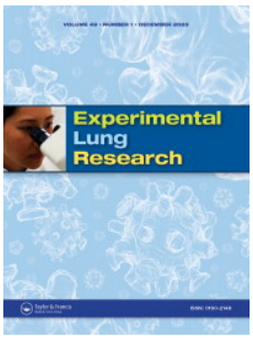

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Effect of mechanical ventilation under intubation on respiratory tract change of bacterial count and alteration of bacterial flora

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ABSTRACT

Background: The most common 'second strike' in mechanically ventilated patients is a pulmonary infection caused by the ease with which bacteria can invade and colonize the lungs due to mechanical ventilation. At the same time, metastasis of lower airway microbiota may have significant implications in developing intubation mechanical ventilation lung inflammation. Thus, we establish a rat model of tracheal intubation with mechanical ventilation and explore the effects of mechanical ventilation on lung injury and microbiological changes in rats. To provide a reference for preventing and treating bacterial flora imbalance and pulmonary infection injury caused by mechanical ventilation of tracheal intubation. **Methods:** Sprague-Dawley rats were randomly divided into Control, Mechanical ventilation under intubation (1, 3, 6 h) groups, and Spontaneously breathing under intubation (1, 3, 6 h). Lung histopathological injury scores were evaluated. 16SrDNA sequencing was performed to explore respiratory microbiota changes, especially, changes of bacterial count and alteration of bacterial flora. **Results:** Compared to groups C and SV, critical pathological changes in pulmonary lesions occurred in the MV group after 6 h ($p < 0.05$). The Alpha diversity and Beta diversity of lower respiratory tract microbiota in MV6, SV6, and C groups were statistically significant ($p < 0.05$). The main dominant bacterial phyla in the respiratory tract of rats were *Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria*. *Acinetobacter radioresistens* in group C was significant, *Megaonas* in group MV6 was significantly increased, and *Parvibacter* in group SV6 was significantly increased. Anaerobic, biofilm formation, and Gram-negative bacteria-related functional genes were altered during mechanical ventilation with endotracheal intubation. **Conclusion:** Mechanical ventilation under intubation may cause dysregulation of lower respiratory microbiota in rats.

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Mechanical ventilation; lung injury; respiratory tract; microecology; microbiota imbalance

Introduction

Mechanical ventilation of tracheal intubation is widely used to manage general anesthesia during surgery, respiratory maintenance in intensive care, and perioperative treatment of critically ill patients. However, mechanical ventilation of tracheal intubation can also lead to lung injury or exacerbate existing lung injury, known as ventilator-induced lung injury (VILI).[1,2] Also, lung inflammation can occur during ventilator therapy. Current studies have found that mechanical ventilation of tracheal intubation induces upregulation of cytokine expression in a pro-inflammatory state in the body. Thus, patients are more susceptible to "second strikes" (prolonged mechanical ventilation, aspiration, shock, sepsis, pulmonary infections).[3] The most common "second strike" in mechanically ventilated patients is pulmonary infections. The effects on the body's immune system from comorbidities and malnutrition in patients receiving mechanical ventilation under anesthesia can also increase the morbidity and mortality of respiratory infections.[4]

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Respiratory microecology is one of the critical factors in the function of the respiratory tract. Studies over the past few years have demonstrated that the lower respiratory tract is not "sterile" and that, in healthy conditions, the lung microbiota is less dense but harbors a remarkable diversity of interacting microbiota. *Actinobacteria*, *Aspergillus*, and ***Bacteroides*** are present in the lungs of healthy individuals.[5–8] Maintaining a dynamic, steady state in healthy individuals may be a process of the continuous influx of microbiota and elimination of adverse growth conditions. Studies have confirmed that the homeostasis of the microbiota is disturbed during lung disease, the lung microbiota changes and pathogenic bacteria exhibit a competitive advantage, resulting in an imbalance of the host immune system.[7,9–12]

The imbalance of respiratory microbiota may lead to local or even systemic bacterial infections, and the microecological regulatory mechanisms are complex. Identifying microbial colonization by alveolar lavage collected primarily from critically ill patients is currently used clinically to guide anti-infection protocols. At the same time, few studies have been reported on the relationship between lung injury and inflammatory response to mechanical ventilation and changes in respiratory microbiota. Therefore, this study investigated the relationship between lung injury and microbiological changes in rats with tracheal intubation and mechanical ventilation and contributed to studying microbiota regulation of the respiratory tract in lung injury and inflammation. Meanwhile, our research group is committed to developing new endotracheal tubes to improve the adverse effects of mechanical ventilation on the respiratory tract.[13–16] This study explored lung injury and microbial changes in intubation and mechanical ventilation rats. We provided new research ideas for developing a new antibacterial endotracheal tube to prevent mechanical ventilation-associated pneumonia.

Materials and methods

To investigate lung injury and microbial changes in intubation and mechanical ventilation rats, we assessed the inflammatory response and microbial changes in the rat airways by pathophysiology and 16SrDNA sequencing.

Animals

Male Sprague-Dawley rats (weighing 230–330 g and aged 8–9 wk) were housed at the Southern Hospital Experimental Center of Southern Medical University under the same temperature and humidity environment and received the same food and water. Animal care and experimental protocols were guidelines by the National Science Council of the Republic of China (NSC, 1997). The animal studies were reviewed and approved by the Ethical Committee on Animal Experimentation of Nanfang Hospital, Southern Medical University, Guangzhou, China (NFYY-2021-0245).

