BMC Infectious Diseases

RESEARCH

Open Access

Lung and gut microbiota profiling in intensive care unit patients: a prospective pilot study

Antonios Kritikos[1,2,3], Eric Bernasconi[4†], Yangji Choi[1†], Valentin Scherz[1], Jean-Luc Pagani[5], Gilbert Greub[1,3], Claire Bertelli[1†] and Benoit Guery[3*†]

Abstract

Background The gut and lung microbiomes play crucial roles in host defense and mayserve as predictive markersfor clinical outcomes in critically ill patients. Despite this, the simultaneous dynamics of lung and gut microbiomes during critical illness remain unclear. This study aims to assess the longitudinal changes in lung and gut microbiota among mechanically ventilated ICU patients with and without infection and to identify microbial features predictive of clinical outcomes, including the development of ventilator associated pneumonia (VAP).

Methods In this prospective observational study, we analyzed 73 endotracheal aspirates (ETA) and 93 rectal swabs collected from 38 ICU patients over multiple timepoints (intubation, infection onset, post-antibiotic, and extubation/discharge). Patients were categorized into three groups: (1) VAP, (2) other infections, and (3) uninfected controls. Lung and gut microbiota were characterized using 16S rRNA gene sequencing. Primary outcomes included microbial diversity and community composition; secondary outcomes included ICU length of stay and ventilator-free days.

Results Alpha diversity declined more significantly in infected patients than in controls during the ICU stay, with the most pronounced changes in lung microbiota. We found an enrichment of *Enterobacteriaceae* and other *Proteobacteria* in the lung microbiome of pneumonia patients, while the gut microbiota remained relatively stable. Relative abundances of key taxa such as *Mogibacterium* were associated with mechanical ventilation duration.

Conclusions This study reveals that distinct microbial patterns in both lung and gut microbiota are associated with infection and clinical outcomes in critically ill patients. Understanding these dynamics is crucial for developing targeted microbiota interventions, potentially improving outcomes such as VAP prevention and management.

Trial registration Ethics Committee of Canton Vaud, Switzerland (2017–01820).

Keywords Gut microbiota, Lung microbiota, Gut-lung axis, Intensive care unit, Ventilation acquired pneumonia, Dysbiosis, Antibiotic

[†]Eric Bernasconi and Yangji Choi contributed equally to this work.

[†]Claire Bertelli and Benoit Guery contributed equally to this work.

*Correspondence:
Benoit Guery
benoit.guery@chuv.ch
[1]Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
[2]Service of Infectious Diseases, HFR Fribourg-Hôpital Cantonal, Fribourg, Switzerland
[3]Service of Infectious Diseases, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
[4]Department of Respiratory Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
[5]Intensive Care Unit, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

Background

Ventilator-associated pneumonia (VAP) is the most frequent hospital-acquired infection in the intensive care unit (ICU) with a reported incidence that can be as high as 40% [1, 2]. VAP is associated with increased morbidity, mortality and an important economic burden [3–5].

In the last decade, culture-independent microbiology allowed the characterization of complex and dynamic bacterial communities [4, 6] and thus rejected the very long-standing misconception regarding the "sterility" of human lungs. Understanding the lung microbiota gave rise to a new concept on VAP physiopathology, in which pathogens compete with endogenous microbiota and the immune system to colonize the lung niche [7]. In this scenario, VAP occurs because of a disruption of the lung microbiota homeostasis, a situation known as "lung dysbiosis" [7]. Recently published data show that lung microbiota profile on admission can predict outcome in ICU patients [8] and that changes in the composition of the lung microbiota have been associated with susceptibility to viral infections or the development of acute respiratory distress syndrome (ARDS) [9, 10]. More specifically, among patients with ARDS, the lung microbiome is enriched with gut-associated bacteria such as *Bacteroides* spp. and species of the *Enterobacteriaceae* family [10], and early enrichment of the lung microbiome with such gut-associated bacteria) is associated with subsequent development of ARDS [11]. While many observational studies have reported associations between the lung microbiota and alveolar inflammation [3, 10, 12, 13], the causal relationship between the lung microbiota diversity and composition with increased alveolar inflammation has only been described recently [14].

On the same time, gut microbiota plays a key role in the pathogenesis of sepsis and ARDS [15] as demonstrated by germ-free or antibiotic suppressed animals in experimental models of sepsis [16–18] and in numerous clinical trials [15, 18, 19]. The gut microbiome is a main enhancer of innate host immunity against infections by the production of antimicrobial peptides (bacteriocins) [20] and by the modulation of the adaptive response via regulation and differentiation of Th17 cells [21].

Despite the increasing body of evidence linking lung and gut microbiota to critical illness outcomes, a comprehensive understanding of the simultaneous dynamics of these two microbiota during severe illness, particularly in relation to infection, remains elusive. Previous studies have mainly focused on either lung or gut microbiota, but few have evaluated both compartments concurrently, especially over the course of a patient's ICU stay [10, 22]. Furthermore, the relationship between microbiota alterations and clinical outcomes, such as the development of VAP or prolonged mechanical ventilation, is not thoroughly studied.

The primary aim of this study is to investigate the longitudinal dynamics of both lung and gut microbiota in critically ill patients, comparing microbiota profiles in patients with VAP, those with other infections, and those without infection. We hypothesize that: (i) lung and gut microbiota diversity will differ between these groups; (ii) the resilience of the lung microbiota will differ from that of the gut microbiota during the ICU stay; and (iii) certain microbiota markers will be predictive of VAP and other clinical outcomes, such as the duration of mechanical ventilation.

To do this, we categorized patients into three distinct groups, those with VAP, those with other infections, and uninfected controls, to capture a broad spectrum of infection-related changes in microbiota composition. Patients with VAP were included to explore the relationship between lung dysbiosis and pneumonia, while those with non-pulmonary infections provided insight into how systemic infections may impact microbiota at distant sites. Uninfected control patients were included to serve as a baseline for comparison, ensuring that observed microbiota changes could be attributed to infection rather than other ICU-related factors.

Methods

Study population

This is a prospective single-center study of mechanically ventilated adult patients. The study was conducted in the 35-bed ICU of Lausanne University Hospital (CHUV) between August 2018 and November 2019. Hospitalized ICU patients were eligible for participation in our study provided they were (i) antibiotic-naïve on admission and (ii) intubated for less than 36 h but with an anticipated length of stay of more than 48 h. Exclusion criteria were: (1) age < 18 years old, (2) antibiotic treatment in the last 30 days, (3) immunosuppression (i.e., solid organ or stem cell transplantation, HIV positive status with detectable viral load, prednisone > 0.5 mg/kg, immunomodulatory treatment or recent chemotherapy) or (4) participation in another ongoing clinical study in the ICU. Patients who died within the first 24 h from inclusion were also excluded as well as those for whom we were not able to obtain a written informed consent. Informed consent was obtained from patients' next of kin and confirmed by the patients themselves when possible after extubation.

The included patients were categorized into three groups according to the occurrence of infections during their ICU stay: (1) **Pneumonia group**: Patients who developed ventilator-associated pneumonia (VAP), (2) **Other infection group**: Patients who developed infections other than VAP, and (3) **Control group**: Patients with no documented infections and no antibiotic treatment administered during their hospital stay. VAP was defined as a clinical suspicion of pneumonia

developing ≥ 48 h after endotracheal intubation as advocated by the presence of new or progressive pulmonary infiltrates on chest radiograph plus at least one of the following: (1) fever, (2) peripheral leukocytosis, (3) purulent tracheal secretions or (4) decline in oxygenation [23]. The "Other infection" group refers to patients who developed primary bacteremia or catheter-related bacteremia, urinary tract infection or nosocomial fever of unknown origin (table S1). Patients were allocated to the three groups based on the diagnosis retained by the ICU physicians. An infectious diseases specialist reviewed patients' charts and medical records to verify allocation based on the afore-mentioned definitions.

Clinical, laboratory and radiological data were collected from the electronic health record. We entered all data in an electronic clinical report form (eCRF) using the REDCap platform (Research Electronic Data Capture v8.5.24, Vanderbilt University, Tennessee, USA) [24].

This project was conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice and the Swiss Human Research Act (HRO). The project received approval from the Ethics Committee of Canton Vaud, Switzerland (2017–01820).

Study design and specimen collection

The ICU nurses in charge of each included patient collected ETA and rectal swabs for microbiota analysis. Initial samples were collected within 48 h of intubation and subsequent sampling was performed on the day of antibiotic introduction (for clinically-suspected or clinically-documented infection), 5 days after, and upon extubation and ICU discharge. ETA were collected using a standardized clinical protocol for VAP diagnosis. In short, a 50-cm, 14-French tracheal suction catheter was introduced through the orotracheal tube until resistance was encountered. The catheter was then pulled back 1–2 cm, the vacuum was released and the probe was delicately removed using turning movements from which the secretion was aspirated into a sterile polypropylene collector tube. The ETA sample was then transferred using a sterile procedure to the dedicated collection tube. Rectal swabs were inserted into the anal canal, beyond the anal verge (± 3 cm). Swabs were rotated gently and then removed.

ETA were stored in DNA/RNA Shield™ collection and lysis tubes (Zymo Research, Irvine, CA, USA) and rectal swabs were collected in DNA/RNA Shield™ collection tubes with swab (Zymo Research, Irvine, CA, USA) and stored immediately at -80 °C for further batched metagenomics analysis. Part of the ETA samples were sent to the microbiology laboratory for routine microbiological workup.

Microbial DNA extraction, library preparation and sequencing

Frozen native tracheal aspirates were thawed and a 10% solution of the reducing agent dithiothreitol (DTT) (AppliChem, Darmstad, Germany) was added (final concentration 1.7% DTT) in order to homogenise mucus. DNA was then extracted using the DNeasy UltraClean microbial kit (Qiagen, Valencia, CA, USA). DNA was extracted from thawed rectal swabs using the MagNA Pure automated platform (Roche, Basel, Switzerland).

