

Temporal dynamics of the oral microbiome after oral care in ventilated ICU patients and associations with outcomes: an exploratory 16S rRNA sequencing study

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Abstract

Objectives

To evaluate oral microbiome alterations in ventilator-associated pneumonia (VAP) patients compared to healthy controls, and explore correlations between dynamic microbiome changes after oral care and clinical outcomes.

Methods

Fourteen VAP patients and seven healthy controls were enrolled. Oral samples were collected and analyzed using 16S rRNA gene sequencing. Eight VAP patients underwent serial oral swab sampling every 4 hours within 24 hours after oral care.

Results

VAP patients showed significantly reduced microbial diversity, with lower abundance of oral commensals such as *Streptococcus*, *Neisseria*, *Prevotella* and with higher abundance of *Klebsiella* and *Acinetobacter*, aligning with sputum culture findings. After oral care, microbiota diversity transiently increased at 0–4 hours, then declined. Dynamic changes of oral microbiota related to VAP outcomes. fatal cases displayed severe microbial dysbiosis, dominated by pulmonary pathogens.

Conclusions

Oral microbiota diversity in VAP patients is markedly reduced, with dynamic fluctuations after oral care. Microbial diversity is associated with clinical outcomes, highlighting the role of oral microbiota dysbiosis in VAP pathogenesis.

Implications for Clinical Practice

Oral microbiota composition and dynamics is related to VAP. Monitoring oral microbiota may help predict VAP risk and prognosis. Standardized oral care procedure may stabilize microbial composition, reduce pathogen overgrowth, and support improved outcomes in ventilated patients.

Introduction

Ventilator-associated pneumonia (VAP) remains the most prevalent nosocomial infection in intensive care units (ICUs), substantially elevating patient morbidity and mortality while prolonging ICU stays and driving up healthcare expenditures. [1]. Given that the oral cavity harbors the second largest microbial

ecosystem in the human body—comprising bacteria, fungi, viruses, and archaea—the translocation of these resident pathogens serves as a primary driver in the multifactorial pathogenesis of ventilator-associated pneumonia (VAP). [2]. While a stable and diverse oral microbiota confers essential colonization resistance against exogenous pathogens, microbial dysbiosis disrupts this ecological equilibrium and is increasingly implicated in both local oral pathologies and systemic disorders, including inflammatory bowel disease and Alzheimer’s disease[3]. The association between the oral microbiota and respiratory diseases has garnered substantial research interest, with mounting evidence demonstrating that inadequate oral hygiene significantly increases susceptibility to hospital-acquired pneumonia. [4, 5], Hospital-acquired pneumonia is predominantly driven by exogenous pathogens that transiently colonize the oropharynx rather than residing there as commensal flora. Key etiological agents include Gram-negative bacilli—such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia spp.*, and *Enterobacter spp.*—alongside *Staphylococcus aureus*. In the context of ventilator-associated pneumonia (VAP), endotracheal intubation bypasses natural upper airway defenses, thereby facilitating the microaspiration and direct translocation of these oropharyngeal organisms into the lower respiratory tract. [6]. Compounding this issue, biofilm formation on the endotracheal tube surface confers marked resistance to host defenses and antimicrobial therapy, thereby establishing a persistent pathogenic reservoir that exacerbates infection risk and complicates clinical management. [7]. Within dual-species biofilm models, recent in vitro evidence demonstrates that the opportunistic oral pathogen *Candida albicans* significantly enhances the bacterial load of key ventilator-associated pneumonia (VAP) pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA) [8]. Inadequate oral hygiene has also been consistently associated with an elevated risk of community-acquired pneumonia, as substantiated by accumulating epidemiological evidence [9, 10]. Critically ill patients admitted to the intensive care unit constitute a highly susceptible cohort, particularly those requiring mechanical ventilation due to unconsciousness or pharmacological sedation—an intervention that substantially elevates the risk of ventilator-associated pneumonia (VAP) [11]. A rapid and marked deterioration in oral health status is consistently observed following admission to the intensive care unit [12]. Among critically ill patients, rapid dental plaque accumulation is associated with a pronounced dysbiosis of the oral microbiome; in those requiring mechanical ventilation, this microbial shift frequently encompasses colonization by potential ventilator-associated pneumonia (VAP) pathogens[13, 14]. In mechanically ventilated patients, accumulating evidence indicates that lower respiratory tract pathogens are frequently genetically indistinguishable from oral isolates of the same species[15]. The absence of adequate oral care, compounded by xerostomia secondary to prolonged mouth opening, exacerbates dental plaque accumulation and precipitates severe soft tissue inflammation. Pre-admission poor oral health is consequently recognized as a specific risk factor for VAP development. Moreover, recent case-control evidence indicates that periodontitis is independently associated with heightened risks of ICU admission, mechanical ventilation requirement, and mortality during the COVID-19 pandemic[16]. Advances in high-throughput microbial DNA sequencing have enabled comprehensive characterization of oral microbiota composition and function. Nevertheless, the specific microbial signatures associated with ventilator-associated pneumonia (VAP) and the optimal timing for initiating oral care in intubated patients remain poorly defined. To address these gaps, we investigated post-intubation microbial

