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Microbial investigation of biofilms recovered from endotracheal tubes using sonication in intensive care unit pediatric patients

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ABSTRACT

Objectives: To compare cultured microorganisms identified on endotracheal tubes biofilms through sonication technique with traditional tracheal aspirate collected at extubation of pediatric intensive care unit patients.
Methods: Demographic and epidemiological data were analyzed to identify factors possibly related with the microbiological profile of the two collection methods. Associations between categorical and continuous variables were analyzed using the chi-square or Fisher's exact test, or Student's *t* test. *p*-Value <0.05 were considered significant.
Results: Thirty endotracheal tubes and tracheal aspirates samples from 27 subjects were analyzed. Only one patient presented the clinical diagnosis of ventilator-associated pneumonia. Overall, 50% of bacteria were Gram-negative bacilli, followed by Gram-positive bacteria in 37%, and fungi in 10%. No statistically significant difference on the distribution of Gram-positive or Gram-negative bacteria ($p = 0.996$), and fungi ($p = 0.985$) were observed between the collection methods. *Pseudomonas* spp. was the most frequent microorganism identified (23.8%), followed by *Streptococcus* spp. (18.5%), *Acinetobacter* spp. (15.9%), coagulase-negative staphylococci (11.2%), and *Klebsiella* spp. (8.6%). Concordant results between methods amounted to 83.3%. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* showed carbapenem resistance in 50% and 43.7% of the isolates, respectively. In general, cultures after endotracheal tubes sonication (non-centrifuged sonication fluid and centrifuged sonication fluid) yielded bacteria with higher rates of antimicrobial resistance compared to tracheal aspirates cultures. Additionally, in 12 subjects (40%), we observed discrepancies regarding microbiologic profiles of cultures performed using the collection methods.

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Conclusions: Our study demonstrated that sonication technique can be applied to ET biofilms to identify microorganisms attached to their surface with a great variety of species identified. However, we did not find significant differences in comparison with the traditional tracheal aspirate culture approach.

Introduction

Nosocomial pneumonia represents approximately one quarter of all nosocomial infections and tracheal intubation increases the risk for infection by six to 20 times higher.[1,2] Among patients under mechanic ventilation on intensive care unit (ICU), ventilator-associated pneumonia (VAP) represents the most frequent type of nosocomial infection, which extends the length of hospitalization, increase mortality and costs of ICU treatment.[1] The clinical diagnosis of VAP has been based upon local and systemic signs and symptoms of infection in addition to radiological criteria.[1,2] Furthermore, bacteriological diagnosis using protected specimen brush (PSB), bronchoalveolar lavage (BAL), and protected endotracheal aspirates have been fully standardized but lack specificity, as it relies upon the identification of microorganisms growing in tracheal secretion.[1–4] Nevertheless, bacteria growing in biofilms attached to the endotracheal tube may also play a role in the pathogenesis of VAP.[5,6] Indeed, the knowledge of biofilm formation by microorganisms in a series of medical devices and its association to difficult-to-treat infections has become a concerning in the nosocomial setting.[7] Therefore, the VAP represents a clear example of multifactorial infection in which the biofilm may be implicated not only on the pathogenesis, but equally on the high prevalence of multiresistant bacteria.[8–12] The endotracheal tube (ET) allows direct entry of microorganisms from the oropharynx environment into the lower respiratory tract, whereas the innate immune system components are ineffective.[12–14] Thus, ET allows microorganisms presenting natural ability to form biofilm to attach on the tube surface through production of a matrix of polysaccharides and proteins that provides protection and consequent survival in the environment.[10] According to previous publications, bacteria within the biofilm can infect the lungs by several ways: through detachment of biofilm portions, thus reaching the lungs and by aspiration into deeper airways of aerosolized planktonic pathogens detached from the biofilm.[13,14]

Recently, microbial diagnosis of implant-associated infections has been incremented by using vortexing and sonication technique applied to dislodge sessile microorganisms attached to a variety of medical implants, by disrupting the polymer matrix on the surface of the biofilm while maintaining the integrity of the pathogen.[15–17] Trampuz et al., Inacio et al., and Vandecandelaere et al. were some of the authors whose studies applied sonication for identifying pathogens from biofilms attached to different human implants.[15,17–20] Despite the lack of data, ET sonication may be an interesting tool to increase the yield of detection of sessile pathogens and its possible effect on the pathogenesis of VAP.

Therefore, we aimed to compare the yield of cultured microorganisms collected by ET centrifuged and non-centrifuged sonication fluid with microorganisms collected by conventional endotracheal aspiration of intubated pediatric ICU patients.

Materials and methods

Study population

We performed a pilot microbiological cross-sectional study comparing the microbial colonization of ET submitted to sonication with the microbial yield of cultures of collected tracheal aspirates at the time of elective extubation. A total of 27 pediatric ICU patients under mechanical ventilation, from December 2012 to June 2014 at the Santa Casa de São Paulo School of Medicine (Brazil) were evaluated. The collection of tracheal aspirates was performed prior to extubation as part of the ICU physiotherapist routine in order to prevent bronchoaspirations. Subjects were excluded when clinical data were unavailable for analysis, when no tracheal aspirates were collected during extubation, or when contamination of ET occurred during extubation, transportation, or processing in the microbiology laboratory. As a cross-sectional study, study participants were not followed-up. The Research Ethics Committee at our institution approved the project and waiver of obtaining an informed consent, as the investigators have neither interfered in patient management nor in the indication of extubation.

Diagnosis of VAP

The diagnosis criteria for VAP were based on the CDC guidelines, in which patients on mechanical ventilation develop pneumonia 48 h after intubation.[21] The diagnostic triad consisted of clinical evidence of pulmonary infection, including fever, purulent secretions, leukocytosis or leukopenia, signs of respiratory distress and worsening gas exchange; radiologic suggestion of pulmonary infection, such as persistence of lung infiltrate, consolidations and cavitations; and microbial evidence of pulmonary infection.[21]

Collection of samples and sonication method

Prior to extubation, the ICU physiotherapy team routinely collects tracheal secretions to prevent bronchoaspiration in pediatric patients. This collected secretion, that otherwise would be discarded, was then used for the microbial analyses of the study. After extubation, the distal 10 cm of the ET were