Journal of Critical Care 37 (2017) 30–37

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.jccjournal.org

Respiratory pathogen colonization of dental plaque, the lower airways, and endotracheal tube biofilms during mechanical ventilation

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ARTICLE INFO

Available online xxxx
Keywords:
Mechanical ventilation
Staphylococcus aureus
Pseudomonas aeruginosa
Endotracheal tube
Endotracheal tube
Ventilator-associated pneumonia

ABSTRACT

Purpose: In mechanically ventilated patients, the endotracheal tube is an essential interface between the patient and ventilator, but inadvertently, it also facilitates the development of ventilator-associated pneumonia (VAP) by subverting pulmonary host defenses. A number of investigations suggest that bacteria colonizing the oral cavity may be important in the etiology of VAP. The present study evaluated microbial changes that occurred in dental plaque and lower airways of 107 critically ill mechanically ventilated patients.
Materials and Methods: Dental plaque and lower airways fluid was collected during the course of mechanical ventilation, with additional samples of dental plaque obtained during the entirety of patients' hospital stay.
Results: A "microbial shift" occurred in dental plaque, with colonization by potential VAP pathogens, namely, *Staphylococcus aureus* and *Pseudomonas aeruginosa* in 35 patients. Post-extubation analyses revealed that 70% and 55% of patients whose dental plaque included *S aureus* and *P aeruginosa*, respectively, reverted back to having a predominantly normal oral microbiota. Respiratory pathogens were also isolated from the lower airways and within the endotracheal tube biofilms.
Conclusions: To the best of our knowledge, this is the largest study to date exploring oral microbial changes during both mechanical ventilation and after recovery from critical illness. Based on these findings, it was apparent that during mechanical ventilation, dental plaque represents a source of potential VAP pathogens.
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1. Introduction

In mechanically ventilated patients, the endotracheal tube (ETT) is an essential interface between the lungs and the ventilator. Unfortunately, the presence of an ETT also impairs pulmonary host defenses and promotes ventilator-associated pneumonia (VAP) through supporting biofilm formation within its inner lumen [1,2]. In addition, the ETT and incomplete mouth closing will alter the oral microenvironment. Ventilator-associated pneumonia is the most common nosocomial infection in critical care with a prevalence of approximately 15%, and prognosis is negatively influenced with involvement of multidrug resistant pathogen containing biofilms [3,4]. The ETT biofilm may serve as a reservoir of respiratory pathogens that are largely protected from host defense mechanisms. In recent years, studies into the origin of VAP causing microorganisms have primarily focused on oropharyngeal sites rather than the gastrointestinal tract [5-8]. As a consequence, a number of recent strategies aimed at preventing VAP have sought to target the oral microbiome [9,10].

Dental plaque was initially considered to be a bacterial construct (*i.e.* a biofilm) in the 1970s [11]. Dental plaque harbors an estimated 500 different bacterial species, with variation in microbial composition occurring between teeth [12]. *Streptococcus* species are recognized as primary pioneer colonizers of teeth and are initiators of dental plaque development [13]. Lazarevic et al [14] analyzed the oral microbiome using molecular methods and reported that up to 70% of sequences belonged to bacteria of the *Streptococcus* and *Neisseria* genera. Saliva plays an important role in modulating dental plaque formation [15,16]. Glycoproteins and proline-rich proteins in saliva will adsorb to tooth surfaces generating an enamel pellicle allowing bacteria to adhere [17].

The oral microbiome could promote VAP in several ways [18]. First, during mechanical ventilation, rapid colonization by potential

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http://dx.doi.org/10.1016/j.jcrc.2016.07.019
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respiratory pathogens including *Pseudomonas, Klebsiella, Staphylococcus aureus,* and *Acinetobacter* can occur and these bacteria may subsequently disseminate to the lung [8,19,20]. Second, commensal oral bacteria may actively promote respiratory pathogen colonization of the ETT lumen, and these bacteria may again translocate to the lower airways leading to VAP [1,21]. Biofilm-mediated infections are difficult to treat, as not only are the cells protected within the biofilm structure, but the microorganisms involved are also frequently inherently less susceptible to antimicrobial agents [22-24].

Colonization of dental plaque by respiratory pathogens is important in VAP etiology, and it is also known that for most critically ill patients, oral hygiene frequently deteriorates during mechanical ventilation [25,26]. Furthermore, not all oral hygiene interventions appear effective at reducing VAP incidence [27,28], and a recent meta-analysis even suggested that accepted oral hygiene treatments, such as use of chlorhexidine, may actually lead to harmful effects [29].

To deliver effective oral care to critically ill patients to reduce VAP, it is important to increase our understanding of the dynamics of the oral microbiome during mechanical ventilation and how this relates to contamination of both the ETT and lower airway. The current study examined the nature of microbial changes in dental plaque and the lower airway, during mechanical ventilation and, in contrast to previous studies, during patient recovery after mechanical ventilation.

2. Materials and methods

2.1. Methods

2.1.1. Patient recruitment

Ethical approval was obtained from the National Research Ethics Service within the Research Ethics Committee for Wales (Ref.: 13/WA/0039). In order for sufficient statistical power (>80%) to observe a 20% change in at least one phylum in microbial profiles and associated downstream high-throughput techniques [30], the minimum number of participants required for the study was 101. Mechanically ventilated patients were eligible for inclusion in the study if they were older than 18 years, they had more than 8 original teeth, their anticipated period of mechanical ventilation was longer than 24 hours, and their expected survival was more than 24 hours. Informed consent for participation in the study was obtained from the next of kin and also taken from the patients if they recovered capacity.