alterations by comparing oral specimens from 14 intubated patients and 7 healthy controls. Utilizing 16S rRNA gene sequencing and downstream bioinformatic analyses, we assessed microbial diversity, taxonomic composition, and functional profiles to evaluate the association between oral care interventions and VAP-related clinical outcomes.

Methods

Participant grouping and sample collection

This study received approval from the ethics committee of Dalian Central Hospital, and written informed consent was obtained from each participant. The methods were carried out in accordance with the approved guidelines (for detailed information, see “Ethics approval and consent to participate”). The experimental group included patients who underwent hematoma removal surgery due to acute cerebral hemorrhage and were transferred to the ICU for rescue and were diagnosed with ventilator-associated pneumonia. Subjects were excluded if they had chronic oral infectious diseases such as chronic periodontal disease and chronic oral ulcers; if they were pregnant with oral care solution or disinfectant for more than six months before the onset of illness; if they experienced acute suppurative oral infection within three months; if they used antibiotics or probiotics within one month; if they were diagnosed with diabetes, coronary heart disease, chronic kidney disease or other systemic diseases, rupture or bleeding of the oral mucosa, or if aspiration occurred during the onset of cerebral hemorrhage, if they experienced aspiration pneumonia or obvious accumulation pneumonia; or if they were diagnosed, with hematogenous infection, chronic bronchitis, bronchiectasis, COPD or other chronic pulmonary infectious diseases. The control group volunteers were instructed to brush their teeth carefully in the morning (for no less than 2 minutes), and then eat normally. They should not brush their teeth at night on the same day. The next morning, oral and lingual swabs were collected on an empty stomach before brushing. All samples were stored in a -80°C medical ultralow temperature freezer within 10 minutes. Eight patients in the oral care group received oral care using the same method, and two oral swabs were taken at seven time points: 0 h, 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h. The sample preservation method was the same as before.

DNA preparation and sequencing

Oral swab genomic DNA was extracted using the Oral Swab Genomic DNA Extraction Kit (TIANGEN, DP322). The V3-V4 hypervariable region of the 16S rRNA gene was analyzed to quantify the bacterial community, and 16SrRNA V3-V4 amplification was performed using Phanta® Flash Master Mix (2×) (Novozan, China). The reaction mixture was as follows: 1 µl of genomic template (20 ng/µl), 1 µl of the front and back primers (10 µM each), 2×Phanta® Flash Master Mix (12.5 µl), and 9.5 µl of double distilled water, (25 µl). Amplification was performed using an Applied Biosystems 2720 thermal cycler, (1 cycle at 95°C for 3 minutes); 25 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30

seconds; 72°C for 10 minutes; and maintenance at 4°C. The amplified product was recovered with a GeneJET gel recovery kit (Thermo Scientific).

