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Microbiological, Immunological and Biochemical Characteristics of the Development of Ventilator Associated Pneumonia

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Abstract

The respiratory microbiome plays a critical role in metabolism, immune system maturation, and protection against pathogens. Traditionally, respiratory microbiology in pneumonia focused on identifying a specific pathogen, often disregarding normal oral flora as contaminants. However, recent research highlights the importance of the pulmonary microbiome's altered composition (dysbiosis) and its association with diseases such as Ventilator-Associated Pneumonia (VAP). The relationship between dysbiosis and immune dysregulation remains unclear, raising questions about whether dysbiosis is a cause or consequence of disease progression. This dataset explores the respiratory microbiome's role in nosocomial lower respiratory tract infections in ICU patients.

This prospective, observational dataset was built in an ICU in Chia, Colombia, focusing on adult patients requiring mechanical ventilation without initial pneumonia. Clinical data and biological samples, including bronchoalveolar lavage, orotracheal secretion, and blood, were collected at multiple time points. Microbiome analysis involved DNA extraction, gene sequencing of the 16S ribosomal unit, and bioinformatic analysis to characterize microbial diversity. Concurrently, pulmonary, and systemic inflammatory markers were quantified using ELISA.

The dataset enrolled 141 patients, generating a comprehensive dataset on demographic, clinical, and microbiome variables. This research not only provides insights into the microbial ecology of VAP but also establishes a repository of data for future studies. The findings underscore the complex interplay between the microbiome and disease in critically ill patients, with implications for improving clinical outcomes in the ICU setting.

Background

The microbiome is essential for metabolism, protection against pathogens, and maturation of the immune system [1]. Until recently, the purpose of studying microorganisms in pneumonia was the identification of a microorganism that could be attributed the role of "pathogen". The identification of microorganisms from the normal oral flora in samples from the lower respiratory tract was considered contaminating and was not taken into account [2].

Today, however, new insights into the role of the pulmonary microbiome are being discovered [3]. For example, altered microbial composition (dysbiosis, decreased biodiversity) is known to correlate with several diseases [4], but the host-microbiome interaction in the context of immune maturation and disease development is unknown, as it is not known exactly whether dysbiosis is a cause or a consequence of immune dysregulation, giving initiation or progression to disease (such as Ventilator-Associated Pneumonia (VAP)) [5]. Other studies suggest that colonization of the oral cavity may be important in the etiology of VAP [6]. Ventilator-associated pneumonia (VAP) is a significant and frequent infection occurring in patients who have been on mechanical ventilation for more than 48 hours. VAP is associated with increased morbidity, mortality, length of hospital stay, and healthcare costs. The pathogenesis of VAP is multifactorial, involving the colonization of the aerodigestive

tract with pathogenic bacteria, which are subsequently aspirated into the lower respiratory tract. Early diagnosis and appropriate antimicrobial therapy are critical for improving patient outcomes, as delayed or inadequate treatment can lead to poorer prognoses [7].

The dynamics of the complete microbial populations in the respiratory tract of intubated patients remains poorly understood and the respiratory tract microbiome has not been studied in depth, evidencing that the composition of the microbiota of patients with VAP is more complex, extensive, and diverse than originally expected [8].

Given the scarcity of data on this highly complex scenario, the data collected in this study will be instrumental in increasing our understanding and improving outcomes for Intensive Care Unit (ICU) patients with nosocomial lower respiratory tract infections (nLRTI) [9].

Methods

Type of study

A prospective, translational (T0-T2), observational dataset was designed, consisting of an initial phase of clinical observation followed by a phase of molecular analysis using human samples collected during the clinical phase. The selected sample included adult patients admitted to the ICU of two clinics in Chía, Colombia, who required invasive mechanical ventilation due to respiratory failure of non-infectious origin, without evidence of pneumonia at the time of admission, and who had approval to participate in the study. It is important to note that, due to the clinical condition of these patients, who were unable to provide informed consent, their relatives or legal representatives were responsible for determining their participation in the study.

In accordance with privacy protection protocols, all dates in the dataset (e.g., date_tracheostomy, intubation_date) have been anonymized; each date has been randomly shifted by a value greater than 365 days, ensuring a shift of at least one year to protect patient privacy. This approach effectively prevents the identification of specific time points while preserving the overall structure and temporal relationships of the data.

Population

The inclusion criteria for this study were: adult patients over 18 years of age; admitted to the ICU who were estimated to require mechanical ventilation for a minimum of 72 hours; who agreed to participate in the study and filled out the informed consent form. The exclusion criteria were patients with a diagnosis of pneumonia on admission to the ICU; patients referred from another institution under mechanical ventilation; patients under mechanical ventilation for more than 12 hours without being evaluated to enter the study; and patients with antibiotic treatment in the 7 days prior to hospitalization.