Amplification of tracheal aspirates was performed using the AccuPrime Taq DNA Polymerase High Fidelity kit (Invitrogen, Carlsbad, CA, USA), using barcoded primers targeting the V1-V2 region. Amplicons were quantified using a LabChip GX instrument with the DNA 1 K kit (Perkin Elmer, Waltham, MA, USA), pooled in equimolar amounts and purified using the AMPure XP bead cleaning system (Beckman Coulter, Brea, CA, USA). For rectal swabs, the V3-V4 hypervariable region of the 16 S rRNA gene was amplified and libraries were prepared according to the 16 S Metagenomic Sequencing Library Preparation protocol (Part. # 15044223 Rev. B). Both ETA and rectal swabs samples were sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Positive controls (ATCC-2002 mock community) and negative controls for DNA extraction and library preparation (DNA free water) were included in each extraction run and each sequencing run for faecal samples. The DADA2-based pipeline zAMP (v.0.9.11) was used for our bioinformatics analyses as previously described [25, 26]. In brief, sequences were attributed to Amplicon Sequence Variants (ASVs) based on DADA2 (1.12.1) [27] and taxonomically assigned using the Ribosomal Database Project classifier [28] in Qiime 1.9.1 [29] against the EzBioCloud reference database (05.2018) [30]. Genera and species identified in our dataset that were described with spaceholders in the 05.2018 release were manually checked for updated taxonomical classification in the database of EzBioCloud, as available online in January 2025.

Rarefaction was done by random subsampling with 10,000 and 100,000 reads set as threshold for lung and faecal samples respectively, after manual examination of rarefactions curves to ensure sufficient sampling of relatively rare taxa for each sample type. Faecal samples with less than 100,000 reads after processing were resequenced once and samples sequenced twice were eventually pooled. Samples with less than 100,000 reads were excluded from further analyses. For ETA samples, we discarded samples with less than 10,000 reads. Sequencing yields for each sample type and rarefaction curves are shown on supplementary material (Figure S1).

Statistical analysis

All statistical analysis were performed in R (v.4.1.1) (https://www.r-project.org/). Shannon and Chao1 indexes were calculated with the Phyloseq R package. NMDS plots were generated using the "vegan" (http://www.cran.r-project.org/package-vegan/) and "phyloseqCompanion" (http://www.cran.r-project.org/package-phyloseqCompanion/) packages, and data separation was tested by a permutation test with pseudo-F ratio (function "Adonis" in "vegan"). For each NMDS plot, ellipses including 95% confidence area based on the standard error of the weighted average of sample coordinates were overlaid. *Betadisper* function was used to assess multivariate homogeneity of group dispersion analysis in beta-diversity analysis. Group differences in alpha diversity and taxon relative abundance were assessed by Wilcoxon or Kruskal-Wallis tests where appropriate. Venn diagrams were generated using the "VennDiagram" package (https://CRAN.R-project.org/package=VennDiagram). D ifferential abundance analysis was performed using negative binomial and zero-inflated mixed model ("nyiuab/NBZIMM" package) [31]. Correlation was assessed by linear regression and calculated by Spearman's coefficient (R). The correlation plot was produced using the "corrplot" and "PerformanceAnalytics" packages. Receiver operating characteristic (ROC) curves were assessed for microbiota features and the optimal cutoffs were calculated based on the best sensitivity and specificity ratios. Sensitivity, specificity, positive and negative predictive values were calculated for the optimal selected cutoff.

As for clinical data, categorical variables are presented as absolute numbers and relative frequencies whereas continuous variables are presented as mean and standard deviation if normally distributed or as median and IQR if non-normally distributed.

Results

Patients' characteristics

Among all prospectively screened patients, forty-four patients fulfilled the inclusion criteria. Six patients were secondarily excluded as shown in the study flowchart (Fig. 1). Among the remaining 38 patients, 9 did not develop any infection nor received any antibiotic treatment (control group), 17 developed VAP (pneumonia group) and 12 developed infections other than VAP (other infection group). In total, 73 ETA and 93 rectal swabs were collected for microbiome evaluation.

Baseline characteristics, summarized in Table 1, did not significantly differ between the three groups of patients besides the length of ICU stay, which was longer for infected patients ($p = 0.003$), and the length of intubation, longer in the pneumonia group ($p < 0.001$). Overall, comorbidities were observed in 58% of the patients. The global mortality at 28 and 90 days were 16% and 19% respectively with no differences between the groups. Table 2 shows the clinical characteristics and laboratory values of patients on admission. The mean APACHE II score was 22, 74% of the patients required an aminergic support, there was no difference between the 3 groups. Pneumonia patients were ventilated with

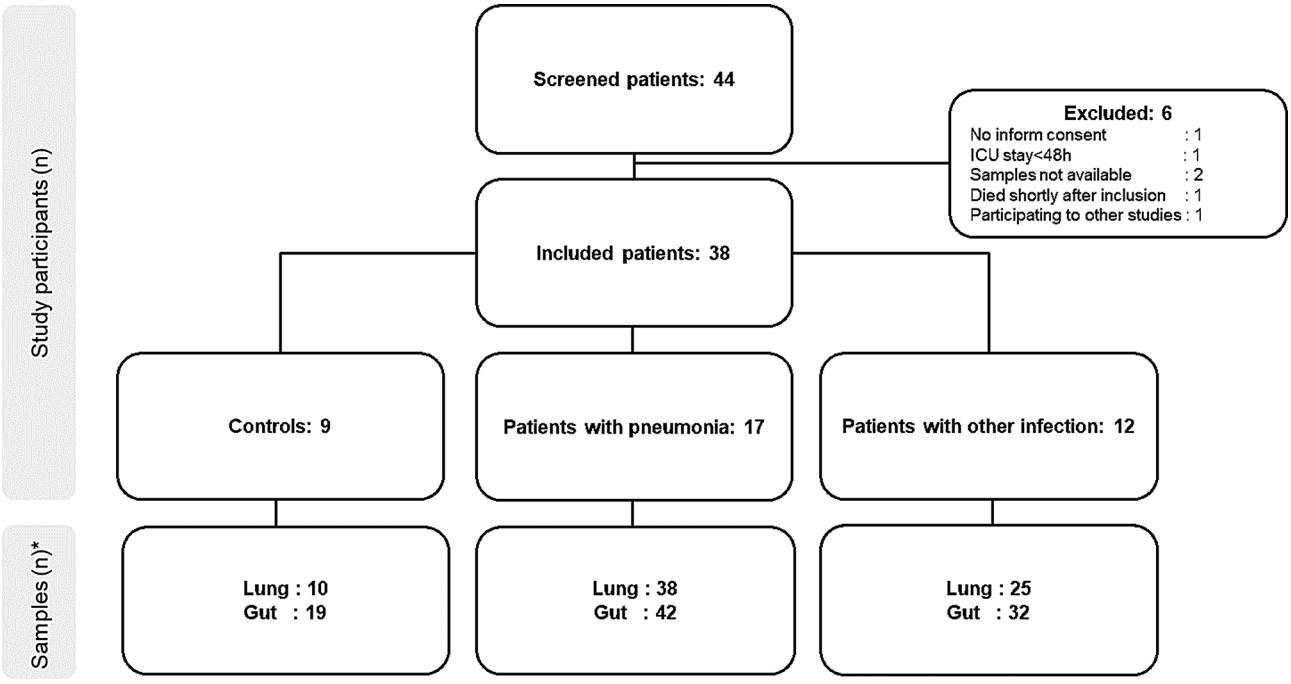

Fig. 1 Flowchart of study participants and taken samples. * Lung samples refer to endotracheal aspirates and gut samples refer to rectal swabs

Table 1 Patients' demographics

Variable	Overall *N* = 38	Study group			*p*-value
		Control *N* = 9	Pneumonia *N* = 17	Other Infection *N* = 12	
Sex					0.65
Male	21 (55%)	4 (44%)	9 (53%)	8 (67%)	
Female	17 (45%)	5 (56%)	8 (47%)	4 (33%)	
Age (years)	63 (51, 69)	68 (51, 76)	60 (49, 65)	65 (52, 68)	0.37
Ethnicity					0.75
Caucasian	28 (74%)	8 (89%)	11 (65%)	9 (75%)	
African	3 (7.9%)	1 (11%)	1 (5.9%)	1 (8.3%)	
Asian	1 (2.6%)	0 (0%)	1 (5.9%)	0 (0%)	
Hispanic	6 (16%)	0 (0%)	4 (24%)	2 (17%)	
Weight (kg)	71 (62, 81)	70 (55, 80)	72 (65, 80)	72 (70, 83)	0.68
Body Mass Index	24 (22, 28)	26 (24, 28)	24 (23, 27)	24 (22, 28)	0.80
Smoker	6 (16%)	1 (11%)	3 (18%)	2 (17%)	0.83
Presence of comorbidities	22 (58%)	5 (56%)	9 (53%)	8 (67%)	0.84
Myocardial infarction	6 (16%)	1 (11%)	4 (24%)	1 (8%)	0.53
Congestive heart failure	5 (13%)	1 (11%)	2 (12%)	2 (17%)	0.99
Peripheral vascular disease	10 (26%)	2 (22%)	4 (24%)	4 (33%)	0.8
Cerebrovascular accident	7 (18%)	1 (11%)	2 (12%)	4 (33%)	0.32
Asthma/COPD	5 (13%)	2 (22%)	1 (6%)	2 (17%)	0.49
Diabetes	5 (13%)	0	2 (12%)	3 (25%)	0.34
Moderate/Severe renal disease	2 (5.3%	1 (11%)	1 (6%)	0	0.71
Length of ICU stay (days)	10 (7, 17)	6 (5, 7)	11 (9, 22)	12 (7, 16)	**0.003**
Length of intubation (days)	7 (5, 12)	4 (2, 6)	10 (7, 16)	6 (6, 12)	**< 0.001**
28 days mortality	6 (16%)	2 (22%)	3 (18%)	1 (8.3%)	0.63
90 days mortality	7 (19%)	3 (33%)	3 (18%)	1 (8.3%)	0.41

Categorical variables are expressed in absolute numbers (%) and continuous variables with median (IQR) values.Comparison among groups were performed with Fisher's exact test or Kruskal-Wallis rank sum test where appropriate

higher positive airway pressure compared to the two other groups ($p = 0.01$). In the pneumonia group, *H. influenzae* was the first pathogen isolated (35%). The clinical characteristics of infectious episodes are shown on table S1. There is no statistically significant difference among both infected groups in terms of total number of received antibiotics or duration of antibiotic treatment. Figure S2 shows the administered antibiotic treatment and reports critical events related to sampling for microbiome evaluation. A monotherapy was proposed in most of the cases initially. The most frequently prescribed molecules were amoxicillin-clavulanate and piperacillin-tazobactam. Only 4 patients received penems.