Twenty-eight male SD rats were randomly divided into seven groups ($n = 4$): control group (group C), mechanical ventilation under intubation for one hour (group MV1), mechanical ventilation under intubation for three hours (group MV3), mechanical ventilation under intubation for six hours (group MV6), spontaneous breathing under intubation for one hour (group SV1), spontaneous breathing under intubation for three hours (group SV3), spontaneous breathing under intubation for six hours (group SV6).

After weighing, we anesthetized the rats with 2% pentobarbital sodium (60 mg/kg) by intraperitoneal injection. We placed our homemade endotracheal tube into the trachea, and the MV group connected a small animal ventilator (SuperV1.0, HYB, China) for mechanical ventilation. A room temperature of 26–28 °C was maintained throughout the procedure. We chose a sterile 16 G venous trocar with the same material as the clinical endotracheal tube, modified to fit the length of rat trachea. The control group was directly executed after tracheal intubation, while the SV group was intubated and reserved for spontaneous breathing. The MV group was mechanically ventilated at 1, 3, and 6 h, respectively. Mechanical ventilation parameters: tidal volume (VT) 7 ml/kg, PEEP = 0 mmHg, respiratory rate 80 breaths/min (adjusted according to respiration during anesthesia), inhalation-expiration ratio (I: E) = 1:2, inhaled gas was air (oxygen concentration 21%). All operations are performed under anesthesia.

Experiments

Rat lung injury assay

Histopathological examination of the lung. To observe the pathological damage of lung tissue at different ventilation durations, we performed HE-stained light microscopy of lung tissue. The anterior lobe of the right lung of rats was fixed in 4% paraformaldehyde solution. After paraffin embedding, sectioning, and HE staining, the lung was placed under a light microscope to observe the histopathological changes. Five high magnification views were randomly selected to observe the lung tissue morphology and the pictures were taken and saved. Scores included lung tissue congestion, edema, erythrocyte infiltration, alveolar cavity destruction, capillary destruction, and other pathological changes, and the mean values were taken after scoring.[17,18]

Bioinformatics analysis

Specimen collection. The trachea and left lungs of 28 SD rats were flash-frozen in liquid nitrogen and stored at −80 °C for 16S rDNA sequencing.

DNA extraction. Microbial DNA was extracted from a mixed homogenate of the left lung and trachea tissue using a DNA Extraction Kit (Magen, Guangzhou, Guangdong, China) according to the manufacturer's instructions. It was detected by a NanoDrop microspectrophotometer and agarose gel electrophoresis.

PCR amplification. The 16S rDNA target region of the ribosomal RNA gene was amplified by PCR (95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 7 min) using primers V3-V4: 341 F CCTACGGGNGGCWGCAG; 806 R GGACTACHVGGGTATCTAAT 15. PCR reactions were performed in a triplicate 50 μL mixture containing 10 μL of 5×Q5 Reaction Buffer, 10 μL of 5×Q5 High GC Enhancer, 1.5 μL of 2.5 mM dNTPs, 1.5 μL of each primer (10 μM), 0.2 μL of Q5 High-Fidelity DNA Polymerase, and 50 ng of template DNA. Related PCR reagents were from New England Biolabs, USA.

DNA sequencing and analysis. Amplicons were extracted and purified from 2% agarose gels using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) and quantified using the ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). The purified amplicons were pooled and sequenced on the Illumina platform (PE250) according to standard protocols. The sequencing data were analyzed for Species Composition, Beta diversity, Linear discriminant analysis effect size (LEfSe), Alpha diversity, and BugBase. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA789199).

Statistical methods

The one-way ANOVA test or the turkey HSD test was carried out to compare the quantitative data. The $\chi 2$ test or Fisher exact test was completed to compare the qualitative data. Strain composition, alpha diversity, beta diversity, and functional analyses were performed by the OmicShare tools, a free online platform for data analysis (http://www.omicshare.com/tools).

Results

Pathological changes in lung tissue

The control group had a normal alveolar structure and a small amount of inflammatory cell infiltration. The lung tissue of SV6 showed apparent hemorrhage, widening of the alveolar septum, lymphocyte infiltration, and destruction of the alveolar structure. Hemorrhages occurred in the MV3 group. MV6 hemorrhage was apparent, the alveolar septum was widened, the exudate in the alveolar cavity increased, the tissue structure was destroyed, and the alveolar fusion occurred. The degree of lung tissue injury increased with time in the SV and MV groups (Figure 1).

Analysis of lower respiratory microbiological changes

Changes in microbiota composition and diversity

The top 10 species with the highest mean abundance ranking in all samples were shown in detail. Other known species were classified as others, and unknown species were marked as unclassified.[19,20] In all lower respiratory tract