A convenience and consecutive sampling were performed, including patients who met the previously mentioned criteria. Demographic data, such as age, sex, initial clinical diagnosis, comorbidities, and antimicrobial therapies of patients were included. ICU admission clinical data and clinical outcomes were collected using the REDcap (Research Electronic Data Capture - Software created by Vanderbilt University) [10] data collection platform.

Case definition

Patients admitted to the ICU for mechanical ventilation must have the following criteria: a new and persistent radiographic pulmonary infiltrate plus at least 2 of the following criteria: a) temperature >38 °C or <36 °C; b) white blood cell count (WCC) >10 or <4 × 10^3 / mm^3; c) purulent tracheal aspirate. Patients diagnosed with VAP should have a new infiltrate on the day of diagnosis and the following days. The diagnostic approach and treatment of patients was performed according to the care guidelines of the participating hospitals and not by the study protocol. The thresholds used for the diagnosis of pneumonia will be 10^5 colony-forming units (cfu)/ml in the sputum culture and 10^4 cfu/ml in the mini-bronchoalveolar lavage (miniBAL) culture.

Procedures

During this study, different biological samples were collected, including: bronchoalveolar lavage (BAL), venous whole blood, rectal swab, orotracheal secretion, urine and nasopharyngeal swab. All samples were recollected per protocol in each patient admitted to the study at day zero (after starting mechanical ventilation), then at day 3 and 7 after intubation, and prior to extubating. In patients who developed pneumonia associated with invasive mechanical ventilation, an additional sample was taken at the time of diagnosis of VAP.

Sample Collection and Initial Processing of Samples

Orotracheal secretion, pharyngeal swab and miniBAL were collected according to protocol.

An equal volume of Sputasol (Thermo Fisher Scientific) was added to each sample and shaken for 15 minutes at 37 °C. Samples homogenized with Sputasol were stored (-80 °C) or mixed with two volumes of RNAlater (Sigma-Aldrich) for DNA extraction and microbiome analysis. Blood samples were collected in serum extraction tubes (Becton Dickinson) and centrifuged at 1300 gravities for 10 min at 18 °C to separate serum to be used for immunological studies; samples were then

stored at -80 °C for subsequent analyses. For rectal swab samples, they were immediately centrifuged at 2500 gravities for 20 minutes. The supernatant was removed and the sediment was resuspended with 0.2 mL of normal saline and stored at -80 °C until analysis. Urine was stored at -80 °C for subsequent analysis.

DNA extraction from bronchoalveolar lavage, orotracheal secretion and nasopharyngeal swab

Samples in RNAlater were centrifuged at 13000 revolutions per minute (rpm) for 10 minutes and the resulting sediments were resuspended in 500 µl of sterile phosphate buffered saline (PBS) (GE Lifesciences) and transferred to sterile bead mill tubes (VWR) containing sterile 1 mm glass beads (Sigma-Aldrich). Homogenization was performed using a VWR homogenizer and DNA was purified using the Roche High Purity PCR Template Preparation Kit (Roche) according to the manufacturer's instructions.

Lung microbiome/bronchoalveolar lavage/orotracheal secretion and nasopharyngeal swab

DNA was extracted using the DNeasy Blood & Tissue DNA Isolation kit [11]. The genetic material was then preserved. Once the sample collection stage was completed, characterization of the 16S ribosomal unit was performed by broad-spectrum gene sequencing and finally bioinformatic analysis was performed.

The purified products will then be visualized using Agilent Bioanalyser, prepared with an Agilent DNA1000 kit (Agilent Technologies). PCR product was made up to 50 µl and subjected to shearing using Adaptive Focused AcousticsTM (Covaris). DNA libraries were prepared using the Gene Read DNA Library I basic kit (Qiagen) according to the manufacturer's instructions. Fourteen cycles of enrichment PCR with index primers were performed according to a protocol adapted from the Multiplex Sample Preparation Oligonucleotide Kit (Illumina). Libraries were quantified with Agilent Bioanalyser, prepared with the Agilent DNA1000 kit (Agilent Technologies). Paired-end sequencing (2 × 101 bp reads) was performed on DNA libraries using the Illumina HiSeq2500 platform.

Inflammatory profiling

We quantified IL-1β, IL-6, and TNF-α cytokines in BALs. To enhance accuracy, 100 µL BAL was mixed with 100 µL sputolysin. The Milliplex® assay (Millipore Corp.) was used following the manufacturer's protocol (High Sensitivity Human Cytokine kit; Millipore Corp., St. Charles, MO, USA, HCYTA-60K).