Bioinformatic analyses

DADA214R language software and the Vsearch tool were used to perform sequence quality control, remove chimeras, and denoise, connect, and cluster the data to obtain amplicon sequence variants (ASVs) and operational taxonomic units (OTUs). Species classification level annotation was performed on ASVs, and OTUs were used for cluster analysis. Based on the abundance distribution of OTUs in different samples, the abundance and diversity indices of OTUs were analyzed, and whether the sequencing depth met the standard was determined through sparse curves. Community structure statistical analysis was performed on each sample (group) at different classification levels, and significant differences between groups were tested. Finally, based on the above analysis, cluster analysis based on OTUs and species composition, as well as statistical comparative analysis using PCoA, PCA, and UPGMA clustering, was conducted to explore the differences in species composition between samples. An interaction correlation network was constructed based on the composition and distribution of species in each sample. Based on the 16S rRNA gene sequencing results, it was also possible to predict the microbial metabolic function of each sample.

Results

Participant characteristics and sequencing coverage

This study involved 14 patients who were diagnosed with ventilator-associated pneumonia due to intracerebral hemorrhage and tracheal intubation and 7 healthy controls. The basic characteristics of all participants are summarized in Table 1, we obtained a high-quality overlapping group. 699409, with a minimum length of 271 bp and a maximum length of 434 bp. The overlapping group with a length of 430 bp was the most common, accounting for 62.6% of the total sequence. By using Vsearch to perform sequence quality control on overlapping groups, connecting and de embedding, clustering was performed to obtain 7043 operational taxonomic units (OTUs).

Table 1
Characteristics of participants.

number	shock	Sputum Culture	antibiotics	outcome
1		<i>klebsiella pneumoniae</i>	Piperacillin tazobactam sodium	Dead
2	none		cefazolin sodium pentahydrate	Discontinues
3		<i>Acinetobacter baumannii</i>	Piperacillin tazobactam sodium	Dead
4		<i>Klebsiella pneumoniae</i>	Piperacillin tazobactam sodium	Dead
5		<i>Klebsiella pneumoniae</i>	Piperacillin tazobactam sodium	Healing
6	none		Ceftriaxone	Dead
7		<i>Klebsiella pneumoniae</i>	Piperacillin tazobactam sodium	Healing
8		<i>Staphylococcus aureus, Klebsiella pneumoniae</i>	Mezlocillin	Healing
9		<i>Klebsiella pneumoniae</i>	Tigecycline, Piperacillin tazobactam sodium	Healing
10		<i>Klebsiella pneumoniae, Acinetobacter baumannii Pseudomonas aeruginosa</i>	Tigecycline, Ceftriaxone	Dead
11		<i>Klebsiella pneumoniae</i>	Tigecycline, cefoperazone sulbactam	Discontinues
12		<i>Klebsiella pneumoniae, Candida albicans, Serratia marcescens</i>	Vancomycin, Meropenem	Dead
13		<i>Acinetobacter baumannii, Staphylococcus aureus</i>	Vancomycin, Meropenem	Dead
14		<i>Pseudomonas aeruginosa, Serratia marcescens</i>	Tazobactam, Tigecycline	Dead

Considerable changes in oral microbial diversity and community structure between the VAP patients and healthy controls

To investigate whether the oral microbial diversity and structure are altered in the VAP group compared with the non-pneumonia control group, we first assessed the microbial (alpha) diversity within each individual. The Chao1 index and the Observed for characterizing richness_Specifications index, representing Faith's index based on evolutionary diversity_ The PD index was $P < 0.05$, indicating a

significant difference between the two groups in terms of species richness and diversity. The VAP group had lower species richness and diversity than did the control group; Pielou's evenness index ($P > 0.05$, indicates) indicated that there was no statistically significant difference in species evenness between the two groups. (Fig. 1a); Beta Diversity analysis: Using QIIME2 (2019) software, a two-dimensional scatter plot was drawn using unweighted UniFrac or unweighted Jaccard principal coordinate analysis (PCoA). It was found that the distance between the experimental group samples was farther and the difference was greater, while the distance between the control group samples was closer and the difference was smaller. The 95% confidence ellipses of the two groups did not overlap or overlapped less, indicating a significant difference in bacterial diversity between the two groups. (Fig. 1b). Taken together, these findings revealed considerable changes in oral microbial diversity and structure in the VAP group.