Lung and gut microbiome alpha-diversity evolution over time

To evaluate alpha-diversity within lung and gut microbial communities among infected and non-infected patients, we first compared the three groups on inclusion. Alpha-diversity did not differ among the three groups on inclusion as expressed by Shannon or Chao1 diversity index for the lung ($p = 0.55$ and $p = 0.89$ for Shannon and Chao1, respectively) and gut microbiome ($p = 0.92$ and $p = 0.88$, respectively) (figure S3). Next, we examined if longitudinal alpha-diversity measurements differed over time within the three groups (Fig. 2). Alpha-diversity decreased over time in all faecal samples, except the control group. Noteworthy, microbial communities in the lung decreased significantly in terms of richness and evenness up to the 5th day of infection in both pneumonia ($p = 0.05$) and patients with other infections ($p = 0.02$) (Fig. 2).

Lung and gut microbiota stability and resilience over time

Beta-diversity analysis on inclusion revealed a rather homogenous microbial profile for the three groups (Fig. 3A and C). The three groups differed however significantly in the gut at discharge with Bray-Curtis dissimilarity index (Fig. 3C). Changes in beta-diversity were mainly driven by the "other infection" group (betadisper ns, adonis $p < 0.001$) that was dominated by the *Bacteroides* genus (Fig. 3D) and other taxa belonging to *Christensenellaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Veillonellaceae* families (depicted as "Other" genera in the graph). A constrained ordination method was subsequently performed to investigate whether the observed microbial variation could be explained by selected constraint variables (sex, body mass index (BMI), number

Table 2 Patients' clinical characteristics upon ICU admission

Variable	Overall *N* = 38	Study group			*p*-value
		Control *N* = 9	Pneumonia *N* = 17	Other Infection *N* = 12	
APACHE II score	22 (18, 28)	24 (23, 25)	20 (18, 25)	19 (17, 30)	0.17
Reason for ICU admission					
Non septic shock	13 (34%)	1 (11%)	7 (41%)	5 (42%)	0.08
Acute respiratory failure	2 (5.5%)	0	2 (12%)	0	0.49
Neurological failure	18 (47%)	6 (67%)	7 (41%)	5 (42%)	0.50
Multiorgan failure	2 (5.5%)	2 (22%)	0	0	0.05
Other	3 (8%)	0	1 (6%)	2 (16%)	0.44
Glascow Coma Scale	4 (3, 4)	4 (3, 4)	4 (3, 4)	4 (3, 4)	0.83
Temperature (°C)	36.4 (35.8, 36.8)	36.7 (36.1, 37.4)	36.5 (35.5, 37.1)	36 (35.8, 36.4)	0.12
Cardiac frequency (bpm)	81 (64, 98)	83 (78, 108)	80 (65, 99)	82 (63, 90)	0.74
Systolic blood pressure (mm Hg)	115 (101, 130)	119 (99, 143)	115 (107, 124)	116 (106, 136)	0.72
Diastolic blood pressure (mm Hg)	67 (53, 77)	67 (54, 75)	65 (52, 76)	73 (60, 80)	0.46
Aminergic support	28 (74%)	7 (78%)	12 (71%)	9 (75%)	> 0.99
Respiratory frequency (per minute)	16 (15, 18)	16 (15, 17)	16 (15, 20)	15 (14, 17)	0.21
FiO2 (%)	40 (31, 58)	35 (30, 40)	40 (40, 60)	50 (34, 60)	0.16
Partial O2 pressure (mm Hg)	85 (76, 138)	92 (81, 135)	83 (70, 144)	84 (73, 96)	0.64
C-reactive protein (mg/L)	24 (7, 69)	13 (6, 46)	26 (8, 70)	31 (7, 76)	0.67
Procalcitonin (mcg/L)	0 (0, 7)	0 (0, 0)	27 (15, 39)	1 (0, 8)	0.062
Lactic acid (mmol/L))	1.9 (1, 3.3)	1.9 (0.8, 3.1)	2.6 (1.3, 3.5)	1.6 (0.9, 2.3)	0.50
Creatinine (mcmol/L)	100 (66, 128)	85 (65, 115)	108 (66, 128)	104 (70, 147)	0.84
White blood cells (G/L)	14 (11, 18)	14 (10, 17)	14 (11, 18)	12 (11, 17)	0.70

Categorical variables are expressed in absolute numbers (%) and continuous variables with median (IQR) values.Comparison among groups were performed with Fisher's exact test or Kruskal-Wallis rank sum test where appropriate. APACHE II: Acute physiology and chronic health evaluation II score

of antibiotics received, days of antibiotic treatment and length of ICU stay). The redundancy analysis failed to explain the sample ordination with Bray-Curtis dissimilarity index, but a few constraint variables such as BMI, days of antibiotic treatment and length of ICU stay, contributed significantly ($p < 0.05$) to the variation when using the Jaccard dissimilarity index (Fig. 4).

The resilience of lung and gut microbiome over time was further investigated by studying ASVs cluster overlap across patients' subsequent samples (Fig. 5). The control group had more shared taxa between inclusion and extubation than infected patients for both lung and gut microbiome. The gut microbiome remained considerably more conserved in successive timepoints than the lung microbiome and both groups of infected patients showed similar trends of decreased shared taxa over time in both lungs and gut as compared to controls (Fig. 5). Interestingly though, a high proportion of shared taxa over time was observed in infected patients, potentially in line with a microbiota decompartmentalization observed in ventilated critically ill patients.

Taxonomic composition and identification of microbiota markers in VAP

After demonstration of heterogeneous lung and gut microbial communities in terms of alpha-, beta-diversity and shared taxa over time, we sought to identify the sources of this microbial profile heterogeneity and detect microbiota markers for early prediction of VAP. We performed a differential abundance analysis using a zero-inflated negative binomial (ZINB) regression model to delineate the community evolution over time. The lung microbiota of pneumonia patients was progressively enriched with *Proteobacteria* (*Neisseria* genus and members of the *Enterobacteriaceae*) as well as members of the *Firmicutes* phylum (*Oribacterium*, *Parvimonas*, *Dialister*) (figure S4). Patients with other infections were progressively enriched with *Bacteroidetes* (*Tannerella*) and *Firmicutes* (*Staphylococcus*, *Dialister*). In the control group, we observed an increase in *Fusobacteria* (*Fusobacterium*), *Firmicutes* (*Shuttleworthia*, *Oribacterium* and *Parvimonas*) and *Bacteroidetes* (*Capnocytophaga*, *Alloprevotella* and *Bergyella*) (figure S4). Regarding the gut microbiome, differences in taxa abundances were mainly driven by dynamic changes of the *Firmicutes* phylum. During the course of the infection and ICU stay, we observed a progressive replacement of taxa belonging to *Veillonellaceae*, *Enterococcaceae*, *Rumminococcaceae*, *Peptoniphilaceae* and *Lachnospiraceae* by other taxa belonging mainly to *Lachnospiraceae*, *Ruminococcaceae* and *Christensenellaceae* (figure S4). Patients with other infections or non-infected patients had moreover a progressive decrease of *Proteobacteria* and *Bacteroidetes* phyla (figure S4).