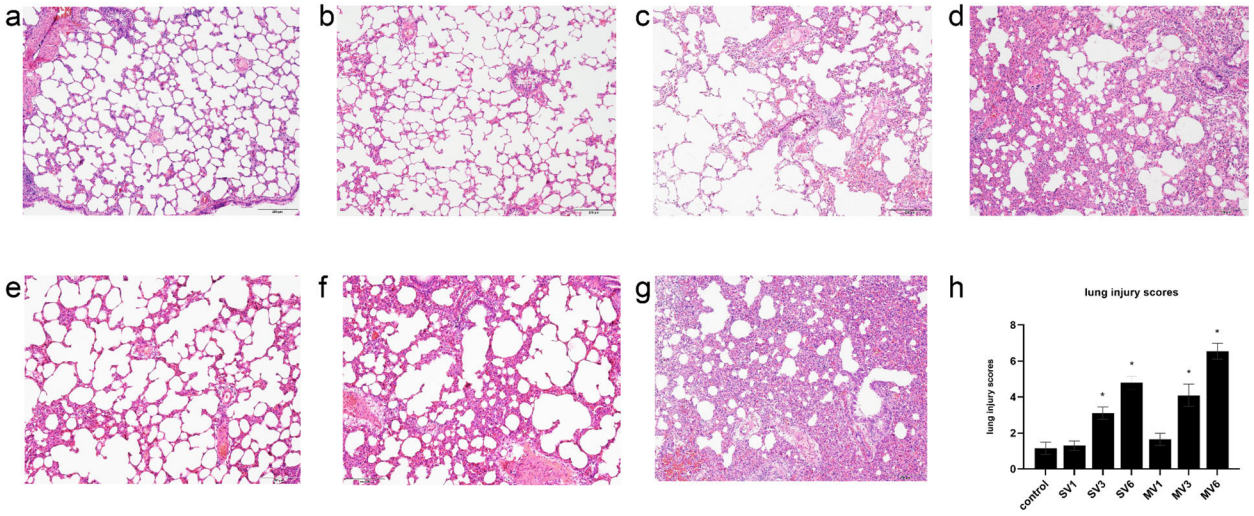

Figure 1. Effect of mechanical ventilation on lung tissue injury from rats. (a) Rats were executed directly after tracheal intubation under anesthesia. (b–d) Rats were kept under anesthesia after tracheal intubation with spontaneous breathing for 1, 3, and 6 h. (e–g). rats were mechanically ventilated for 1, 3, and 6 h after tracheal intubation. (h) Lung tissue injury score. Data are representative of at least four different experiments. Results are mean ± S for four rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with control animals or between mechanical ventilation for six hours and spontaneous breathing for six hours.

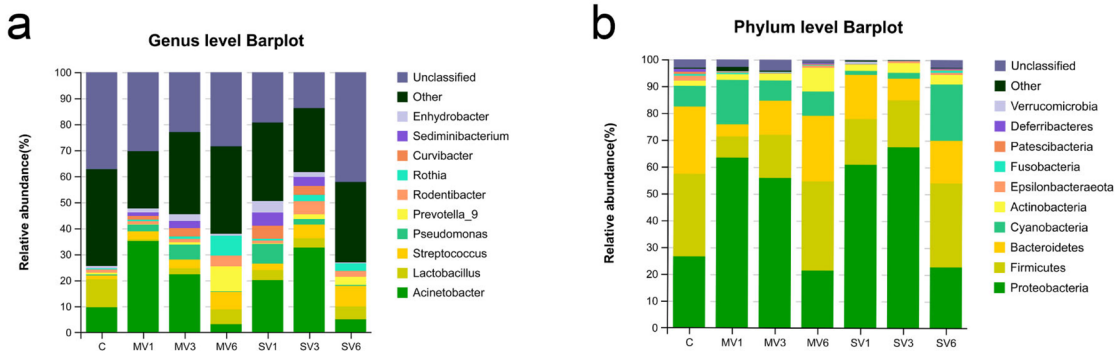

Figure 2. Changes in the lower respiratory tract microbiota of rats during mechanical ventilation. (a) At the genus level, the main dominant genera were *Acinetobacter*, *Lactobacillus*, and *Staphylococcus*. (b) At the phylum level, the main dominant phyla were *Proteobacteria, Firmicutes, Bacteroidetes, Cyanobacteria*, and *Actinobacteria*. Tracheal intubation and mechanical ventilation caused changes in rats' lower respiratory tract microbiota ratio.

samples, the bacterial community was mainly composed of *Acinetobacter, Lactobacillus*, and *Streptococcus*. At the phylum level, *Proteobacteria, Firmicutes, Bacteroidetes*, and *Cyanobacteria* were the main ones. *Acinetobacter's* high relative sequence abundance was observed in the MV1, MV3, SV1, and SV3 groups, respectively (Figure 2a). At the phylum level, species relative abundance analysis showed that *Proteobacteria* had the highest relative abundance in the SV3 group (67.46%), followed by MV1 and SV1. In comparison, *Firmicutes* had the highest relative abundance in MV6 (33.26%) (Figure 2b).

Two levels of cluster analysis were performed to observe different bacterial distributions between samples and differences in bacterial community composition between samples. The hierarchical clustering results showed that the distribution of microbial communities in the lower respiratory tract of rats in each group was relatively different. The bacterial community may be affected by mechanical ventilation under intubation. Cluster analysis of bacterial community structure in each experimental group yielded major clusters that differed from those in the control group. Principal coordinate analysis (PCOA) and non-metric