Phylogenetic alteration of the oral microbiota

Krona software was used to create a species composition map and interactively display the community taxonomic composition. In the control group, the proportion of *Firmicutes* (58%) exceeded half (mainly *Streptococcus* accounted for 43%), followed by *Proteobacteria* (25%, *Neisseria* accounted for 13%, and *Haemophilus* accounted for 6%) and *Bacteroidetes* (12%), with the least being *Actinobacteria* (3%), *Clostridium* (2%), and *Spirochetes* (0.6%), which is similar to the results reported in the literature (Fig. 2a). In the experimental group, *Proteobacteria* (41%) was the most abundant (*Acinetobacter* accounted for 14%, *Pseudomonas* accounted for 11%, and *Klebsiella* accounted for 11%), *Firmicutes* (27%, *Enterococcus* 9%, *Streptococcus* 4%, *Gemella* and *Vibrio* all accounted for 3%) was the second placemost abundant phylum, followed by *Bacteroidetes* (24%), while *Spirochetes* (4%), *Actinobacteria* (3%), and *Clostridium* (0.9%) were the least abundant. (Fig. 2b). A Venn diagram was created for two groups of ASV/OTU abundance tables, revealing a total of 467 ASV/OTUs shared by the two groups, accounting for only 9.61% (Fig. 2c). At the phylum level, the two common species were mainly *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, while at the genus level, they were mainly *Streptococcus*, *Acinetobacter*, *Alloprevotella*, *Pseudomonas*, and *Neisseria*. (Fig. 2d). Heatmap showed that the VAP group had significant differences in microbial distribution compared to the control group. The abundance of *Klebsiella*, *Escherichia*, *Eikenella*, *Bacteroidales*_[G-2], *Selenomonas*, and *Pseudomonas* in the VAP group was significantly higher than that in the control group, while the abundance of *Prevotella*, *Cupriavidus*, and *Aggregatibacter* in the experimental group was relatively lower than that in the non-pneumonia control group. (Fig. 2e). The Manhattan plot suggests that the control group significantly upregulated *Actinobacteria*, *Firmicutes*, *Clostridium*, and *Proteobacteria* at the phylum level, with *Firmicutes* having the highest abundance; At the order level, *Actinobacteria*, *Bacillus*, *Lactobacillus*, *Vibrio*, *Clostridium*, *Neisseria*, and *Pasteurella* are significantly upregulated. The experimental group also observed a significant upregulation of *Actinobacteria* and *Firmicutes*, but at the order level, they were significantly upregulated in the order of *Actinobacteria* and *Mycoplasma*, respectively. (Fig. 2f).

Oral microbial dynamics after oral care

Microbiome analysis of the oral swabs taken every 4 hours from 8 patients in the VAP oral care group, we found that oral microbiome composition changes drastically after oral care. In genus level, microbiome composition changes, but with different patterns. In VAP survival cases (case1, 3, 4, 8), two dynamic change patterns observed. In case 1 and 3, community diversity decreased after oral care, and the relative abundance of *prevotella* and *Alloprevotella* increased, *Alloprevotella* became the dominance taxa in oral microbiome, followed by *prevotella*, *streptococcus*, and other oral anaerobic commensals. In case 4 and 8 another pattern observed, community structure had dynamic changes, but with a relative higher community diversity. In case 4 and 8, oral microbial diversity changes, but microbial community was not dominated by single genus. The community have relative high abundance of *streptococcus*, *Alloprevotella* and other commensals (Fig. 3a-d). In VAP dead cases (case2, 5, 6, 7), the oral microbial diversity decreases 4hours after oral care, and the relative abundance of pathogens and pathobionts increased. In case 2, the relative abundance of *Klebsiella* and *Acinetobacter* gradually increased after oral care, and oral microbiota is dominated by *Klebsiella*, *Acinetobacter* and *Alloprevotella*. In case 5, the abundance of oral anaerobic commensal increased after 4 hours, then the abundance of *Acinetobacter* and *enterococcus* increases. In case 6, the abundance of oral commensal microbiota rapidly decreased after oral care, and *enterococcus* became the dominant genus of oral microbiota. In case 7, the oral microbiota is dominated by *alloprevotella* and *Acinetobacter*. (Fig. 3e-h)