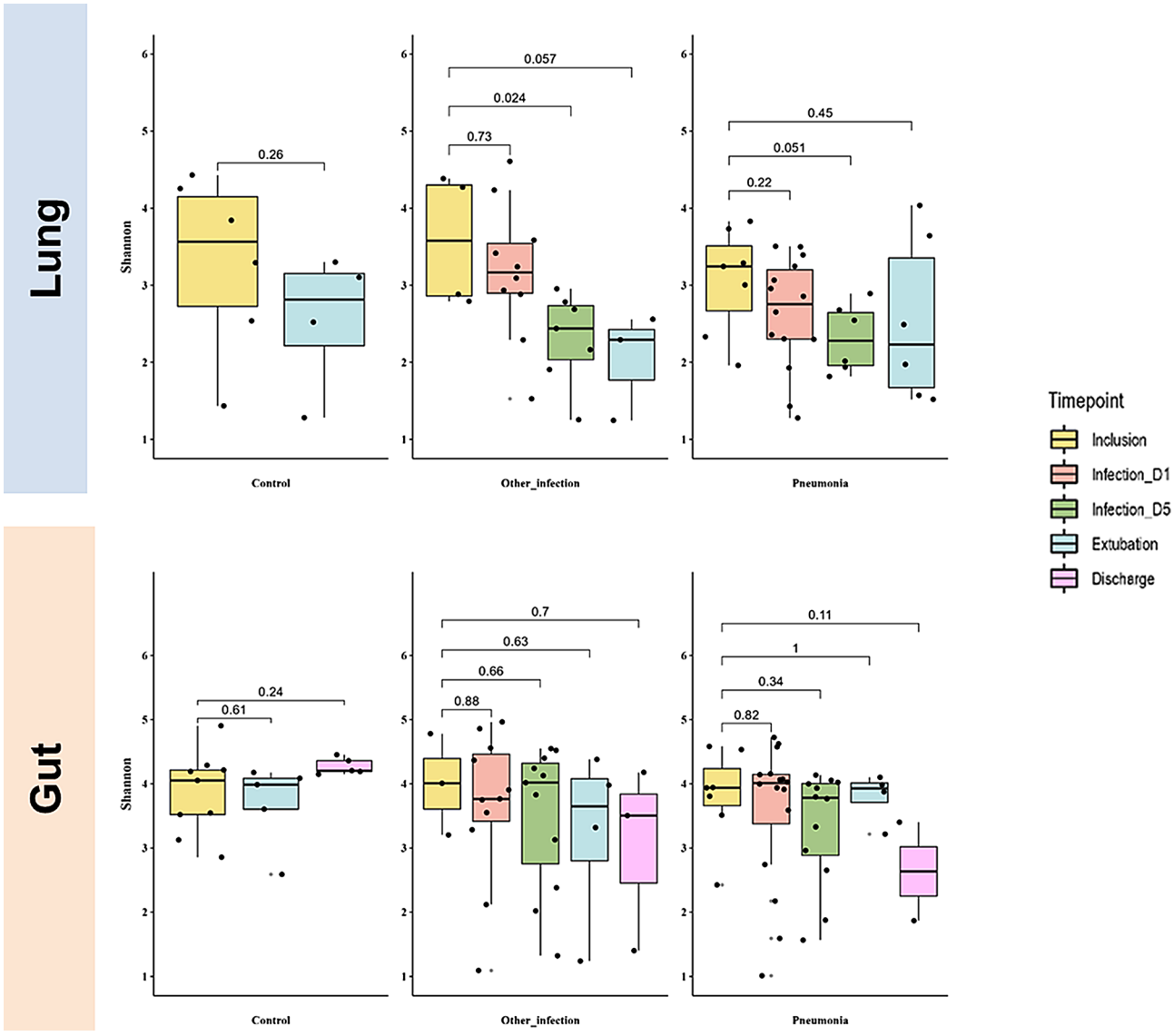

Fig. 2 Alpha diversity evolution among patient's groups over time

We next aimed to identify metataxonomic markers of VAP early in the course of ICU stay. Abundance of certain taxa belonging to *Bacteroidetes*, *Firmicutes* and *Actinobacteria* phyla were enriched in both lung (*Bergeyella*) and faecal samples (*Actinotignum*, *Eubacterium*, *Butyricimonas* and other members of *Christensenellaceae* or *Lachnospiraceae* families) of non-pneumonia patients on inclusion (Fig. 6). On the other hand, certain *Actinobacteria* (*Rothia* and *Corynebacterium*) and *Firmicutes* (*Mogibacterium*, *Caproiciproducens* etc.) were enriched on inclusion in the lungs and faecal samples of pneumonia patients, respectively (Fig. 6).

Correlation of microbiota features with clinical outcome

We finally tested whether identified key features of the microbiome would predict the various clinical outcomes in patients with VAP. Indeed, many microbiota and metataxonomic markers showed a correlation with patients' outcome. Lung alpha diversity as expressed by Shannon index on the first day of infection showed a statistically significant negative correlation with intubation length (Fig. 7). Metataxonomic features of the lung microbiome on inclusion such as the *Firmicutes* phylum relative abundance, the *Actinobacteria/Bacteroidetes* ratio and the *Firmicutes/Bacteroidetes* ratio showed an inverse correlation with arterial partial pressure of oxygen on the first day of infection (Fig. 7). Moreover, metataxonomic markers of the gut microbiome such as the relative abundance of *Mogibacterium* genus in the gut were also positively correlated with intubation or ICU length (Fig. 7).

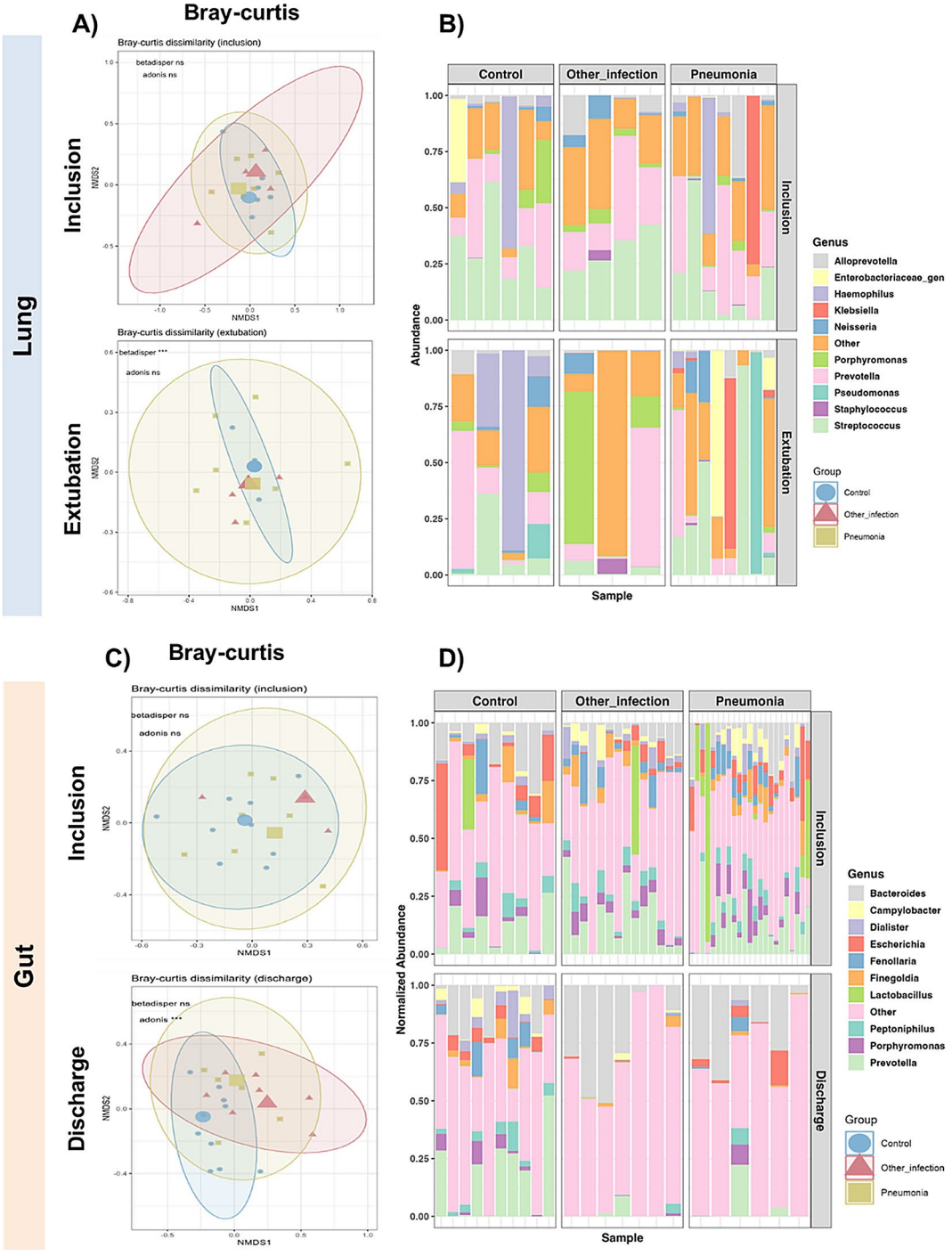

Fig. 3 Beta-diversity comparison among patients' groups. This figure shows beta-diversity (Panels **A** and **C**) and taxonomic composition of the 10 most abundant genera (Panels **B** and **D**) among groups on inclusion and extubation/discharge. The left panel shows the lung microbiome data and the right panel the gut data. Betadisper (betadisper function) refers to multivariate homogeneity of group dispersions analysis and adonis (adonis function) refers to permutational multivariate analysis of variance. The bigger highlighted symbols in the graphs represent median values. (ns= not significant, * = p<0.05, ** = p<0.01, *** = p<0.0001)

Next, we evaluated the performance of the microbiota features correlated with intubation length to predict the duration of intubation in patients with VAP as compared to the APACHE II clinical score. To do this, we stratified patients with VAP into two groups based on intubation length. Those with a shorter or equal duration of intubation with the median duration of intubation of VAP patients (≤ 10 days) and those with a duration

