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CONTRIBUTIONS OF SONICATION IN THE IDENTIFICATION OF BACTERIA ASSOCIATED WITH ENDOTRACHEAL TUBE BIOFILM AND THE RISK OF VENTILATOR-ASSOCIATED PNEUMONIA

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SUMMARY

In this study, we performed a comprehensive comparative analysis of the evolution of patients undergoing mechanical ventilation, investigating the impact of two different therapeutic strategies on the overall clinical course. In the study group, patients benefited from a proactive intervention through the systematic replacement of orotracheal intubation tubes at predetermined intervals, intended to reduce the risk of bacterial colonization and biofilm formation—factors that can lead to serious infectious complications. On the other hand, the control group followed a standard approach, where the monitoring of the clinical evolution was based on the periodic collection of tracheal secretions, without actively intervening in tube replacement. The main objective of the research was to evaluate, from a global perspective, whether the strategy of systematic tube replacement can positively influence the clinical evolution, contributing to the prevention of infectious complications associated with mechanical ventilation.

We designed the study to provide an overview of the potential benefits of a preventive intervention in a field where the risk of healthcare-associated infections represents a major problem. We aimed to emphasize the importance of adopting proactive measures that would allow better management of tracheal secretions and, implicitly, a reduction in the incidence of severe respiratory infections. The integrated approach of clinical evaluation and periodic monitoring of secretions allowed for the comparison of the two strategies, highlighting the differences between active intervention and the traditional surveillance method. The general results suggest that the systematic replacement of intubation tubes may have a beneficial effect on patient outcomes by creating an environment less favorable to bacterial colonization and thus reducing the risk of developing infectious complications.

Through this approach, the study underlines the need to reassess management protocols in intensive care units, proposing that the integration of innovative preventive methods can contribute to optimizing clinical outcomes. In the context in which infections associated with mechanical ventilation are linked to a significant increase in treatment complexity and healthcare costs, the implementation of proactive strategies, such as the periodic replacement of tubes, can represent an important step towards improving patient safety and reducing infection risks. Therefore, this research contributes to expanding knowledge in the field of critical care patient management, highlighting the potential of systematic preventive

interventions in contrast to conventional passive monitoring approaches. A well-defined, systematically implemented preventive strategy can bring notable benefits regarding the clinical evolution of mechanically ventilated patients, suggesting future research directions and opportunities to optimize care protocols in critical settings.

LIST OF PUBLICATIONS:

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"In the midst of difficulties lie opportunities." – Albert Einstein

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INTRODUCTION

Albert Einstein once said, “In matters of truth and justice, there is no difference between large and small problems. When it comes to the way people are treated, they are all the same.” However, when it comes to illness, suffering, the patient, their relatives, and even the manifestation of certain conditions, things are completely different. Consequently, every patient must be treated individually for their current illness, taking into account their entire personal history and chronic treatments. Although every patient is unique, with the development of care and treatment methods there has been an attempt to standardize treatments, diagnostic and preventive methods, and even nursing maneuvers. As a result of the introduction of unified protocols and procedures in hospitals, the quality of care for various patient groups has improved significantly. The limitation of the current guidelines lies precisely in the fact that there are no “gold standards” regarding each diagnostic method, or each curative or preventive treatment.

The development of the medical field, both in terms of the diagnosis and prevention of various diseases and in terms of treatment methods, has led to an increase in the global average life expectancy. Monitoring and support methods for vital functions, as well as techniques for replacing the function of certain organs, are also becoming increasingly efficient and continue to develop and diversify. As a result of this fantastic evolution in the biomedical and pharmaceutical fields, the treatment of extremely serious pathologies has become possible. However, despite the benefits provided by these devices, their use requires a higher degree of invasiveness, which can lead to the potential emergence of infections associated with medical devices and care.