Principal component analysis (PCA) and Principal coordinate analysis (PCoA) was used to assess the composition change after oral care, showed the first turning point at the 4th hour after oral care, and then microbiota underwent compositional change. 24 hours after oral care, the microbiota underwent a drastic change again (Fig. 3i-k)

Characteristics of the oral microbiota and clinical prognosis in patients with VAP

A comparative analysis was conducted on the top 20 bacterial genera in the 24-hour oral swab samples of 14 patients in the experimental group and 7 patients in the non-pneumonia control group. The relative abundance of each bacterial genus is represented by the height of the bar chart, indicating that *Streptococcus*, *Neisseria*, *Prevotella*, *Vibrio* and *Haemophilus* were the dominant bacterial genera in the non-pneumonia control, and the diversity of the oral microbiota was relatively high; The diversity of the oral microbiota in the VAP patients was significantly reduced, with a significant decrease in the relative abundance of the aforementioned genera. The abundance of opportunistic pathogenic bacteria such as *Prevotella*, *Klebsiella*, *Acinetobacter*, and *Pseudomonas* increased significantly, consistent with the findings for pathogenic bacteria cultured in the lungs. In dead cases (case2,5,6,7,10,12,13,14), Severe damage to the diversity of the oral microbiota was observed and pulmonary pathogenic bacteria became the absolute dominant bacterial genus in the oral cavity. In the oral care group, the diversity of the oral microbiota in deceased patients was severely disrupted, and the diversity of the oral microbiota gradually decreased within 24 hours after oral care (Fig. 4).

Functional metabolic analysis

The core of the KEGG database is the Biological Metabolic Pathway Analysis Database (KEGG Pathway Database), <http://www.genome.jp/kegg/pathway.html>). Among these pathways, metabolic pathways are divided into six categories: metabolism, genetic information processing, environmental information processing, cellular processes, organic systems, and human diseases. Each category of metabolic pathways was further divided into multiple levels. At present, the second level includes a total of 45 subfunctions of metabolic pathways. We calculated the abundance of secondary functional pathways in the KEGG database, and no significant differences were found between the two groups. (Fig. 5a-b).

Discussion

Existing evidence shows that poor oral hygiene and dysbiosis of the oral microbiota contribute to healthcare-associated infections and impact clinical outcomes [17, 18]. We confirmed that the oral microbiome in patients with VAP significantly differs from non-pneumonia healthy controls. This distinction could have implications for clinical outcomes. To confirm the relationship between oral microbiota dysbiosis and the onset of pneumonia, we discovered that, in addition to the significant impact pneumonia has on the microbiota of stroke patients, various interventions, such as antibiotic treatment, invasive airway manipulation, oral hygiene maintenance, and time spent in the ICU, can also influence the microbial composition. Therefore, it is probable that this dysbiosis occurs due to stress caused by factors related to illness, invasive treatments, and pharmacological interventions. In our study, oral microbiota characteristics were significantly correlated with VAP. Compared with those in the non-pneumonia control group, the abundances of *Streptococcus oralis* and *Neisseria* in the VAP group decreased, suggesting that they were healthy core flora, which was consistent with previous reports [19]. In previous studies, oral *Streptococcus* was considered a normal commensal microorganism in stroke patients. Patients in the intraoral streptococcal enrichment group had greater species diversity and fewer systemic infections, and *Streptococcus salivarius* considered to be probiotic that can maintain the balance of oral flora by producing bacteriocin [20]. The oral cavity is the organ with the greatest diversity of *Prevotella* [21]. In previous studies, *Prevotella* was thought to be associated with poor oral hygiene and involved in oral diseases such as gingivitis and periodontitis [22, 23]. *Prevotella* forms a *Streptococcus*-like filamentous chain that promotes the formation of biofilms by *Actinomyces* through interspecific molecular regulation [24] and is therefore considered a risk factor for poststroke pneumonia. However, in our study, a decreased abundance of *Prevotella* was associated with one of the hallmarks of poor prognosis in patients with VAP. *Veillonella* and *Streptococcus* are important early oral colonizing bacteria, that establish a stable symbiotic relationship through glucose metabolism, signal exchange, gene regulation and other pathways. *Streptococcus* colonizes through binding to the acquired pellicle receptor that covers the tooth surface by binding to adhesin, and copolymerizes with other early and late colonizing bacteria, especially *Veillonella*, which acts as a "bridge" [25, 26]. We found that *Veillonella*, in combination with *Streptococcus*, can be used as a risk factor for predicting the prognosis of patients with VAP. In critically ill patients, the combined effects of invasive mechanical ventilation, broad-