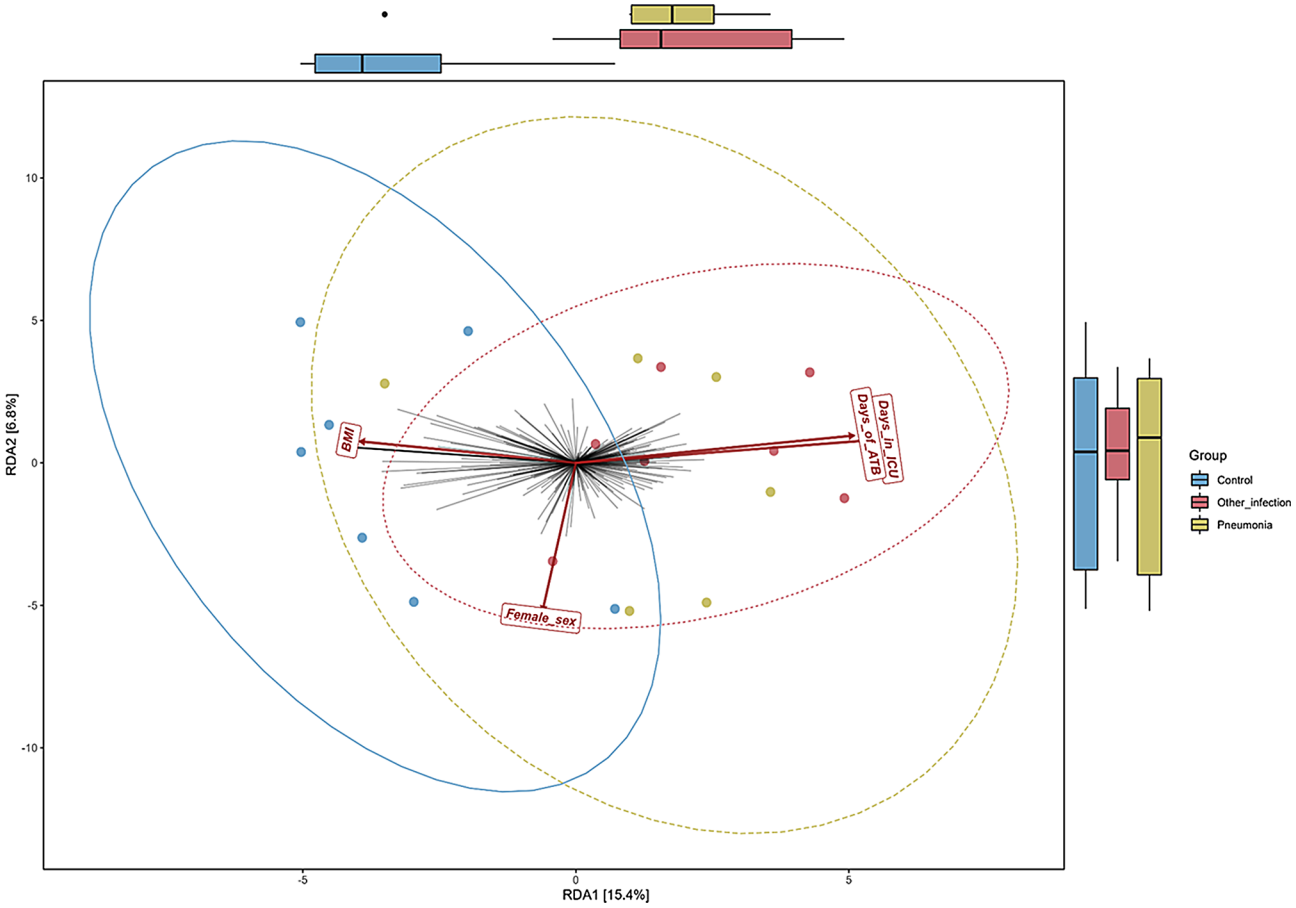

Fig. 4 Constrained ordination (RDA) displaying Jaccard dissimilarity of the gut samples at discharge, as explained by taxa variation (at the genus level) and by selected constraint variables. Redundancy analysis (RDA) of the gut data at discharge. Dots represent samples, black lines the contribution of the different taxa (unlabeled here for clarity reasons) and the red lines with the arrows depict the contribution of statistically significant ($p<0.05$) selected constraint variables. Percentages along the axes indicate fractions of total inertia (departure from sample homogeneity). The boxplots along the axes show the median values, the IQR and the range of the ordination variation

longer than the median duration of intubation of VAP patients (>10 days). The microbiota features were compared to the APACHE II score, which has already been previously used to predict the outcome of mechanical ventilation and length of ICU stay [32, 33]. The ROC curves of the performance of microbiota features and APACHE II score are displayed in Fig. 8. The area under the curve (AUC) was 0.53, 0.76 and 1 for APACHE II score, Shannon diversity index and gut *Mogibacterium* relative abundance, respectively. Optimal performance for microbiota features was obtained at a cutoff of 2.5 for Shannon diversity index, and 0.00032 of relative abundance in the gut for *Mogibacterium* genus (Fig. 8). Finally, we modified the existing APACHE II score to consider the Shannon diversity index and the relative abundance of *Mogibacterium* in the gut. The weight of those variables was assigned based on the AUC of each variable as shown in Fig. 8. The new APACHE II score was therefore calculated by adding 10 points if the Shannon diversity index was <2.5 and then by multiplying the score by a factor of 2 if the relative abundance of *Mogibacterium* in the gut was >0.00032. The performance of this "modified APACHE II score" is shown in Fig. 8. The modified APACHE II score outperformed the normal APACHE II score to predict intubation length, with an AUC of 0.77 instead of 0,53. The sensitivity, specificity, positive and negative predictive values for the modified APACHE II score, for an optimal cutoff of 27.5, was 83%, 45%, 45% and 83% respectively.

Discussion

We describe here the longitudinal dynamics of lung and gut microbiota in a cohort of previously antibiotic-naïve mechanically ventilated patients. To our knowledge, this is the first clinical study that examines the concomitant composition of the lung and gut microbiota of critically ill patients. We aimed to characterize temporal evolution trends and/or compositional changes over time of these two sites and evaluate if VAP patients present a distinct microbiota signature. Finally, we tried to discover

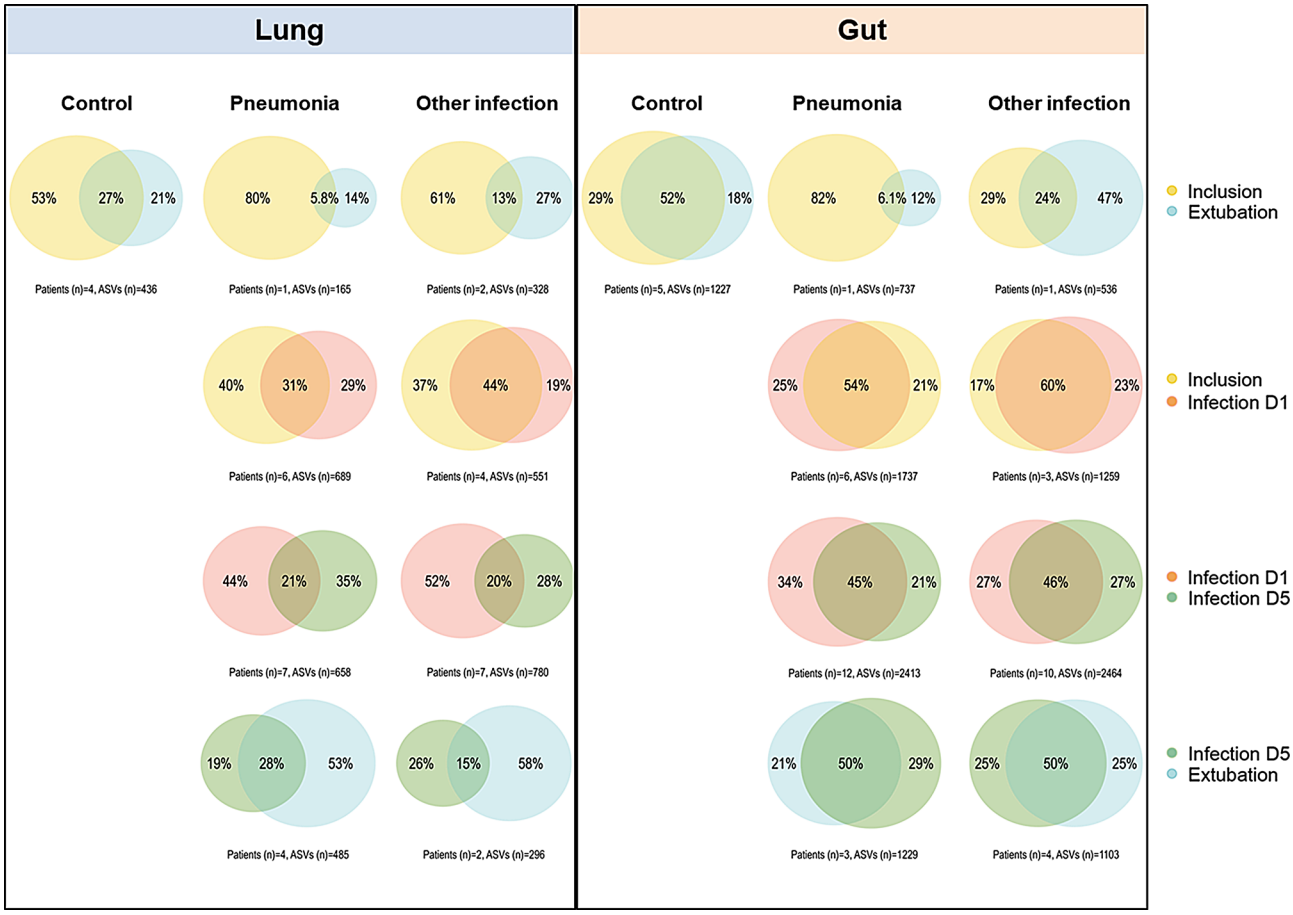

Fig. 5 Venn diagram of shared species among patients' groups over time. Venn diagrams depicting percentages of unique and shared ASVs among the three groups during their hospital stay and the course of infection

microbiota markers predictive of clinical outcome in VAP patients.

In line with previous studies [2, 3], mechanical ventilation was associated with a decrease in alpha diversity of the lung microbiota. Interestingly, in our study, the gut microbiota showed similar trends over time although the decrease in alpha diversity was less pronounced as compared to the lung microbiota. Prior studies reported a more profound decrease of alpha diversity in critically ill patients than controls [34, 35] and in pneumonia compared to non-pneumonic patients [3, 36, 37]. In our study, the control group also had higher baseline alpha diversity and although a slight decrease was noted over time this was not statistically significant. Noteworthy, alpha diversity in the gut of control patients did not show any statistically significant variation during the ICU stay. Both groups of infected patients showed a decrease in alpha diversity in both sites (lung and gut) with greater impact on the lung microbiome irrespectively of the type of infection, which can likely be partly explained by the administration of antibiotics to both groups. Evenness of the lung microbiota significantly decreased between the 1st and the 5th day of infection in both groups of infected patients and was then partially restored in some patients later until discharge. This is an important finding of our study challenging the so far existing knowledge suggesting that the lung microbiome of pneumonia patients is more susceptible to changes than in non-pneumonic patients [3, 36, 37]. In our study, the lung microbiome of non-pneumonia infected patients was disrupted as well as the lung microbiome of pneumonia patients suggesting a major impact, of not only mechanical ventilation, but systemic inflammation and/or antibiotic therapy, in shaping the microbiome.

Lung and gut microbiota beta-diversity did not differ significantly among groups on inclusion. The gut microbiome evolved differently and was yet dissimilar upon discharge between control and other infection group in both beta diversity analysis and in the number of shared taxa within the three groups over time. Infected patients present a distinct microbiota profile that differentiates them from control over time.