Within 6 hours of critical care admission, a critical care mouth plan was completed to determine the level and frequency of oral care required. Oral care included toothbrushing 4 times a day with sterile water, and moistening of the oral cavity and lips. Antiseptic mouthwashes were not used. Additional care was provided for denture wearers. Ventilator-associated pneumonia was diagnosed using the existing Clinical Pulmonary Infection Score calculated using parameters of temperature, white blood cell count, partial pressure of arterial oxygen/fraction of inspired oxygen ratio, the presence of tracheal secretions, and changes on chest radiograph. Quantitative microbiological culture (>10^3 CFU/mL) of the lower airways by bronchoalveolar lavage [BAL]/nondirected bronchoalveolar lavage (NBL) was undertaken if the Clinical Pulmonary Infection Score was higher than 6. Bronchoalveolar lavage was performed bronchoscopically by an attending clinician, whereas NBLs were undertaken by the bedside nurse inserting a suction catheter through the catheter mount into the lung parenchyma and flushing and withdrawing sterile saline [32-36].

2.1.2. Decayed, missing, and filled teeth score

The decayed, missing, and filled teeth (DMFT) score was recorded (by a dental professional). The DMFT score is a measurement of dental caries status and is therefore an indicator of prior longer-term oral hygiene levels [31]. Each incidence of a tooth recorded as decayed, missing, or filled, results in a score of 1 to generate a score of between 0 to 28, with higher scores representing poor oral health. The DMFT scores, although not reflective of the remaining dentition, may indicate prior differences in oral hygiene maintenance within the patient cohort and therefore the level of risk for dental plaque changes.

2.1.3. Dental plaque collection

Subgingival and supragingival plaque was collected using paper points (Quality Endodontic Distributors; QED; Peterborough, UK; size 40) and dental examination kits (Minerva Dental Ltd, Cardiff, UK) [37]. Collection was performed on 3 occasions during the first week of admission to critical care and then weekly. The fist sample was collected within 24 hours of the start of mechanical ventilation. A total of 9 paper points, sampling 3 teeth per area (front, middle, and back), were used per collection. In cases where the patient did not have sufficient teeth for the above protocol, plaque was taken from the closest areas. Plaque specimens were suspended in transport medium [38] and processed using microbial culture on the day of collection.

2.1.4. Collection of subglottic aspirations, NBLs, BALs, and ETTs

Subglottic secretions were collected through a subglottic port in the ETT using a syringe and transferred into sterile universals. Subglottic aspirates and NBLs were aseptically transferred into universal containers, and ETTs were collected and placed in sterile bags when available for transport to the microbiology laboratory.

2.1.5. Identification of respiratory pathogens

Clinical specimens were processed within a Class II safety cabinet. Dental plaque was vortex-mixed and spread-plated onto appropriate selective agar media for detection of *S aureus* (MSA; mannitol salt agar) and *Pseudomonas aeruginosa* (Pseudomonas agar base; Lab M Ltd., Heywood, Bury), which are prevalent VAP pathogens [39-41]. Agar media were incubated at 37°C under aerobic conditions for 5 days.

NBLs and BALs were centrifuged for 3 minutes at 10 000*g*, and the pellet resuspended in 1 mL of phosphate-buffered saline. A 50-μL volume was spread-plated on the previously described selective agar. Growth of *P aeruginosa* and *S aureus* was recorded, and colonies of presumptive respiratory pathogens identified by biochemical testing. *S. aureus* colonies were subcultured on mannitol salt agar for 18 to 24 hours at 37°C and tested for catalase and coagulase activity [42]. Colonies of *P. aeruginosa* were subcultured on *Pseudomonas* agar for 18 to 24 hours at 37°C and tested for oxidase activity.

Definitive identification of *S. aureus* and *P. aeruginosa* was by species-specific polymerase chain reaction (PCR; Table 1) [43,44]. DNA extraction used a commercially available DNA extraction kit (Qiagen Ltd., Manchester M15 6SH, United Kingdom). Polymerase chain reaction was performed in a total reaction volume of 50 μL containing 2 μL of DNA template. Thermal cycling parameters for *S. aureus* detection were an initial 5 minutes at 94°C, followed by 35 cycles of 94°C for 40 seconds, 50°C for 40 seconds, and 72°C for 1 minute, with a final elongation step of 72°C for 10 minutes. For *P. aeruginosa* PCR, there was an initial denaturation step of 95°C followed by 35 cycles of 94°C for 45 seconds, 58.4°C for 45 seconds, and 72°C for 1 minute, ending with 5 minutes at 72°C.

2.1.6. Antimicrobial susceptibility profiling

S. aureus and *P. aeruginosa* were cultured on Mueller Hinton agar at 37°C for 18 to 24 hours. A 0.5 McFarland standard (10^8 cells/mL) was prepared to create an inoculum for antimicrobial sensitivity testing. A sterile swab was used to homogenously inoculate the 0.5 McFarland standard across the agar. Cefoxitin disks were used to identify methicillin-resistant *S. aureus* (MRSA), whereas sensitivity profiles of *P. aeruginosa* and *S. aureus* were tested against 6 to 12 antimicrobials disks (selected based on previous administration to patients and according to frequent antibiotics used for these microorganisms). Agars were incubated for 18 to 24 hours at 37°C and subsequent zones of inhibition (ZOI) measured, and categorization of isolate susceptibility (sensitive, resistant, and intermediate resistant) was done according to British Society for Antimicrobial Chemotherapy guidelines. For

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