Metabolomic profiles

BAL samples were centrifuged, and metabolites were extracted using methanol: chloroform. GC-TOF-MS analyzed plasma samples post-derivatization. An Agilent GC system coupled to a QTOF 7250 was used. Data processing included deconvolution, alignment, integration, and normalization. Metabolite identification utilized a metabolomics library. Supplementary materials provide details about sample preparation, analysis, and data processing.

Data Description

The following is a comprehensive list of variables contained in the database, each of which is further explained in detail in the "MicroNAV_Data_Dictionary.csv" document. This file provides a description of the contents and structure of each dataset, including an overview of key columns and their applications.

The database 'MicroNAV_Dataset.csv' was recorded in REDCap and contains patient clinical data, including demographic and clinical information. The dataset consists of 141 records that met the inclusion criteria, and includes a list of identification variables that were anonymized, along with hospital admission dates, COVID-19 diagnostic status, and patient outcomes, specifically whether they were discharged or deceased during hospitalization

Clinical data:

- Baseline
- 72-hour follow-up
- 5^{th} day follow-up
- 7^{th} day follow-up
- Ventilator associated pneumonia diagnosis
- Extubating and discharge
- Secondary infection

The database "MicroNAV_OTU.csv" contains clinical and microbiological data from the included patients, who had various types of samples (TS, BAL, NASAL) collected at different timepoints for analysis (Baseline, Follow-up). Additionally, it includes other variables related to their hospital stay and microbial profiles.

Microbiome (OTU):

- Specimen
- Taken samples
- Type of sample
- Diagnosis and tracheostomy status
- Hospital length of stay
- Mortality
- Identified OTU (Operational Taxonomic Unit): Operational Taxonomic Units (OTUs) are groups of similar 16S or 18S rRNA gene sequences, used to represent types of microorganisms in studies of microbial communities. By grouping sequences based on similarity, OTUs help researchers estimate and compare the diversity of microbes in different environments [12]. In this database, each row represents an Operational Taxonomic Unit (OTU) identified by a unique ID (e.g., Otu00001, Otu00002). These IDs correspond to clusters of similar microbial sequences, representing different microbial taxa.

This cohort includes a total of 141 patients, of whom 94 (66.7%) did not develop VAP, while 47 (33.3%) did. The median age was similar between groups, with 49.5 years in the non-VAP group and 50 years in the VAP group. Body mass index (BMI) was also comparable, with median values of 24.96 and 25.35, respectively. The proportion of males was slightly higher in the non-VAP group (69.1%) compared to the VAP group (59.6%), though this difference was not statistically significant.

Regarding follow-up assessments, the VAP group showed a higher percentage of adherence at 72 hours, five days, and seven days (97.9%, 95.7%, and 85.1%, respectively) compared to the non-VAP group (72.3%, 61.7%, and 42.6%), with these differences reaching statistical significance ($p < 0.001$). No significant differences were observed in ICU or hospital mortality between the groups. However, secondary infections were significantly more common in the VAP group (51.1%) than in the non-VAP group (20.2%; $p < 0.001$).

Usage Notes

The dataset acquisition pipeline was developed using REDCap software, and the data is provided in a CSV format, which makes it compatible with a variety of data analysis tools and software packages.

To date, the dataset has been primarily utilized for analyses within the local region. However, no publications have yet emerged from these analyses. Given the observational, translational, and prospective design, a repository of valuable data has been established for future investigations. The amassed dataset includes both initial clinical information and molecular analyses, thereby augmenting the caliber and breadth of data accessible for subsequent studies. It is important to acknowledge that this data collection's single-center nature may limit research aiming to encompass a broader population.

Please carefully review the annotation guidelines before using this dataset. Users interested in replicating or extending the analysis of the collected data are encouraged to consult the GitHub repository associated with this project [13]. The code used in the analysis is available in this repository, allowing researchers to review, modify, and utilize the techniques and methods employed in the study. To access the code, simply visit the link provided in the "GitHub Repository" section and explore the available files and scripts. If you have any questions or need more information on how to use the code, feel free to contact the project team.

Release Notes

1.0.0. Initial release of the database.

Ethics

Regarding the ethics considerations, this study is thought to have minimum risk given the sample collection process with the requirement of informed consent from every patient in order to be included in the study. The protocol was reviewed and approved by the IRB "Subcomisión de Investigación y Ética en Investigación sobre Calidad Científica e Integridad Ética" (Clínica Universidad de La Sabana's Ethics Committee) during IRB session Number 017 held on september 3rd, 2019.

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Conflicts of Interest

None of the authors have any financial, commercial, legal, or professional relationships with other organizations or individuals that could influence this research.

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