A study on the contributions of sonication to the identification of bacteria associated with intubation cannula biofilm and the risk of ventilator-associated pneumonia

Ioana Codru (ioanacodru.ic@gmail.com)
Lucian Blaga University

Mihai Sava
Lucian Blaga University

Bogdan Ioan Vintilă
Lucian Blaga University

Alina Simona Bereanu
Lucian Blaga University

Victoria Bîrluțiu
Lucian Blaga University

Study protocol

Keywords: ventilator-associated pneumonia, biofilm, sonication

Posted Date: April 24th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2736125/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Abstract

Ventilator-associated pneumonia is one of the most severe complications of critically ill patients that need mechanical respiratory support, as it poses a significant risk of prolonging hospitalization, disability, and even death. This is why physicians worldwide target newer methods for prevention, early diagnosis, and early target treatment for this condition. There are few methods for a quick etiological diagnosis of pneumonia, especially point of care, and most are only readily available in some intensive care units. This is why a new, simple, and cheap method is needed for determining the bacteria that might be infectious in a particular patient. The method in question is sonication.

Method: In this prospective, observational, single-center study, endotracheal cannula specimens will be collected from a minimum of 100 patients in our intensive care unit. This specimen will be submitted to a specific sonication protocol for bacteria to dislodge the biofilm inside the cannula. The resulting liquid will be seeded on growth media, and then a comparison will be made between the germs in the biofilm and the ones in the tracheal secretion of the patient. The primary purpose is to determine the bacteria before the appearance of a manifest infection.

Introduction

The most prevalent infection in the intensive care unit (ICU) is the hospital–acquired pneumonia (HAP) (1, 10). This group of conditions encompasses two different entities: ventilator-associated pneumonia (VAP) and high-severity pneumonia developed during hospitalization. VAP is a type of pneumonia that develops in mechanically ventilated patients. The reported incidence of VAP is extensive, ranging from 1.9–3.8 per 1000 days of mechanical ventilation in the USA to more than 18 per 1000 days in Europe (1). The incidence of this type of pneumonia in Romania is probably even higher, but retrospective or prospective multicentric studies have not quantified its prevalence (2).

VAP occurs after 48 hours of mechanical ventilation. The onset relative to hospital admission discriminates early pneumonia (under five days) from late pneumonia (over five days) (3, 12).

VAP diagnosis is made on clinical, paraclinical, and radiological criteria. For the radiological criterion to be met, two successive chest radiographs showing new or progressive lung infiltrates or a single chest radiograph without a medical history of underlying heart or lung disease are required. It should be accompanied by at least one of the following: new onset fever (without any other cause) or changes in the leukocytes number (≤ 4000/mm$^3$ or ≥ 12000/mm$^3$) and at least two of the following signs: purulent sputum, (cough, dyspnoea – for HAP), declining oxygenation, increased oxygen requirements.

The most common causes of VAP are bacteria, but viruses are increasingly recognized. Fungal pathogens are uncommon except for immunocompromised patients (5) and patients treated with wide-spectrum or long-term antibiotic treatments. The frequency of different pathogens can vary according to geographic region and according to hospital and hospital clinical activity. The bacteria most frequently isolated from patients with VAP are aerobic gram-negative bacteria (Klebsiella pneumoniae,
Acinetobacter species, Pseudomonas aeruginosa, Enterobacter spp, Stenotrophomonas maltophilia, Serratia marcescens) in more than 60% of the cases (6, 7, 10) and gram-positive cocci (Staphylococcus aureus, particularly MRSA). The rates of polymicrobial infections vary even more and are usually associated with aspiration, and the incidence is higher in adults with acute respiratory distress syndrome (ARDS) (8). The viral etiology of hospital-acquired pneumonia appears most commonly seasonal, including influenza, parainfluenza, adenovirus, and respiratory syncytial virus (8). Sars-Cov2 has become one of the most prevalent viruses to be isolated in hospitalized patients in the last four years.

The complications associated with ventilation-associated pneumonia are multiple. Prolonged antibiotic therapy and mechanical ventilation increase the risk for serial colonization and reinfection with pathogens: recurrent pneumonia or Clostridium difficile colitis (5). A possible risk is the cardiac decompensation triggered by the combination of hypoxemia and increased metabolic demands due to infection. It can manifest by acute ischemia, exacerbation of heart failure, and new onset arrhythmias (9).