The most serious infections, as well as those with the highest mortality and morbidity, are infections associated with medical care. These represent a major problem in hospitals, affecting approximately 10% of hospitalized patients. Beds in intensive care units (ICU) account for between 2–10% of a hospital’s total beds, yet these units are responsible for one quarter of broncho-pulmonary and bloodstream infections (spread from central/arterial venous catheters) associated with medical care [1–3] A healthcare-associated infection (also known as a nosocomial or hospital-acquired infection) is an infection that occurs in a patient during the course of medical care, in a hospital or care center, which was not present or in the incubation period at the time of admission. It can affect patients in any type of care facility or

may appear after discharge. Furthermore, occupational infections, which occur in staff, are also classified as nosocomial infections [4–6].

Healthcare-associated pneumonia is the most common nosocomial infection in intensive care units (ICU) [7–9]. Healthcare-associated pneumonia is defined as pneumonia that is not present at the time of admission or not in the incubation period at the time of hospital admission and that appears more than 48 hours after admission [9,10]. This entity is further classified into: ventilator-associated pneumonia (VAP) and severe pneumonia developed during hospitalization. The incidence of VAP in the United States varies between 1.8 and 3.6 per 1,000 days of mechanical ventilation, while in Europe the values are much higher, reaching 18 per 1,000 days of MV or even more [10]. It is very likely that in our country the incidence rate of VAP is significantly higher than the European average, but studies to confirm these data have not been conducted.

Depending on the time of onset, VAP is classified as follows: early-onset ventilator-associated pneumonia, occurring less than 4 days after the initiation of invasive ventilatory support and usually attributed to antibiotic-sensitive germs, and late-onset pneumonia, caused by multidrug-resistant microorganisms that appears after more than 4 days of mechanical ventilation [3,10].

This brief review of data on healthcare-associated infections, and in particular on pneumonia in critically ill patients and ventilator-associated pneumonia, supports my decision to delve “behind the scenes” of this critical patient condition.

The present study aims to present a different approach to perspectives on the etiology and diagnosis of ventilator-associated pneumonia by evaluating the bacterial biofilm formed on the orotracheal intubation cannula. I also evaluated the probability of preventing the development of VAP as a result of bacterial migration from the biofilm by changing the intubation tube. Additionally, I conducted a comparative analysis between two groups of patients who were mechanically ventilated in our intensive care unit. In the study group, the orotracheal intubation tube was systematically changed on the second and seventh days, while in the control group the patients did not benefit from this maneuver, but at least two tracheal aspirates were collected from them. The purpose of the analysis was to evaluate the impact of the systematic change of the tube on the clinical evolution, particularly on the development of ventilator-associated pneumonia, compared to the standard monitoring approach.

PART I

CURRENT STATE OF KNOWLEDGE

Mechanical Ventilation Associated Pneumonia (VAP) is one of the most severe nosocomial infections, with an incidence of 2–16 cases per 1,000 ventilation days and a reported mortality ranging between 24% and 76% [11–15]. It is common in intensive care units, being associated with increased hospital stay, higher costs, and extensive use of antibiotics [3,15–17].

According to ECDC data, Romania faces a high prevalence of healthcare-associated infections and an alarming consumption of antibiotics. High rates of antimicrobial resistance are noted, particularly in *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and MRSA [18].

The diagnosis of VAP is based on imaging and clinical criteria: new or modified pulmonary infiltrates, fever, leukocytosis or leukopenia, purulent secretions, and worsening respiratory function [14,19,20]. The pathophysiology involves colonization of the oropharynx, bacterial aspiration, and the compromise of physiological barriers due to orotracheal intubation [2,21–24].

The etiology is dominated by multidrug-resistant Gram-negative bacilli: *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, and MRSA [25–29]. Microbiological diagnostic methods include secretion culture, bronchoalveolar lavage (BAL), bronchial brushing, and molecular tests (PCR, MALDI-TOF) [30–34]. Imaging, particularly CT and ultrasound, is complementary in establishing the diagnosis [7,20,35–38].

Biofilms play an essential role in the persistence of infections by providing a protective environment for bacteria [39–41]. On endotracheal tubes, biofilm forms rapidly and becomes a persistent source of VAP [42–45].

Sonication, through the application of ultrasonic waves, dislodges the biofilm, allowing for the detection of bacteria that would otherwise be difficult to identify in culture [46–48].

