than 8 days represents a risk factor for developing VAP [34]. Wilson *et al.* prove that advanced biofilm stage (maturation or mature stage) is associated with pneumonia, while duration of intubation, patient age and hospital stay are not related and cannot predict the biofilm stage [35]. Bardes *et al.* and Cairns *et al.* also showed that there was no rela-

tionship between duration of intubation and number of identified bacterial species [7, 9]. Researchers even demonstrate that ET biofilm might always be present in intubated patients, whatever the duration of intubation and cannot be removed by rinsing due to strong adhesion [36].

It seems that VAP might be more related to the ET presence than to the mechanical ventilation *per se*. In 2009 Pneumatikos *et al.* suggested the replacement of the term „ventilator-associated pneumonia” with the term “endotracheal tube-associated pneumonia” in order to better describe its pathogenesis [3].

■ ANTIBIOTIC RESISTANCE OF BIOFILMS

Biofilms represent a persistent source of organisms causing recurrent infections [11, 25]. Moreover, it was proven that these microorganisms exhibit significantly greater antibiotic resistance than their tracheal counterparts [37]. There are three mechanisms described to be responsible of decreased antibiotic susceptibility: the biofilm resides in an air-filled lumen with no host defense mechanisms, it protects the sessile forms so that the antibiotic cannot penetrate or deactivate them due to impaired diffusion and also offers slow growing or dormant state to the sessile bacteria [11].

Due to the temporal biofilm dynamics VAP development within the first 2-5 days after intubation is more likely to be caused by antibiotic-sensitive bacteria as methicillin-sensitive *Staphylococcus aureus*, with a better prognosis, later occurring VAP (5 or more days after initiation of mechanical ventilation) involving frequently multidrug resistant pathogen like methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and extended spectrum β -lactamase producing Enterobacteriaceae, with higher morbidity and mortality [24].

In subjects who experienced a successfully treated episode of VAP, the responsible bacteria were still present in the biofilm [36, 25]. Gordon Sahuquillo *et al.* even observed on a small sample of patients, that neither the use of systemic nor inhaled antibiotics influenced the persistence of variable and potentially infectious microorganisms in ET biofilm after VAP [4]. This resistance frequently implies the necessity of device withdrawal in order to achieve clinical and microbiological cure [7], but usually the elective ET change during mechanical ventilation is not recommended [18, 38].

■ PREVENTION AND TREATMENT OF BIOFILM FORMATION

Due to the high prevalence of ET biofilm in mechanically ventilated patients and the microbial dynamic link between airway colonization, biofilm formation and VAP development, biofilm-directed interventions seem to be a high priority [12]. In order to prevent ET biofilm formation, knowledge of the source of involved microorganisms is important [7].

Respiratory pathogens are not usually found in the oral microbiota of healthy people, but hospitalized patients are susceptible to oral biofilm colonization by these microorganisms [39]. Improved oral hygiene has been proven to be an effective strategy of VAP prevention, taking into consideration that normal oral microflora may represent pioneering colonizing species and potential respiratory pathogen may be isolated from dental plaque's biofilm [7, 39]. Chemical control of oral pathogens seems to be more effective than mechanical removal [39].

Biofilm formation prevention on the ET surface can be also achieved by the use of specific antiseptic (silver-coated, chlorhexidine, gentidine) or antibiotic (sulfadiazine) impregnated ET. Care must be taken to the fact that, although long-term use of antimicrobial-impregnated central venous catheters has shown no selection of bacterial resistance, biofilm formation has been associated with antibiotic-resistant pathogen and lack of antimicrobial penetrability into ET biofilm [40].

Another mechanical removal technique of ET biofilm is the mucus shaver. It consists of an inflatable silicone rubber (introduced into the ET in a deflated and extracted in an inflated status), which extracts the accumulated material on its inner surface. Published studies report encouraging results and it is recommended by VAP prevention guidelines [3, 38].

One recently described non-invasive treatment method is the photodynamic therapy. In vitro models, which used spraying of small amounts of a photosensitizer solution into the ET lumen followed by light exposure, resulted in reduced number of biofilm microorganisms after a single treatment [41].

There are a lot of guidelines recommended strategies for VAP prevention. Some of them avoid or diminish the ET biofilm consequences. One recommended prevention strategy is to avoid the use of ET if possible. The use of noninvasive ventilation can decrease the tracheal intubation rates and even mortality, the avail-

able evidence suggesting a clear benefit in terms of a lower VAP risk [3]. Early extubation policies should be a standard procedure and implemented in all the hospitals. Some researchers suggest that a solution to overcoming the disadvantages of ET long-term use can be early tracheostomy, which significantly reduces the duration of mechanical ventilation and length of ICU stay. But biofilms can also form on the surface of tracheostomy tubes, even though the cleaning and maintenance methods are easier, with several disinfecting solutions available [42].

■ CONCLUSIONS

Ventilator-associated pneumonia (VAP) is a common and major nosocomial infection in mechanically ventilated patients. Two major mechanisms involved in airway colonization lead to VAP development: microaspiration and ET biofilm formation. Oral and ET biofilms play a major role in lung infection development, promotion of treatment resistance and infection recurrence in mechanically ventilated patients. As a result, biofilm formation control, either on the ET or in the oropharyngeal cavity is an important strategy for VAP prophylaxis. Will the term “ET-associated pneumonia” soon be transformed into “biofilm-associated pneumonia”? Further studies on larger populations need to be conducted to better characterize the biofilm-associated microorganisms, their origins, the timeline of biofilm formation and prevention strategies.

■ CONFLICT OF INTEREST

None to declare.

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