According to a pooled analysis of randomized studies, attributable mortality from ventilator-associated pneumonia is estimated to be about 10% (10). About one-third of HAP develop in ICU, with Ventilator Acquired Pneumonia (VAP) accounting for 90% of cases. Ventilator-Associated Pneumonia occurs in 9–40% of intubated patients, representing the most frequent ICU-acquired infection (11). The 28-day mortality rate for hospital-acquired pneumonia is around 30% among patients admitted to the ICU (12).

Along with the increased morbidity and mortality associated with VAP, this kind of healthcare-associated infection is of concern as it poses a substantial economic burden. The Society of Healthcare Epidemiology of America (SHEA) conducted a matched cohort study of the Premier database. It evaluated the impact of VAP on the length of stay (LOS) in the hospital and ICU, duration of mechanical ventilation, and hospital costs. Regarding expenses, SHEA demonstrated an increase in hospitalization costs by almost 40% (13).

Currently, few measures try to prevent ventilator-associated pneumonia and even fewer cheap and accessible means of early diagnosis of this condition. Early diagnosis is essential as it prompts targeted antibiotic therapy and a faster resolution of pneumonia.

**Methods**

The present study is a prospective, observational, single-center study. It aims to isolate the bacteria in the biofilms of the tracheal cannulas of mechanically ventilated patients. The purpose of bacterial isolation is not only to compare with the bacterial load from tracheobronchial secretion but also to determine the optimal replacement time of the endotracheal cannulas in patients with prolonged mechanical ventilation.

It is estimated that more than 100 mechanically ventilated patients will be enrolled in the study over 12 to 18 months. The study will be located in the intensive care unit of Sibiu County Clinical Emergency Hospital, Romania. This ICU is a level I intensive care as it can assist a large variety of pathology: medical
and surgical (general, thoracic and vascular surgery, neurosurgery, ENT, urology, trauma, burns). It is organized on two floors with 12 single-bed rooms on each floor and 4 double-bed rooms for post-anesthesia care / intermediary care.

This study will not affect the patient's treatment plan and will be performed according to the attending physician's indications. The endotracheal cannulas will be replaced if the patient's clinical assessment allows this maneuver without endangering the well-being of the ill.

**Aims Of The Study:**

- Detection of the bacteria in the biofilm formed inside the endotracheal cannulae
- Detection of the mean period during which the biofilm organizes in the lumen of the cannulae
- Determination of the optimal time to replace the intubation cannulae in patients that need mechanical ventilation for more than 48 hours so that the contamination of the lower respiratory airways by dislodging the biofilm following suction manoeuvres is prevented.
- Comparison of the microorganisms from the patient's respiratory secretions with the bacterial load in the biofilm to initiate an early targeted antibiotherapy. Furthermore, it should be determined whether a clean cannula with a sterile biofilm can prevent the onset of VAP.
- Development of new methods to prevent VAP.
- **Final aim**: decreasing the morbidity and mortality of critically ill patients by preventing or early and targeted treating VAP, reducing the LOS in the ICU and in the hospital with a secondary lowering of costs associated with hospitalization.

**Population Description:**

Patients admitted to our ICU, aged between 18 and 100 years old, are mechanically ventilated for more than 48 hours. Among the causes of respiratory failure are pulmonary infections (bacterial, viral, or fungal pneumonia, bronchopneumonia, COPD exacerbations due to respiratory tract infections), aggravated lung diseases (severe asthma attack, status asthmaticus), or extrapulmonary factors (polytrauma after traffic accidents, falls, neurological or neurosurgical patients with deteriorated consciousness and alterations of the airway reflexes).