All the above highlight once again the important role of systemic inflammation and sepsis on the disruption of

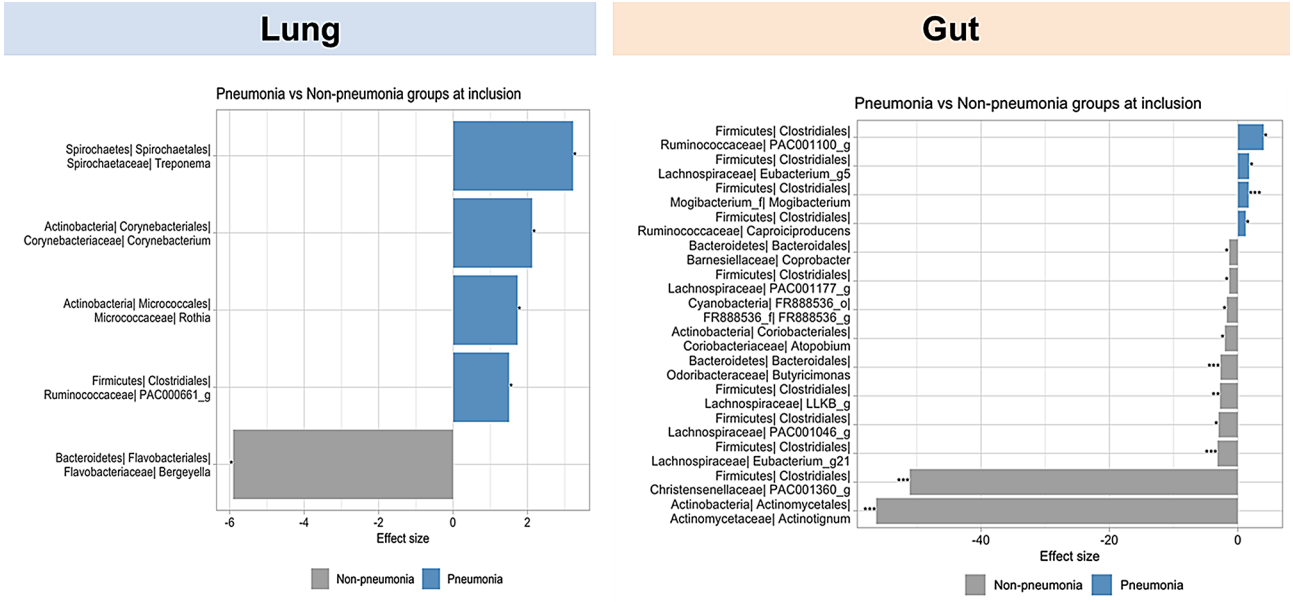

Fig. 6 Differential abundance analysis comparing taxonomic composition of pneumonia vs. non-pneumonia patients on inclusion. Differential abundance analysis using a zero-inflated regression model to compare taxonomic differences of pneumonia versus non-pneumonia patients on inclusion. Only statistically significant differentially abundant taxa are shown here. Overrepresented taxa for each group are shown based on the color code of the legend. Only taxa with a minimum prevalence of 30% were taken into account. (* p<0.05, ** p<0.01, *** p<0.001)

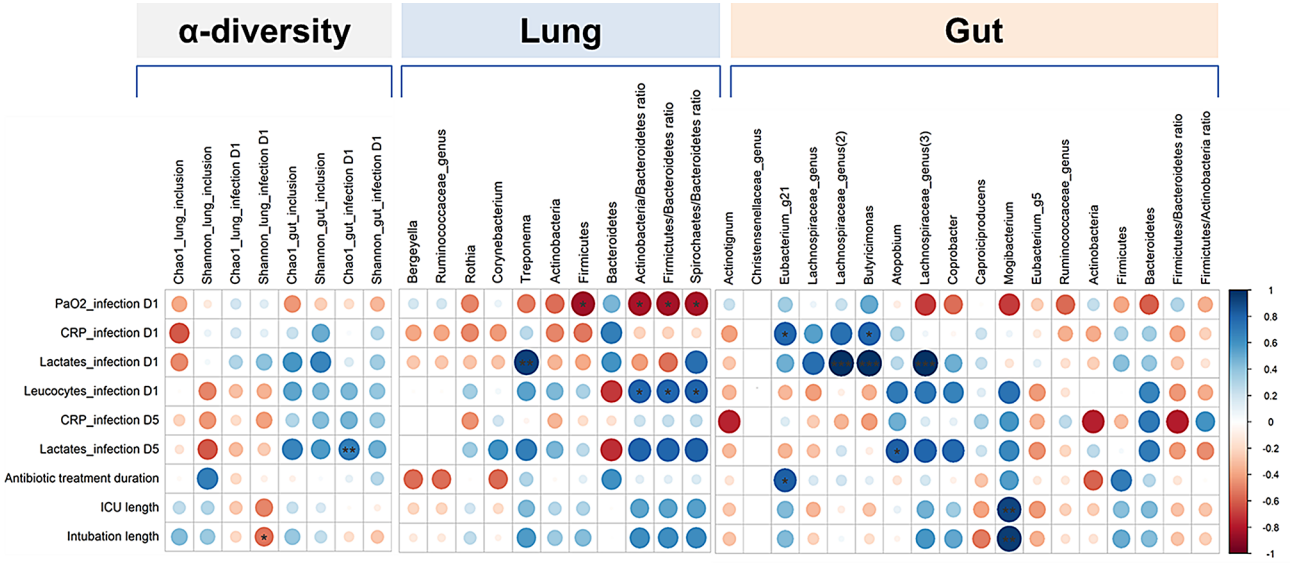

Fig. 7 Spearman's correlation analysis of selected microbiota and taxonomic key features on inclusion with selected metadata. This figure shows correlation between taxonomic features on inclusion and metadata. The color of the circles shows the direction of the correlation based on the heatmap on the right part of the graph, and the intensity of the color and the size of the circles represent the strength of the correlation. Statistically significant correlations are depicted with stars in the middle of the circles. (D1= day 1, D5= day 5, PaO_2= partial pressure of oxygen, CRP= C-reactive protein, * p<0.05, ** p<0.01, *** p<0.001)

microbiome, as well as antibiotic therapy. It is important to note however that among patients with other infections there were two bacteremia cases presumably of abdominal origin (the first due to *E. faecalis* and the second due to *E. aerogenes*) thus potentially explaining the persistent alterations observed in the gut. The dynamics of changes in microbiota profile among controls and pneumonia patients have also been previously demonstrated [2, 38]. Nevertheless, existing literature report conflicting results. A recent study by *Emonet et al.* [2] did not show significant differences in the lung microbiome in terms of beta-diversity between pneumonia patients and controls. Interestingly, in another study using a murine model of Gram-negative pneumonia-derived

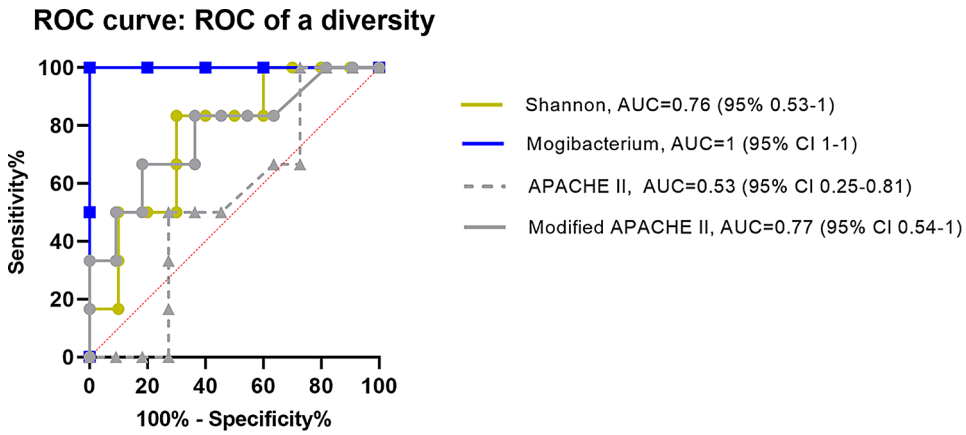

Fig. 8 Receiver operating curves (ROC) for the prediction of duration of mechanical ventilation in VAP patients. This figure shows the receiver operating characteristic (ROC) curves of the Shannon diversity index on the 1st day of infection, the gut relative abundance of *Mogibacterium* genus on inclusion (n=2 in intubation length >10 days group and n=5 in intubation length ≤10 days group) and the APACHE II or the Modified APACHE II score to predict the duration of intubation in ICU patients with VAP

sepsis, gut microbiota showed important changes in terms of beta-diversity over time [22].

To identify the compositional changes most contributing to microbial heterogeneity of our study, we highlighted the dominance of supraglottic predominant taxa (*Veillonella*, *Campylobacter*, *Treponema*, *Granulicatella*, *Rothia*) in the lungs of pneumonia patients early in the course of ICU stay, and the progressive enrichment by *Proteobacteria* (*Neisseria genus* and members of the *Enterobacteriaceae*) and *Firmicutes* (*Oribacterium*, *Parvimonas*, *Dialister*). Previous studies have demonstrated the role of a pulmonary "supraglottic dominant phenotype" (including *Rothia* and *Veillonella*) in the expression of alveolar inflammatory cytokines and a pro-inflammatory phenotype characterized by elevated Th17-lymphocytes [39]. The presence of such bacteria in the lungs suggest microaspiration or inefficient microbial clearance, which is common in patients with VAP. The basal level of pulmonary mucosal immune activation is therefore associated with local lung bacteria, which in some cases seem to be delivered from the upper respiratory tract [39]. The cross-talk between the gut and the lung microbiota, the so called gut-lung axis, has been largely described so far [18, 40–42] although very few studies explicitly studied in parallel the two sites [10, 22]. The dynamics of the aerodigestive tract are thought to become inverted during critical illness and microbiota translocation from gut to lungs is enhanced [1]. Whereas in healthy subjects, the oropharynx is the primary source of microbiota for the lungs and stomach, the overgrown microbial reservoir of the gut become the primary microbial source for the oropharynx and lungs in critically ill patients [1]. Numerous publications so far have described and associated the early enrichment of the lung microbiome by *Pseudomonadaceae*, *Enterobacteriaceae* or *Lachnospiraceae* with a hyperinflammatory state and with acute respiratory distress syndrome [8, 10, 43]. Early enrichment with *Enterobacteriaceae* and *Lachnospiraceae* was also observed in our study.