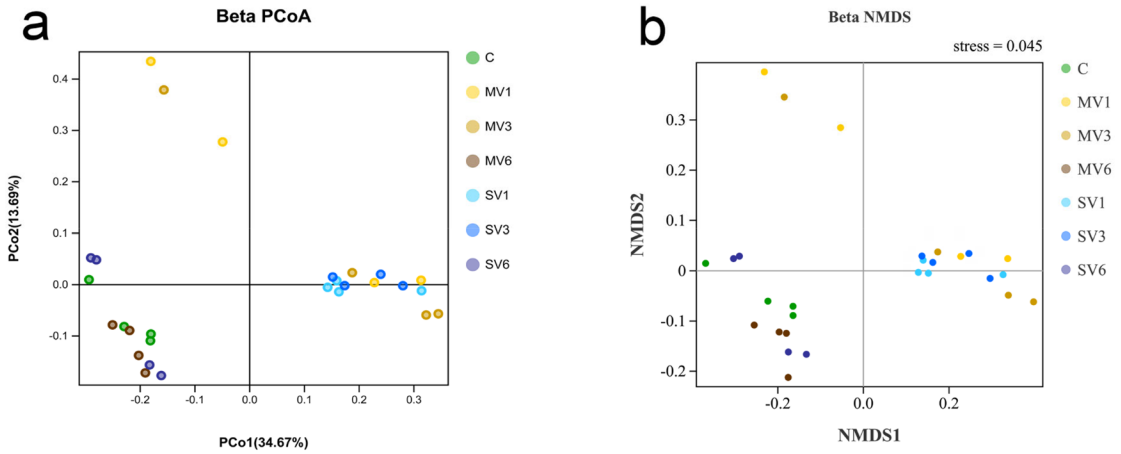

Figure 3. The sample taxonomic analysis revealed the microbial community ranking map of 16S rDNA bacteria: a scatter plot and PCoA ranking based on phylogenetic distance (PCoA1 vs. PCoA2) (a). UniFrac unweighted non-metric multidimensional scale-metric multidimensional scaling (NMDS) (b).

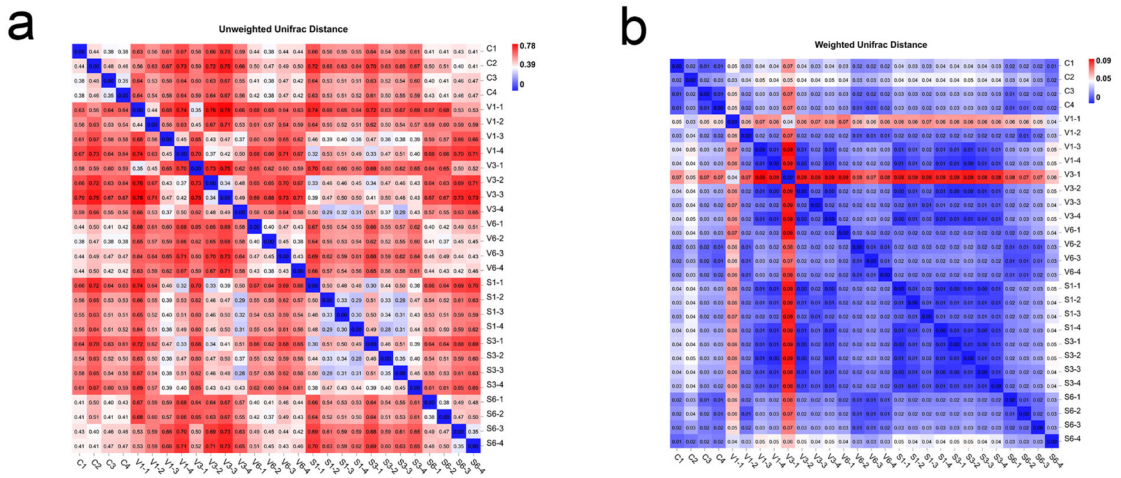

Figure 4. Beta diversity analysis heatmap. Unweighted distance matrix (a): rats in the control group and each experimental group showed a clustering trend, suggesting that there were Specific differences in the species of lower respiratory tract microbiota between rats in the control group and each experimental group. Weighted distance matrix (b): the samples of the control group and each experimental group did not show an apparent clustering trend, suggesting that there was no significant difference in the diversity of bacterial microbiota among each group.

multidimensional scales (NMDS) also confirmed other general differences in bacterial community composition (16S rDNA gene sequencing) between different samples.[21] The contribution rates of PCOA 1 and PCOA 2 were 34.67% and 13.69%, respectively (Figure 3a). There were differences between the MV groups and control groups. The bacterial communities in the 1-h and 3-h groups were well-grouped and had apparent effects. NMDS based on Unweighted UniFrac distances between samples also revealed differences between experimental and control groups (Figure 3b).

A heatmap of the Beta diversity index was used to measure the coefficient of difference between two samples, with unweighted and weighted UniFrac distances indicating proximity between duplicate samples and overall differences in bacterial communities.[22] In Unweighted UniFrac (Figure 4a), rats in both the control and experimental groups showed a clustering trend under Unweighted analysis, suggesting some differences between the bacterial communities in the control and experimental groups. In Weighted UniFrac (Figure 4b), the difference in bacterial community clustering between groups was

insignificant, considering OTU abundance's effect on each group.