Inclusion criteria:

- Age between 18 and 100 years old
- Romanian citizenship
- Patients or legal representatives are informed and consent in writing to be part of the study
- Patients that require more than 48 hours of mechanical respiratory support

Exclusion criteria:
- Under-aged patients
- Patients who do not consent or the consent could not be obtained
- The non-compliant collection of biological samples in patients
- Clinically unstable patients, so the samples could not be collected.
- Less than 48 hours of mechanical ventilation
- Non-Romanian nationality

Eligible patients for the proposed research topic will be divided into two groups according to the cause of respiratory failure:

- Group 1 – respiratory failure due to pulmonary infection – confirmed bacterial pneumonia, bacterial bronchopneumonia, or with a high degree of clinical and biological suspicion (purulent tracheal secretions, paraclinical or imaging investigations highly suggestive of respiratory infection)
- Group 2 – respiratory failure secondary to non-infectious pulmonary conditions (severe asthma attack, pulmonary fibrosis) or extrapulmonary causes that need mechanical ventilation (polytrauma secondary to traffic accidents, falls, neurological or neurosurgical patients, patients with deteriorated consciousness that need airway protection)

Data Collected:

After admission to the ICU, a quick but rigorous clinical exam is performed to stabilize or correct the issues that can immediately endanger the patient’s life, according to the ABC rule (airway, breathing, circulation). If the patient is already mechanically ventilated, the patency of the tube is checked, and specific ventilatory parameters will be set to ensure adequate ventilation and respiration of the critically ill. If the patient breathes spontaneously but needs respiratory support, orotracheal intubation will be performed, and mechanical ventilation will be initiated. The same measures are taken in the case of the patient that is already in the ICU but has deteriorated.

Data:

- Age, gender, BMI
- Length of stay in hospital
- Length of stay in ICU
- Number of days of stay in ICU before intubation
- Outcome
- Mortality at 28 days
Diagnostic criteria for ICU admission

Cause of the respiratory failure

Associated conditions

Sickness severity scores (SOFA, APACHE II)

Pneumonia severity scores (PSI / Pneumonia severity index; SMART-COP Score; CURB-65)

Blood work (including inflammatory panel and blood gas analysis)

Monitoring curves (blood pressure, heart rate, oxygenation, ventilation, diuresis, GFR, creatinine clearance)

Antibiotherapy

Newly onset organ dysfunction/worsening of a preexisting organ dysfunction/organ failure

**Microbiological Specimens – The Timing Of The Sampling, Specimen Manipulation, Sonication Protocol**

- $T_0$: tracheal aspirate collection in the first 2 hours after the patient's admission to the intensive care unit or after tracheal intubation and initiation of the invasive respiratory support
- Collection of a second tracheal aspirate 48–72 hours after $T_0$
- Replacement of the endotracheal cannula at 48–72 hours from $T_0$ and collection of a cannula specimen. The specimen will be sonicated according to an established protocol for bacterial sonication, and the sonication fluid will be inoculated onto bacterial culture media.
- Collection of a third tracheal aspirate at 168–192 hours from $T_0$ if the patient requires prolonged mechanical ventilation or required reintubation less than 24 hours from the time of extubation
- Change of the endotracheal cannula at 168–192 hours from $T_0$ and the collection of the cannula specimen, sonication, and seeding of the fluid onto growth media.

Sonication protocol: Orotracheal intubation cannula specimens will be sonicated for 30 minutes using an ultrasonic bath (BactoSonic14.2, Bandelin GmbH, Berlin, Germany) at a frequency of 42kHz with a power of 0.22W/cm². The resulting sonication liquid is then homogenized, and 5–10 ml is centrifuged for 5 minutes at 2500 rpm. The resulting precipitate will be inoculated onto culture media and incubated at 37 degrees to inspect them for bacterial growth.

**Ethics and personal data protection**

This study has obtained ethics approvals from the ethical committee of the County Clinical Emergency Hospital of Sibiu and the Lucian Blaga University of Sibiu. To be enrolled in the study, the patient or the
legal representative must sign an informed consent. At any point, the consent can be withdrawn without impacting the patient’s treatment and care.

According to Romanian law, the information collected for this study will be confidential, and patient data will not be published. Access to personal data is provided only to the research team involved in the study. However, the control committees will be granted access to the initial patients’ data to verify compliance with the study procedures. However, they will not be allowed to make the data public.

**Discussions**

Since critically ill patient represents an enormous challenge no matter the admission diagnosis, the primary aim of the healthcare systems is to decrease the mortality and morbidity of the patients and shorten the length of stay in intensive care units. By achieving these targets, the total costs of care will be significantly lower. More than other complications, VAP can extend the length of ICU stay and pose the patient with a significant risk of death. Many studies focus on preventing VAP, early diagnosis, and targeted treatment.