Gut microbiome alterations in patients with respiratory tract infections have also been studied previously [44]. Although there is a considerable heterogeneity among studies, a systematic review showed a depletion of *Lachnospiraceae* and *Ruminococcaceae* and enrichment of *Enterococcus* in patients with respiratory tract infections. In our study we observed mainly modifications within the *Firmicutes* phylum (mostly interesting the *Lachnospiraceae* and *Ruminococcaceae* families) in the gut of pneumonia patients. Gut microbiome can derive short chain fatty acids (SCFAs), in combination with host-derived cytokines and chemokines travel through the bloodstream and lymphatic system to enhance a protective immune response [45]. *Lachnospiraceae* and *Ruminococcaceae* are a common key component of the gut microbiota that produce SCFAs and therefore are essential for pathogen protection [44].

Finally, we sought for microbiota markers predictive of the clinical outcome in critically ill patients. Alpha-diversity based on Shannon index on the 1st day of infection and *Mogibacterium* genus relative abundance in the gut were both associated with a prolonged mechanical ventilation, while the *Firmicutes* relative abundance in the lungs or the *Firmicutes* to *Bacteroidetes* ratio were associated with the arterial partial pressure of oxygen on the first day of infection. Previous publications have also linked low alpha diversity with prolonged mechanical ventilation [8, 38, 43]. While a low gut *Firmicutes*/*Bacteroidetes* ratio has been linked to the development or the severity of respiratory tract infections in many publications [44, 46], a predominance of *Firmicutes* in the lung has been associated with immune-mediated phenomena such as bronchiolitis obliterans

in solid organ transplant recipients [47]. The role of *Firmicutes/Bacteroidetes* ratio in the lungs of critically ill patients remains to be elucidated with further studies, as well as the underlying mechanisms of host-microbe interaction. Our data also revealed a strong association of clinical outcome with *Mogibacterium* genus in the gut, an obligate anaerobic Gram-positive bacterium from the family of *Anaerovoracaceae*. *Mogibacterium* spp. has mostly been linked with periodontitis and subgingival plaque [48], but recent reports also highlight the presence of *Mogibacterium* in the lungs of pneumonia patients [49–51]. While this is the first study to our knowledge that associates gut *Mogibacterium* to VAP, previous publications report its role in altered lipid metabolism in the gut [52] and these bacteria might therefore play a role in SCFAs-mediated immune response or in the pharmadynamic of antibiotics.

In this study we used microbiota key features to predict the length of intubation of pneumonia patients. The limited patient sample size does not allow us to draw very robust results, nevertheless this is a proof of concept that microbiota related markers could be integrated and improve existing scores to predict patients' outcome. Further studies with independent samples should be carried out to confirm the interest of a combined microbiota and clinical-derived score to predict clinical outcomes such as length of ventilation.

We must acknowledge a few limitations to our study. First, because of sample availability, we used endotracheal aspirates and not bronchoalveolar lavage samples, which is not the ideal sample to study infection of bronchia and alveolar spaces, as already raised in previous studies [3]. Trachea represents an island in-between the mouth and the lungs and is considered the most appropriate sample to investigate the influence of mechanical ventilation and respiratory microbiome in the absence of bronchoalveolar lavage samples [3]. Second, we used primers targeting the V1-V2 hypervariable regions for the lung microbiome samples and V3-V4 hypervariable regions for the faecal samples. Previously published systematic comparisons of 16 S hypervariable regions indicated that V1-V3 hypervariable regions were the best surrogates for oral or airway microbiome analysis [53, 54], while V3-V4 hypervariable regions usually outperform other combinations for human faecal samples [55, 56]. We performed an in silico analysis (unpublished data) to compare taxonomic assignment of microbiome samples based on the two targeted variable regions (V1-V2 vs. V3-V4) and we found an overall agreement of 95%. V1-V2 performed better in identifying streptococci at species level. Nevertheless, and besides the observed high correlation rates, differences in metataxonomic techniques and 16 S rRNA gene sequencing parameters could make direct comparison of samples in gut and lung sites difficult to evaluate. Finally, we note that this is a real-life pragmatic study and thus definition of infection might have been blurry, especially in the other infection group. Therefore, we cannot exclude that some of the patients with fever of unknown origin might have had an involvement of the respiratory tract despite they did not fulfill criteria for pneumonia.

Conclusion

This prospective study helps elucidate the dynamic changes of gut and lung microbiota compartments in critically ill patients. Further larger studies with comparable methodologies are yet required to better characterize the role of different actors across the interplay of dysbiosis, inflammation and clinical outcome in critically ill patients.

Abbreviations

VAP	Ventilator associated pneumonia
ICU	Intensive care unit
ETA	Endotracheal aspirate
BAL	Bronchoalveolar lavage
ARDS	Acute respiratory distress syndrome
ASVs	Amplicon sequence variants

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10825-6.

Supplementary Material 1

Acknowledgements

We would like to thank all the technical staff of the Microbiology Institute of Lausanne University Hospital and in particular Sebastien Aeby for providing administrative support with the study's samples and for sequencing the gut microbiota samples. We thank all the nursing staff of the intensive care unit for their valuable help in collecting patients' samples. Finally, yet importantly, we would like to thank the Department of Medicine of Lausanne University Hospital for awarding our project with a scholarship. All of the above contributed significantly to our project and without their valuable help, its realization would not have been possible.

Author contributions

BG conceived, designed the study and provided scientific counselling. AK was responsible for the administrative management of the study, collected patients' data and revised patients'charts, performed literature research, analyzed data, performed statistical analyses, drafted and revised the article. EB sequenced lung samples, analyzed data and performed statistical analyses for part of the lung microbiome samples. YC analyzed data and performed statistical analyses for part of the gut microbiome samples. VS analyzed sequenced samples with our in-house bioinformatics pipeline and provided scientific input. JLP participated in administrative management of the study in ICU and screened eligible patients. GG provided scientific counseling and provided essential reagents and materials, and revised the article. CB provided scientific counseling and supervised bioinformatics and statistical analyses of the study.

Funding

Open access funding provided by University of Lausanne
This research was funded by a scholarship provided to AK by the Department of Medicine of Lausanne University Hospital. Part of the analysis was supported as a part of NCCR Microbiomes, a National Centre of Competence in Research, funded by the Swiss National Science Foundation (grant number 180575).

Data availability
16 S amplicon sequences of samples analyzed in the present study are available from the European Nucleotide Archive (ENA): PRJEB82425/ERP166112.

Declarations

Ethics approval and consent to participate
This project was conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice and the Swiss Human Research Act (HRO). The project received approval from the Ethics Committee of Canton Vaud, Switzerland (2017–01820). All patients or their legal representatives (in case of patient's inability) provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Received: 29 July 2024 / Accepted: 19 March 2025
Published online: 05 April 2025