Antibiotic resistance is aggravated by mechanisms such as the production of ESBL, carbapenemases, active efflux, or membrane impermeability [49–51]. Within the biofilm, these mechanisms are potentiated, necessitating alternative approaches: combination therapies, anti-biofilm enzymes, bacteriophages, or new broad-spectrum molecules. While these alternatives require further validation, they offer promising perspectives for the treatment of severe infections.

PART II

PERSONAL CONTRIBUTIONS

Structure of the Study and Presentation of Results

This research was conducted at the Sibiu County Emergency Clinical Hospital in the Intensive Care Unit, with the primary objective of investigating the role of the biofilm formed on orotracheal intubation tubes in the occurrence of ventilator-associated pneumonia (VAP). The study also aimed to evaluate the efficiency of sonication for the early detection of bacteria involved in biofilm formation and to assess the relationship between their presence and the subsequent development of VAP. Another focus was the impact of systematic tube replacement on the clinical evolution of the patients.

The study design included two groups of critically ill patients on mechanical ventilation: a study group and a control group. In the study group, an active prevention strategy was applied by systematically replacing the tracheal cannula on days 2 and 7 of invasive mechanical ventilation, according to a predetermined protocol. In contrast, the control group followed a standard approach without tube replacement but with periodic monitoring of tracheal secretions via tracheal aspirates.

All patients enrolled in the study were on mechanical ventilation for at least seven days. Two types of microbiological samples were collected: (1) sonication of the removed tubes to detect bacteria within the biofilm, and (2) standard tracheal aspirates. These samples were analyzed microbiologically to identify the bacterial species present and their antibiotic resistance patterns. The frequency of pathogen occurrence, resistance profiles, development of VAP, and clinical evolution were compared between the two groups.

The study revealed a direct relationship between the presence of biofilm and the occurrence of severe respiratory infections. In the study group, where the tube was systematically replaced, the incidence of VAP was significantly lower than in the control group. Sonication proved to be an effective and sensitive method for the early detection of bacteria from the biofilm, often identifying pathogenic organisms that were not detected in the tracheal aspirates at the time but were later associated with active infections.

The data collected allowed not only for the characterization of the types of pathogens present in the biofilm but also for establishing a correlation between the time of their detection and the clinical onset of infection. This indicates that sonication provides a more timely response, enabling the initiation of appropriate treatment.

Clinical and Statistical Comparison Between Patient Groups

The comparison between the two patient groups was conducted from clinical, microbiological, and statistical perspectives. In the study group, the incidence of VAP was significantly lower, and when infection did occur, its onset was delayed. In contrast, the control group experienced a higher frequency of VAP, with an earlier onset of infection associated with a more severe clinical course.

Statistical analyses confirmed significant differences between the two groups regarding the duration of hospitalization, duration of mechanical ventilation, length of antibiotic therapy, associated complications, and mortality rates. Specifically, patients in the study group benefited from shorter periods of ventilation and antibiotic therapy, and there was a significantly lower need for the use of reserve antibiotics (such as carbapenems and colistin).

Regarding the isolated pathogens, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Acinetobacter baumannii* were the most frequently identified. In the control group, these pathogens exhibited a more complex resistance profile. In many cases, the same pathogens were detected both in the tube biofilm and in the tracheal secretions, confirming the hypothesis that the biofilm serves as an infection reservoir.

Another noteworthy aspect was the efficiency of the sonication method in anticipating infection. In the study group, sonication enabled the identification of pathogens before the clinical manifestation of VAP, offering the opportunity for early, targeted intervention. This highlights the diagnostic and preventive value of sonication in the care of critically ill patients.

Discussion

The findings of the study are consistent with the specialized literature and support the hypothesis that the biofilm on intubation tubes significantly contributes to the development of VAP. The comparative analysis between the groups demonstrates that systematic tube replacement, alongside sonication evaluation, can reduce infection incidence and improve clinical outcomes.