Comparison of biomarkers of the microbiota

LEfSe was used to quantify microbiota biomarkers in each group and to elucidate further possible interactions of the identified bacterial affiliations in the rat's lower respiratory tract.[23] By detecting significant differences in the abundance of different bacterial biomarkers within the group (linear discriminant analysis (LDA) > 2; $p < 0.05$). Among all categorical levels of C-MV1, 159 biomarkers were associated with C, and 32 were associated with the MV1 group. *Firmicutes* with higher abundance (LDA 5.09, $p = 0.02$), including *Lactobacillus* and *Megasphaera*, were significantly enriched in group C (Figure 5a). *Proteobacteria* was a highly abundant biomarker in the C-SV1 dataset (LDA 5.27, $p = 0.02$). Species including *Pseudomonadales, Alphaproteobacteria, Gammaproteobacteria, and Moraxellaceae*were significantly abundant in the SV1 group (Figure 5b). In the experimental three-hour groups, the primary biomarker of MV3 was *Alphaproteobacteria* (LDA 4.57, $p = 0.02$), and *Proteobacteria* was the primary biomarker of SV3 (LDA 5.26, $p = 0.02$) (Figure 5c,d). The maximum number of discriminative clades was reduced in groups MV6(9) and SV6 (10). In the MV6 group, *Megamonas* could be a potential taxonomic indicator under the experimental conditions, with the LDA score of 3.89 and $p = 0.02$ (Figure 5e). *Parvibacter* was probably the main taxonomic indicator in the SV6 group (LDA 2.78, $p = 0.01$) (Figure 5f).

Effects of mechanical ventilation on the diversity of respiratory microbiota

In this study, there were differences in bacterial Alpha diversity among the groups.[24,25] Microbial differences in rat lower respiratory tract samples under mechanical ventilation under intubation were indicated by comparing richness and diversity indices. The MV6 group (Chao1 988.17; Ace 1027.20) had the highest richness index, significantly higher than the short-term mechanical ventilation group. According to Shannon's estimation of OTU diversity, the control group (6.69) and MV6 group (6.12) had higher bacterial diversity than the other treatment groups. At the same time, the Simpson index was not significantly different among the groups. In that study, we also noted a decreasing trend in the richness and Shannon index of the microbiota in the one-hour and three-hour groups (Table 1). These results indicate that mechanical ventilation under intubation affects the changes in bacterial diversity in the lower respiratory tract of rats.

Predicted functional categories

To further interpret the function of the rat lower respiratory tract microbiota, we utilized BugBase. This bioinformatics tool can infer the phenotype of the entire community from the predicted metagenome.[26] BugBase found that lower respiratory tract samples enriched gene functions associated with anaerobiosis. As shown in Figure 6a,b, the species determining anaerobic function changed at different time points of the experiment. Proteobacteria was the dominant bacteria of anaerobic functional phenotype in the MV1 group, but this dominant position showed a downward trend over time. Biofilm formation and Gram-negative phenotypes are bacterial genus phenotypes frequently associated with respiratory pathogenicity. Our study showed that the biofilm formation phenotype was greatly enhanced during the early stages of tracheal intubation and mechanical ventilation (1–3 h), and Proteobacteria were the main cause of the altered biofilm formation phenotype (Figure 6c,d). The present study enhanced genetic phenotypes associated with biofilm formation in rats receiving mechanical ventilation by tracheal intubation. In addition, the phenotypes of Gram-negative bacteria were not significantly different between groups.

Discussion

As one of the most frequently contacted parts of the human body, the respiratory micro-ecosystem is essential in maintaining human health and is most vulnerable to damage. In recent years, the lower respiratory tract microbiota has been extensively studied and reported to be similar to the oropharynx.[1,2,27] However, studies exploring the relationship between mechanical ventilation and