spectrum antimicrobial exposure, and hyperoxia drive a niche transition in the oral and upper airway microbiota from communities dominated by obligate-anaerobic commensals (e.g., *Prevotella*, *Veillonella*, *Fusobacterium*) toward pathogen-enriched assemblages composed of facultative aerobes and classical respiratory pathogens (e.g., members of *Proteobacteria*, *Staphylococcus aureus*, *Pseudomonas spp.*). The accompanying loss of alpha diversity and “colonization resistance” implies attenuation of acidification, bacteriocin activity, and metabolite-mediated buffering within the upper airway–lung axis, culminating in a low-diversity, pathogen-enriched dysbiosis that is plausibly linked to adverse outcomes[27–29].

At the population level, a multicenter, large Invasive mechanical ventilation cohort with longitudinal, multi-compartment profiling (oral–lung–gut) demonstrated time-dependent declines in obligate-anaerobic commensals and concomitant increases in potential pathogens in both oral and pulmonary sites. Notably, low-diversity, pathogen-enriched clusters in the lower respiratory tract independently predicted survival beyond conventional clinical risk factors, while synchronous depletion of oral anaerobic commensals was observed at the mouth niche [27]. In a nested case–control study of IMV-associated pneumonia, low-diversity clusters were enriched for facultative anaerobes and classical pathogens and were associated with worse 60-day survival, whereas assignment to high-diversity clusters in oropharyngeal swabs correlated with improved survival—consistent with preservation of oral commensal anaerobes aligning with better prognosis [2]. Earlier work likewise linked combined dysbiosis across oral and tracheal sites—characterized by low diversity and reduced abundance of protective oral taxa—to worse 30-day survival and delayed liberation from mechanical ventilation after multivariable adjustment [30]. Collectively, these data support that depletion of oral commensal anaerobes is not merely a marker of dysbiosis but is robustly associated with clinically important outcomes.

From an immunological and functional perspective, commensal oral obligate anaerobes (e.g., *Prevotella*, *Veillonella*) have been associated with more favorable outcomes in lower respiratory infections and can engage multiple mucosal defense pathways (including epithelial barrier homeostasis, innate immune modulation, and metabolite signaling). Their depletion may therefore weaken local immunoregulation and facilitate pathogen colonization and ascension[28, 29]. These considerations suggest that preserving indigenous oral anaerobic commensals is clinically important for VAP prevention. Chlorhexidine (CHX) is an antiseptic used for oral care in intubated patients in many ICUs and has been demonstrated to reduce the incidence of VAP [31, 32]. On the other hand, similar to antibiotics, CHX induces bacterial resistance and hypersensitivity [33, 34]. Our dynamic microbiota monitoring found obvious turning points of oral microbial composition at 4 h and 24 h after oral care. The relatively stable microbial structure between the two time windows indicates that the timing of CHX-based oral care can be optimized focusing on the 4-hour and 24-hour nodes. This finding provides a theoretical basis to rethink the conventional oral nursing schedule performed every 6–8 h in routine ICU practice, implying that the frequency and intervention window of chlorhexidine wiping may need individualized adjustment to match the rhythm of oral microbiota fluctuation.