References

1. Papazian L, Klompas M, Luyt CE. Ventilator-associated pneumonia in adults: a narrative review. Intensive Care Med. 2020;46(5):888–906.
2. Emonet S, Lazarevic V, Leemann Refondini C, Gaia N, Leo S, Girard M, Nocquet Boyer V, Wozniak H, Despres L, Renzi G, et al. Identification of respiratory microbiota markers in ventilator-associated pneumonia. Intensive Care Med. 2019;45(8):1082–92.
3. Zakharkina T, Martin-Loeches I, Matamoros S, Povoa P, Torres A, Kastelijn JB, Hofstra JJ, de Wever B, de Jong M, Schultz MJ, et al. The dynamics of the pulmonary Microbiome during mechanical ventilation in the intensive care unit and the association with occurrence of pneumonia. Thorax. 2017;72(9):803–10.
4. Martin-Loeches I, Dickson R, Torres A, Hanberger H, Lipman J, Antonelli M, de Pascale G, Bozza F, Vincent JL, Murthy S, et al. The importance of airway and lung Microbiome in the critically ill. Crit Care. 2020;24(1):537.
5. Vincent JL, Sakr Y, Singer M, Martin-Loeches I, Machado FR, Marshall JC, Finfer S, Pelosi P, Brazzi L, Aditianingsih D, et al. Prevalence and outcomes of infection among patients in intensive care units in 2017. JAMA. 2020;323(15):1478–87.
6. Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The Microbiome and the respiratory tract. Annu Rev Physiol. 2016;78:481–504.
7. Fernandez-Barat L, Lopez-Aladid R, Torres A. Reconsidering ventilator-associated pneumonia from a new dimension of the lung Microbiome. EBioMedicine. 2020;60:102995.
8. Dickson RP, Schultz MJ, van der Poll T, Schouten LR, Falkowski NR, Luth JE, Sjoding MW, Brown CA, Chanderraj R, Huffnagle GB, et al. Lung microbiota predict clinical outcomes in critically ill patients. Am J Respir Crit Care Med. 2020;201(5):555–63.
9. Bos LD, Weda H, Wang Y, Knobel HH, Nijsen TM, Vink TJ, Zwinderman AH, Sterk PJ, Schultz MJ. Exhaled breath metabolomics as a noninvasive diagnostic tool for acute respiratory distress syndrome. Eur Respir J. 2014;44(1):188–97.
10. Dickson RP, Singer BH, Newstead MW, Falkowski NR, Erb-Downward JR, Standiford TJ, Huffnagle GB. Enrichment of the lung Microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. Nat Microbiol. 2016;1(10):16113.
11. Panzer AR, Lynch SV, Langelier C, Christie JD, McCauley K, Nelson M, Cheung CK, Benowitz NL, Cohen MJ, Calfee CS. Lung microbiota is related to smoking status and to development of acute respiratory distress syndrome in critically ill trauma patients. Am J Respir Crit Care Med. 2018;197(5):621–31.
12. Bernasconi E, Pattaroni C, Koutsokera A, Pison C, Kessler R, Benden C, Soccal PM, Magnan A, Aubert JD, Marsland BJ, et al. Airway microbiota determines innate cell inflammatory or tissue remodeling profiles in lung transplantation. Am J Respir Crit Care Med. 2016;194(10):1252–63.
13. Das S, Bernasconi E, Koutsokera A, Wurlod DA, Tripathi V, Bonilla-Rosso G, Aubert JD, Derkenne MF, Mercier L, Pattaroni C, et al. A prevalent and culturable microbiota links ecological balance to clinical stability of the human lung after transplantation. Nat Commun. 2021;12(1):2126.
14. O'Dwyer DN, Ashley SL, Gurczynski SJ, Xia M, Wilke C, Falkowski NR, Norman KC, Arnold KB, Huffnagle GB, Salisbury ML, et al. Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. Am J Respir Crit Care Med. 2019;199(9):1127–38.
15. Dickson RP. The Microbiome and critical illness. Lancet Respir Med. 2016;4(1):59–72.
16. Souza DG, Vieira AT, Soares AC, Pinho V, Nicoli JR, Vieira LQ, Teixeira MM. The essential role of the intestinal microbiota in facilitating acute inflammatory responses. J Immunol. 2004;173(6):4137–46.
17. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa e Melo F, Roelofs JJ, de Boer JD, Hoogendijk AJ, de Beer R, de Vos A, Belzer C, et al. The gut microbiota plays a protective role in the host defence against Pneumococcal pneumonia. Gut. 2016;65(4):575–83.
18. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieers G, Guery B, Delhaes L. The Gut-Lung axis in health and respiratory diseases: A place for Inter-Organ and Inter-Kingdom crosstalks. Front Cell Infect Microbiol. 2020;10:9.
19. Silvestri L, de la Cal MA, van Saene HK. Selective decontamination of the digestive tract: the mechanism of action is control of gut overgrowth. Intensive Care Med. 2012;38(11):1738–50.
20. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell. 2012;148(6):1258–70.
21. Alverdy JC, Luo JN. The influence of host stress on the mechanism of infection: lost microbiomes, emergent pathobiomes, and the role of interkingdom signaling. Front Microbiol. 2017;8:322.
22. Wolff NS, Jacobs MC, Wiersinga WJ, Hugenholtz F. Pulmonary and intestinal microbiota dynamics during Gram-negative pneumonia-derived sepsis. Intensive Care Med Exp. 2021;9(1):35.
23. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, Napolitano LM, O'Grady NP, Bartlett JG, Carratala J, et al. Management of adults with Hospital-acquired and Ventilator-associated pneumonia: 2016 clinical practice guidelines by the infectious diseases society of America and the American thoracic society. Clin Infect Dis. 2016;63(5):e61–111.
24. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inf. 2019;95:103208.
25. Scherz V, Nassirnia S, Chaabane F, Castelo-Szekely V, Greub G, Pillonel T, Bertelli C. zAMP and zAMPExplorer: Reproducible Scalable Amplicon-based Metagenomics Analysis and Visualization. bioRxiv. 2025. https://doi.org/10.1101/2025.03.09.633768
26. Scherz V, Caruana G, Taffe P, Brouillet R, Bertelli C, Jaton K, Fougere Y, Posfay-Barbe KM, Mornand A, Rochat-Guignard I, et al. Unexpected associations between respiratory viruses and bacteria with pulmonary function testing in children suffering from cystic fibrosis (MUCOVIB study). J Cyst Fibros. 2022;21(2):e158–64.
27. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from illumina amplicon data. Nat Methods. 2016;13(7):581–3.
28. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261–7.
29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6.
30. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol. 2017;67(5):1613–7.
31. Zhang X, Yi N. NBZIMM: negative binomial and zero-inflated mixed models, with application to microbiome/metagenomics data analysis. BMC Bioinformatics. 2020;21(1):488.
32. Matic I, Titlic M, Dikanovic M, Jurjevic M, Jukic I, Tonkic A. Effects of APACHE II score on mechanical ventilation; prediction and outcome. Acta Anaesthesiol Belg. 2007;58(3):177–83.
33. Takekawa D, Endo H, Hashiba E, Hirota K. Predict models for prolonged ICU stay using APACHE II, APACHE III and SAPS II scores: A Japanese multicenter retrospective cohort study. PLoS ONE. 2022;17(6):e0269737.
34. Kyo M, Nishioka K, Nakaya T, Kida Y, Tanabe Y, Ohshimo S, Shime N. Unique patterns of lower respiratory tract microbiota are associated with

inflammation and hospital mortality in acute respiratory distress syndrome. Respir Res. 2019;20(1):246.
35. Schmitt FCF, Lipinski A, Hofer S, Uhle F, Nusshag C, Hackert T, Dalpke AH, Weigand MA, Brenner T, Boutin S. Pulmonary Microbiome patterns correlate with the course of the disease in patients with sepsis-induced ARDS following major abdominal surgery. J Hosp Infect 2020.
36. de Steenhuijsen Piters WA, Huijskens EG, Wyllie AL, Biesbroek G, van den Bergh MR, Veenhoven RH, Wang X, Trzcinski K, Bonten MJ, Rossen JW, et al. Dysbiosis of upper respiratory tract microbiota in elderly pneumonia patients. ISME J. 2016;10(1):97–108.
37. Dickson RP, Martinez FJ, Huffnagle GB. The role of the Microbiome in exacerbations of chronic lung diseases. Lancet. 2014;384(9944):691–702.
38. Fromentin M, Ricard JD, Roux D. Respiratory Microbiome in mechanically ventilated patients: a narrative review. Intensive Care Med. 2021;47(3):292–306.
39. Segal LN, Clemente JC, Tsay JC, Koralov SB, Keller BC, Wu BG, Li Y, Shen N, Ghedin E, Morris A, et al. Enrichment of the lung Microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. Nat Microbiol. 2016;1:16031.
40. Zhang D, Li S, Wang N, Tan HY, Zhang Z, Feng Y. The Cross-Talk between gut microbiota and lungs in common lung diseases. Front Microbiol. 2020;11:301.
41. Dumas A, Bernard L, Poquet Y, Lugo-Villarino G, Neyrolles O. The role of the lung microbiota and the gut-lung axis in respiratory infectious diseases. Cell Microbiol. 2018;20(12):e12966.
42. Stavropoulou E, Kantartzi K, Tsigalou C, Konstantinidis T, Voidarou C, Konstantinidis T, Bezirtzoglou E. Unraveling the interconnection patterns across lung microbiome, respiratory diseases, and COVID-19. Front Cell Infect Microbiol. 2020;10:619075.
43. Kitsios GD, Yang H, Yang L, Qin S, Fitch A, Wang XH, Fair K, Evankovich J, Bain W, Shah F, et al. Respiratory tract dysbiosis is associated with worse outcomes in mechanically ventilated patients. Am J Respir Crit Care Med. 2020;202(12):1666–77.
44. Woodall CA, McGeoch LJ, Hay AD, Hammond A. Respiratory tract infections and gut Microbiome modifications: A systematic review. PLoS ONE. 2022;17(1):e0262057.
45. Shi HY, Zhu X, Li WL, Mak JWY, Wong SH, Zhu ST, Guo SL, Chan FKL, Zhang ST, Ng SC. Modulation of gut microbiota protects against viral respiratory tract infections: a systematic review of animal and clinical studies. Eur J Nutr. 2021;60(8):4151–74.
46. Khan M, Mathew BJ, Gupta P, Garg G, Khadanga S, Vyas AK, Singh AK. Gut dysbiosis and IL-21 response in patients with severe COVID-19. Microorganisms 2021, 9(6).
47. Ubags NDJ, Marsland BJ. Mechanistic insight into the function of the Microbiome in lung diseases. Eur Respir J 2017, 50(3).
48. Chen C, Hemme C, Beleno J, Shi ZJ, Ning D, Qin Y, Tu Q, Jorgensen M, He Z, Wu L, et al. Oral microbiota of periodontal health and disease and their changes after nonsurgical periodontal therapy. ISME J. 2018;12(5):1210–24.
49. Bahrani-Mougeot FK, Paster BJ, Coleman S, Barbuto S, Brennan MT, Noll J, Kennedy T, Fox PC, Lockhart PB. Molecular analysis of oral and respiratory bacterial species associated with ventilator-associated pneumonia. J Clin Microbiol. 2007;45(5):1588–93.
50. Chen C, Shen T, Tian F, Lin P, Li Q, Cui Z, Zhang Y, Xue M, Ye J, Guo X, et al. New microbiota found in sputum from patients with community-acquired pneumonia. Acta Biochim Biophys Sin (Shanghai). 2013;45(12):1039–48.
51. Shen Y, Yu F, Zhang D, Zou Q, Xie M, Chen X, Yuan L, Lou B, Xie G, Wang R, et al. Dynamic alterations in the respiratory tract microbiota of patients with COVID-19 and its association with microbiota in the gut. Adv Sci (Weinh). 2022;9(27):e2200956.
52. Yu J, Cheng Q, He F, Meng F, Yu Y, Xu C, Wen X, Hong L, Gao J, Li J, et al. Altered intestinal microbiomes and lipid metabolism in patients with prolonged disorders of consciousness. Front Immunol. 2022;13:781148.
53. Wang Z, Liu H, Wang F, Yang Y, Wang X, Chen B, Stampfli MR, Zhou H, Shu W, Brightling CE, et al. A refined view of airway Microbiome in chronic obstructive pulmonary disease at species and Strain-Levels. Front Microbiol. 2020;11:1758.
54. Zheng W, Tsompana M, Ruscitto A, Sharma A, Genco R, Sun Y, Buck MJ. An accurate and efficient experimental approach for characterization of the complex oral microbiota. Microbiome. 2015;3:48.
55. Abellan-Schneyder I, Matchado MS, Reitmeier S, Sommer A, Sewald Z, Baumbach J, List M, Neuhaus K. Primer, Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing. *mSphere* 2021, 6(1).
56. Chen Z, Hui PC, Hui M, Yeoh YK, Wong PY, Chan MCW, Wong MCS, Ng SC, Chan FKL, Chan PKS. Impact of Preservation Method and 16S rRNA Hypervariable Region on Gut Microbiota Profiling. *mSystems* 2019, 4(1).

Publisher's note