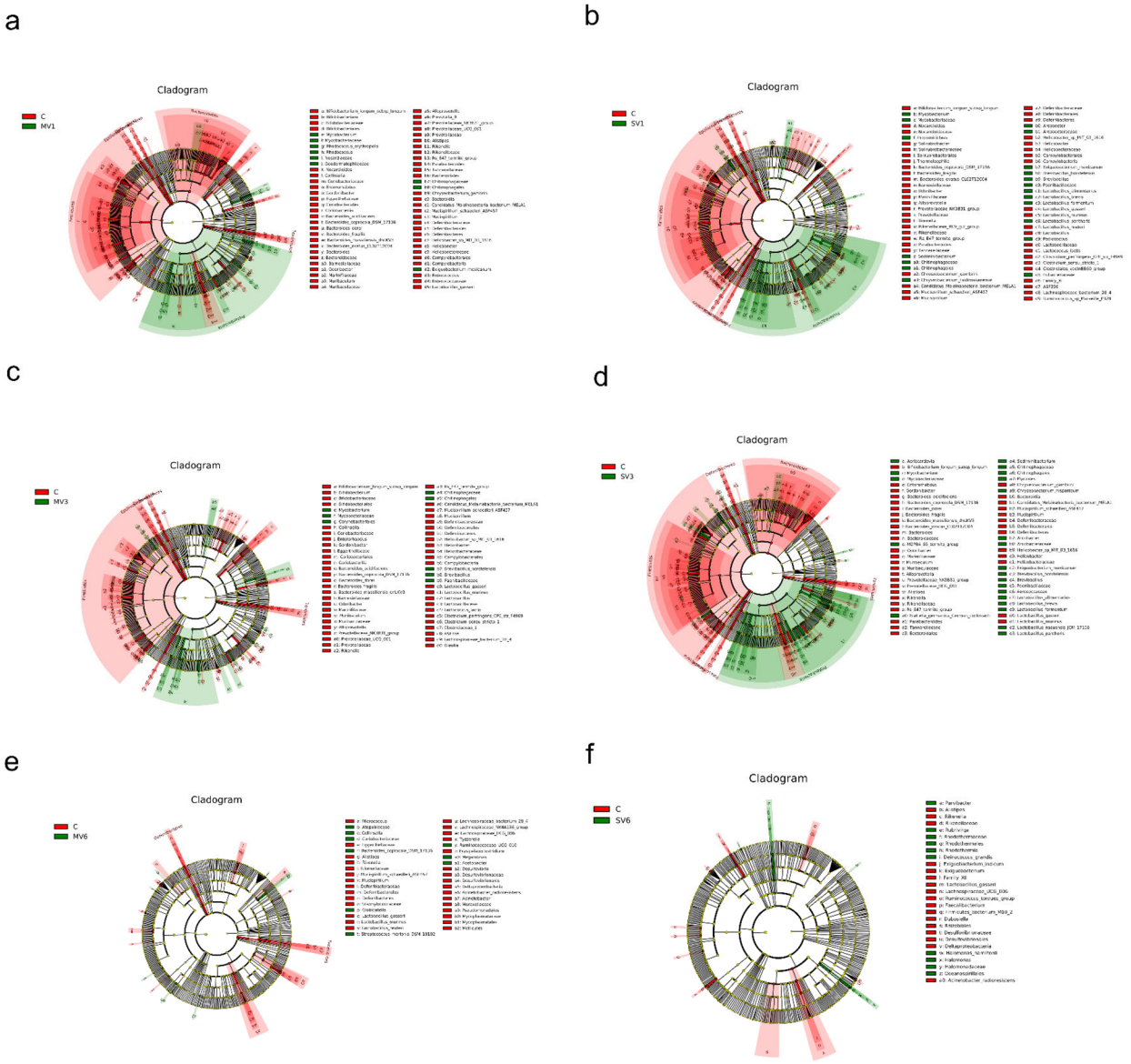

Figure 5. LEfSe Comparative analysis plot: categorical representation indicating statistical and biological agreement and differences in the identified biomarkers between the experimental groups. (a) control versus mechanical ventilation under intubation for one hour (C-MV1); (b) control versus spontaneous breathing under intubation for one hour (C-SV1); (c) control versus mechanical ventilation under intubation for three hours (C-MV3); (d) control versus spontaneous breathing under intubation for three hours (C-SV3); (e) control versus mechanical ventilation under intubation for six hours (C-MV6); (f) control versus spontaneous breathing under intubation for six hours (C-SV6). the colored part represents the significantly different taxonomic trends of the bacterial microbiota. Red or green shading depicts bacterial taxa significantly higher in each experimental group, while yellow represents species that did not differ significantly.

Table 1. Alpha diversity of rat lower respiratory tract microbiota.

		Mechanical ventilation			Spontaneously breathing		
	Control	1h	3h	6h	1h	3h	6h
Chao1	925.1499	566.0260	586.2087	988.1747	561.3573	543.2223	939.8622
ACE	973.9671	604.9513	619.7504	1027.2041	582.9082	574.4539	985.4881
Shannon	6.6898	4.7184	5.7610	6.1168	5.5315	5.2578	5.9806
Simpson	0.9715	0.8758	0.9539	0.9538	0.9526	0.9339	0.9369
PD-whole tree	169.7246	125.2935	97.9107	169.7123	92.0423	90.5608	173.9426
Good's coverage %	0.9983	0.9990	0.9990	0.9982	0.9989	0.9990	0.9981