At this point, though, there is no prophylactic measure that is 100% efficient, and the rapid diagnostic methods (PCR methods for bacterial isolation) are not readily available in all intensive care units. This is the main reason for using sonication as a new prevention and early diagnosis method. Sonication is cheap, and there is no need for highly trained personnel or expensive reactants. This kind of method was used in clinical practice sparingly. Dentistry is the main branch that uses sonication to determine the bacteria in the biofilms of dental prostheses. In the last years, though, steps in using sonication were made in orthopedics, when this method was used to isolate bacteria from infected hip or knee prostheses. Because the traditional methods could not isolate any microorganism, the biofilm was dislodged with the help of the ultrasounds, and a rare bacteria were isolated: Ralstonia Piketty. The patient with the hip infection with R. Piketty could be treated with targeted antibiotics after the bacteria responsible for the infection was finally isolated (14).

As with any diagnostic method, sonication has its limitations. First, a cannula specimen is needed to have the sonication fluid. If the patient is not stable enough (there are difficulties in maintaining proper oxygenation or the cardiovascular system needs increasing vasoactive or positive inotrope support), the tracheal cannula cannot be changed in the proposed timeline without endangering the ill. In this scenario, the cannula will stay on the spot, and the patient will be excluded from the study.

Another area for improvement regarding this study is the impossibility of isolating certain species of bacteria. Bacteria that require special growth media can be included in this category: atypical bacteria, mycobacteria, and anaerobic bacteria.

The proposed number of patients to be included in the study is at least 100. This figure might not be achieved if the number of intubated and ventilated patients in our ICU is lower than the number proposed...
or if other exclusion criteria are met on the way (withdrawn consent, less the 48 hours of mechanical ventilation).

**Abbreviations**


**Declarations**

**Ethics and personal data protection. Consent to participate**

This study has obtained ethics approvals from the ethical committee of the County Clinical Emergency Hospital of Sibiu (no. 320/09.01.2020) and the Lucian Blaga University of Sibiu (no. 7125/11.12.2019). Both documents are available for verification at any time. To be enrolled in the study, the patient or the legal representative must sign an informed consent. At any point, the consent can be withdrawn without impacting the patient’s treatment and care.

I can confidently assure you that all procedures will be carried out in strict accordance with relevant guidelines, such as the Declaration of Helsinki, to ensure that the highest ethical standards are maintained throughout the process.

**Consent for publication**

the legal representative will sign an informed consent form before collecting biological material. The current study will not change any patient’s clinical and therapeutical approach, and the lack of signed informed consent represents an exclusion criterion. The consent form in the native language is readily available and can be offered to the editorial board at any time.

**Availability of data and materials**

all data generated and analyzed during this study are included in this published article.

**Competing interest**

there is no conflict of interest to declare by any of the authors.

**Funding**
No funding was received by the authors.

Authors’ contribution


Acknowledgments

not applicable

References

2. ECDC SURVEILLANCE REPORT Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011–2012
4. Leone, Marc; Bouadma, Lila; Bouhemad, Bélaïd; Brissaud, Olivier; Dauger, Stéphane; Gibot, Sébastien; Hraiech, Sami; Jung, Boris; Kipnis, Eric; Launey, Yoann; Luyt, Charles-Edouard; Margetis, Dimitri; Michel, Fabrice; Mokart, Djamel; Montravers, Philippe; Monsel, Antoine; Nseir, Saad; Pugin, Jérôme; Roquilly, Antoine; Velly, Lionel; Zahar, Jean-Ralph; Bruyère, Rémi; Chanques, Gérald. Hospital-acquired pneumonia in ICU. Société française d’anesthésie et de réanimation (Sfar). Published January 31, 2018. Volume 37, Issue 1. Pages 83-98.
5. Hospital-Acquired and Ventilator-Associated Pneumonias Elsevier Point of Care. It was updated June 22, 2022.


13. Marin H. Kollef, MD; Cindy W. Hamilton, PharmD; Frank R. Ernst, PharmD, MS: Economic Impact of Ventilator-Associated Pneumonia in a Large Matched Cohort. Infection control and hospital epidemiology march 2012, vol. 33, no. 3