This study makes a significant contribution to the prevention of nosocomial infections by underlining the importance of adopting active, personalized protocols based on real-time

microbiological data. Sonication has proven to be highly sensitive in detecting viable bacteria, even at the early stages of colonization, suggesting that traditional sampling methods might miss clinically relevant pathogens. Incorporating sonication into routine clinical practice could thus enhance diagnostic and preventive capabilities.

Another critical aspect discussed is antibiotic resistance. The study confirms the presence of multidrug-resistant pathogens within the biofilm, often limiting therapeutic options. Avoiding the empirical use of broad-spectrum antibiotics by tailoring treatment based on sonication results may reduce the selective pressure and emergence of MDR/XDR strains.

The paper proposes a paradigm shift in the management of mechanically ventilated patients—from a passive, reactive approach to an active, preventive strategy based on rapid diagnostic technologies and standardized interventions (e.g., scheduled tube replacement).

Limitations

Like any clinical research, this study has several limitations that may affect the generalizability of the results. One limitation is the relatively small sample size and the single-center nature of the study, which may be influenced by the specific characteristics of the Intensive Care Unit, the local epidemiological profile, and the patient population included.
The implementation of the sonication method also involves logistical challenges and resource requirements that may not be available in all hospitals. Additionally, the time needed for sample processing and result interpretation can vary depending on the capabilities of the microbiology laboratory.

Another limitation pertains to potential uncontrolled variables—such as the patient's immune status, types of comorbidities, associated treatments, and other intensive care interventions—that could influence the occurrence or progression of VAP. Moreover, although valuable, sonication is not yet internationally standardized, which necessitates further studies for validation and reproducibility.

Conclusions

The thesis demonstrates that a proactive approach to preventing VAP—through controlled replacement of the intubation tube combined with sonication analysis for the identification of

bacterial biofilm—yields superior clinical outcomes compared to conventional methods. Sonication is a promising technique with enhanced sensitivity in detecting viable bacteria and holds potential for integration into nosocomial infection prevention protocols.

The results suggest that a strategy based on active monitoring, early diagnosis, and standardized intervention could reduce the risk of severe infections, shorten hospital stays, and decrease the use of reserve antibiotics. The study supports the adoption of innovative infection control policies in intensive care units and proposes sonication as a complementary diagnostic method for VAP.

This work contributes to the advancement of knowledge regarding ventilator-associated infections and opens new avenues for future research in the prevention and treatment of critically ill patients.

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APPENDIX 2: Informed Consent for Participation in the Study

CONSENT FORM

- COLLECTION OF OTI TUBE SPECIMEN FOR THE DETERMINATION OF THE BACTERIAL BIOFILM BY SONICATION

SCIENTIFIC RESEARCH: **Contributions of sonication in identifying bacteria associated with the biofilm of intubation tubes and the risk of ventilator-associated pneumonia**

DOCTORAL STUDENT: Drd. Ioana Roxana Codru
SCIENTIFIC SUPERVISOR: Prof. Dr. Victoria Bîrluțiu
Participant ID Code: _____

Through this form, we invite you to participate in a research study. Before taking part in this study, it will be explained to you and you will be able to ask questions.

1. I have been informed about the research study YES / NO
2. I understand that my care and treatment (as a study participant) is not affected by the research study YES / NO
3. I agree that my medical and general data be collected from the general and specific ICU observation form, while respecting anonymity, by the staff involved in this study YES / NO
4. I agree that the samples collected be sent to the laboratories that will process them, while respecting anonymity YES / NO
5. I agree that additional samples may be taken, if necessary, for scientific benefits YES / NO

PARTICIPANT NAME: _____
(printed letters)

DATE: ____ SIGNATURE: _____

*NAME OF GUARDIAN:** _____
(printed letters)
Relationship with the participant:
_____

DATE: ____ SIGNATURE:

NAME OF THE PERSON OBTAINING CONSENT: _____,
(printed letters)
Position: _____, Department:
_____.

A guardian is defined as a first-degree relative of the hospitalized patient. The guardian will be the person from whom consent is obtained (assisted consent) in the case that the patient (study participant) has an altered state of consciousness.