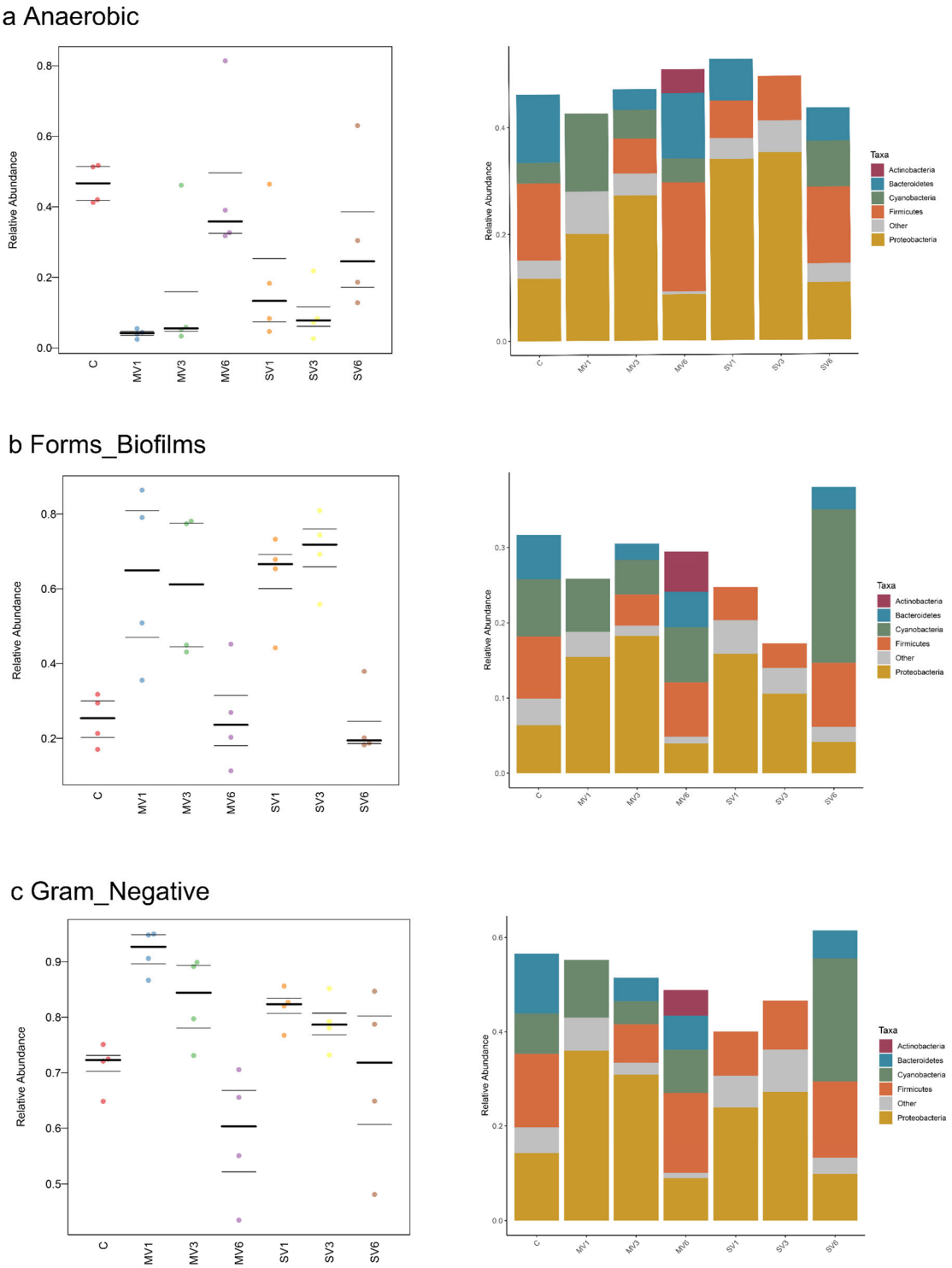

Figure 6. Bugbase analysis of rat lower respiratory tract microbiota. (a,b) With the associated representative genera changes anaerobic activity is diminished at the beginning of mechanical ventilation. (c,d) Biofilm formation is enhanced at the beginning of mechanical ventilation, and the associated representative genera are altered. (e,f) Gram_negative formation did not vary much between groups; it slightly increased at the beginning of the experiment, and the proportion decreased with the prolongation of the experiment.

lower airway microecology have mainly explored the level of ventilator-associated pneumonia (VAP).[28–31] Changes in lower airway microecology during the initial phase of mechanical ventilation have not been reported. To investigate the *in vivo* response of tracheal intubation and mechanical

ventilation on lower respiratory microecology in SD rats in this study, we obtained pathological findings of lung inflammation and injury and data on bacterial community structure.

Rats' lower respiratory tract bacterial diversity and tracheal intubation ventilation

This study examined the lower respiratory tract's inflammatory response and microbial community structure over six hours in mechanical ventilation of tracheal intubation and Spontaneous respiration under tracheal intubation rats. The ongoing clinical studies have demonstrated that *Actinobacteria, Proteobacteria, Bacteroidetes*, and *Firmicutes* constitute the adult bronchoalveolar lavage fluid (BALF) of most bacteria strains.[5–8] The rat strains in the study were comparable to human strains. They covered the major human strains, indicating that it is feasible for SD rats to mimic the human clinical tracheal intubation environment. The mechanical ventilation for respiratory tract flora is due to the influence of mechanical ventilation in lower respiratory tract flora richness and diversity index of the effect. We observed that 3 h after tracheal intubation and mechanical ventilation, the lung tissue began to appear with bleeding points, the alveolar septum widened, and many exudates in the alveolar cavity, and the destruction of the alveolar structure changed, which aggravated with time. Correspondingly, the percentage of Proteobacteria was highest in the experimental and control groups. At the start of mechanical ventilation, the rate of Proteobacteria in the lower respiratory tract increased more than 2-fold compared with the control group. At the same time, the number of Firmicutes and Bacteroidetes decreased by about 4-fold. After 6 h of mechanical ventilation, the number of actinomycetes was more than four times that of the control group. It is suggested that mechanical ventilation can cause lung tissue damage and change the microecological structure. However, further studies on the interaction between microbiota changes and tissue damage are needed, and whether this change also occurs in clinical patients undergoing surgery under general anesthesia is the focus of our subsequent studies.

Acinetobacter may be one factor in mediating the proinflammatory phase of lung tissue injury.[32] Overgrowth of this pathogen may promote airway injury and exacerbate microecological dysbiosis. In all experimental groups analyzed, bacterial community diversity decreased at the beginning of the experiment, especially in species richness (Ace, Chao) and bacterial diversity as measured by the Shannon diversity index (Table 1). The above findings suggest that tracheal intubation ventilation perturbs the ecological balance of the lower airway in rats, thereby perturbing the steady state of bacterial diversity. In mechanical ventilation, within 6 h, bacterial diversity increases gradually. However, its flora composition and health rats differences still exist, perhaps due to pathogens' excessive growth and stimulation of the host immune system to further promote disease progression.[33] Our results demonstrate the potential impact of microecological imbalance during mechanical ventilation, especially in the early stage of ventilation, suggesting whether patients may experience similar situations during mechanical ventilation in clinical practice. If the microecological changes are monitored more closely, potential adverse events may be detected and treated early.

Changes in composition and functional analysis of active microbial communities

At the phylum level of the lower respiratory tract community, *Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria* were the dominant bacteria (relative abundance of species) in each experimental group. Meanwhile, PCoA and NMDS analyses showed that bacterial community structure was significantly altered under mechanical ventilation. Furthermore, in the LEfSe analysis, we found that significant biomarkers emerged in the comparison of C with MV1, C with SV1, C with MV3, C with SV3, C with MV6, and C with SV6 in the case of comparison with the control group (Figure 5). Lower respiratory tract biomarkers were significantly present in the MV1(32 clades), SV1(52 clades), MV3(30 clades), SV3(77 clades), MV6(9 clades), SV6(10 clades) groups compared with the control group. The presence of these biomarkers and the differences

in biomarkers between the different experimental groups show the potential function and activity of the lower respiratory tract microbiota during mechanical ventilation. In particular, *Firmicutes, Proteobacteria*, and *Actinobacteria* may play an important role in mechanical ventilation and the diversity of microbial community composition and function of the lower respiratory tract. These changes in microbiota may be associated with the microecological imbalance caused by mechanical ventilation and the underlying inflammatory state of the lung. These results remind us that in the subsequent clinical practice, monitoring the microbial community's change and related biomarkers' existence may contribute to early detection and intervention in patients with respiratory complications.

Influence of component transfer on predicted metabolic function

Clinical studies have shown that lower respiratory tract microbes are essential in regulating the inflammatory response in various respiratory diseases, such as asthma,[34] chronic obstructive pulmonary disease (COPD),[35] cystic fibrosis,[36] and respiratory tract infections. Microecological dysbiosis in these diseases can lead to pathogen overgrowth and a reduction in the diversity of commensal microorganisms, which can induce an inflammatory response in the host. The key findings of this study are that the stimulation of endotracheal intubation and mechanical ventilation in the lower respiratory tract causes ecological balance destruction, loss of symbiosis of microbial diversity, and early inflammatory pathological changes in lung tissue. With the prolonged duration of mechanical ventilation, inflammatory cascades and excessive activation of proinflammatory cytokines may occur, further aggravating lung tissue injury.

Interestingly, our BugBase functional analysis found that genes associated with anaerobes were functionally enriched in lower respiratory samples. During the early stages of mechanical ventilation, the function of genes associated with anaerobes decreased (C vs. MV1 $p = 0.013$), and the main functional bacteria were also changed. At the same time, Biofilm formation and Gram-negative phenotypes are usually associated with respiratory pathogens. Our study suggests a remarkable increase in biofilm formation phenotypes in the early stages of mechanical ventilation, and Proteobacteria become the dominant species of the phenotype. There were no significant differences in Gram-negative bacterial phenotypes between groups. After six hours of mechanical ventilation, the biofilm phenotypes were more diverse, and bacteria associated with biofilm formation phenotypes were observed in the predicted genomes of all groups. These findings indicate that mechanical ventilation may cause further epithelial cell damage, make the defense mechanism, and cause the body's inflammatory response. Biofilms are known to exhibit remarkable antimicrobial tolerance and can evade attack by the host immune system and persist in the host.[37,38] In the present study, rats mechanically ventilated by endotracheal intubation showed an enhanced phenotype of biofilm-associated genes, consistent with past studies' results. This phenomenon may be related to the damage of airway epithelial cells and the destruction of defense mechanisms caused by tracheal intubation and mechanical ventilation. It is important to note that respiratory epithelial cells are the first line of defense against potentially harmful environmental stimuli, and maintaining immune homeostasis is essential.[39,40] Epithelial cell function obstacles might be in various airway and pulmonary inflammatory disease occurrences and progress of the critical influence. In addition, it has been shown that Nf-κb, TINCR, and PGLYRP4 (rs3006458) are involved in regulating the airway immune mechanism.[41,42] Future studies will further explore the relationship between mechanical ventilation status and epithelial changes during tracheal intubation in depth to more fully understand the effects of mechanical ventilation on the microbiome and immune response in the lower respiratory tract. These findings may provide necessary theoretical support and guidance for improving the treatment and care of mechanically ventilated patients.

In addition, our study also with the concept of intestinal lung microbiome – axis.[43] Important microbiota in this study, such as Firmicutes and Bacteroidetes, produce short-chain fatty acids

(SCFA) and are also important flora in the gut. SCFAs produced by these microbiotas plays a role as a bridge between the immune system and microbiota, which may significantly impact maintaining the body's immune balance.[44,45] Our study suggests that intubated mechanical ventilation may lead to changes in the lower respiratory tract microbiota. However, whether there are corresponding changes in gut microbiota in this environment is the focus of our subsequent research. We will continue to explore the relationship between the gut-lung microbiome axis and the connection between these microbiota changes and the mechanisms triggered by endotracheal intubation and mechanical ventilation, so as to provide useful information for improving the prognosis of patients.

Conclusion

In summary, we found that tracheal intubation and mechanical ventilation may cause changes in the structure and function of the microbial community in the lower respiratory tract of rats. At present, mechanical ventilation technology has been widely used in clinical practice. This study provides some theoretical data for preventing pneumonia response under mechanical ventilation. Future studies hope to develop the effectiveness of novel antibacterial endotracheal tubes in preventing endotracheal intubation-related airway diseases during or after short-term surgery.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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