The optimal timing and frequency of oral care for patients who undergo endotracheal intubation remain controversial. Through sequencing technology, we detected changes in the oral microbiota of the experimental group over time. We found that the composition of the oral microbiota after oral care showed the first turning point at the 4th hour and again at the 24th hour; however, the structure of the microbiota in between, these two hours is relatively stable, and the optimal timing of oral care can be presumed to be within 4 hours after intubation and at 24 hours, For the frequency of traditional care [35], labor costs can be reduced, but the optimal frequency of oral care still needs to be confirmed by case–control studies with large samples in the later stage. This was a longitudinal prospective study in which the impact of initial treatment on microorganisms; decreased in patients in the inclusion group. For the first time, the correlation between dynamic changes in oral microecology and the prognosis of VAP patients was explored in detail, and it was proposed that the oral microbiota could be used as a potential marker for the early identification of VAP and guidance for treatment.

Limitations

There were several limitations to our study. First, this is a single-center exploratory study without an external validation cohort, which only preliminarily verified the dynamic alterations of oral microbiota. Second, subjects enrolled were poststroke ICU patients; these patients cannot take regular oral feeding, receive intensive antibiotic therapy and mechanical ventilation. Such ICU treatment and inpatient environment can inherently cause drastic shifts in indigenous microbiota independent of VAP occurrence, which may interfere with result interpretation. Besides, urgent clinical treatment also precluded standardized professional oral score assessment in enrolled individuals. Third, the sample size was relatively small, and we did not recruit intubated ICU patients without VAP as the optimal control group. Further multicenter, large-cohort studies incorporating this reasonable control group are required to validate our findings in future.

Conclusion

The diversity of the oral microbiota in VAP patients significantly reduced, and *Proteobacteria* is the primary dominant taxa, indicating that the oral microbiota composition and diversity are related to VAP. Within 4–8 hours after oral care, the diversity and abundance of the oral microbiota briefly increase, followed by rapid growth of dominant bacteria and a decrease in microbial diversity. Approximately 24 hours after oral care, the composition of the microbiota undergoes drastic changes again. In dead VAP cases, diversity of the oral microbiota deceased severely, and the abundance of anaerobic bacteria such as *Prevotella* and *Vibrio* reduced. The diversity of the oral microbiota and the proportion of anaerobic bacteria are related to the prognosis and outcome of VAP patients. Impaired microbial diversity and high abundance of pathogenic bacteria is related to poor prognosis.

Abbreviations

ICU

intensive care unit
VAP
ventilator-associated pneumonia
ASVs
amplicon sequence variants
OTUs
operational taxonomic units
PCA
Principal component analysis
PCoA
Principal coordinate analysis
CHX
Chlorhexidine

Declarations

Ethics approval and consent to participate

The clinical protocols involving the patients and the informed consent form were approved by the Ethics Committee of Dalian Central Hospital (No. YN2017-030-01). All participants provided written informed consent. All methods were performed in accordance with the relevant guidelines and regulations in ethics approval and consent to participate.

Clinical Trail

The observational clinical trial was registered on September 21, 2018, at the Chinese Clinical Trail Registry (ChiCTR) (<https://www.chictr.org.cn/>), registration number: ChiCTR1800018502.

Consent for publication

Not applicable.

Competing interests

All authors declare no competing interests.

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Author Contribution

Conceptualization: S.C., Y.Z.; analysis and interpretation of data: S.C., T.J. Y.Z.; project administration: N.Z.; methodology: S.C., T.J.; writing—original draft preparation: T.J., N.Z., J.Z; writing—review and editing: T.J., N.Z., J.Z.; visualization: T.J., J.Z; supervision: N.Z.; data curation: T.J., N.Z.; funding acquisition: S.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability

Sequencing datasets generated in current study are not publicly available but can obtain from corresponding authors on reasonable request.

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Figures

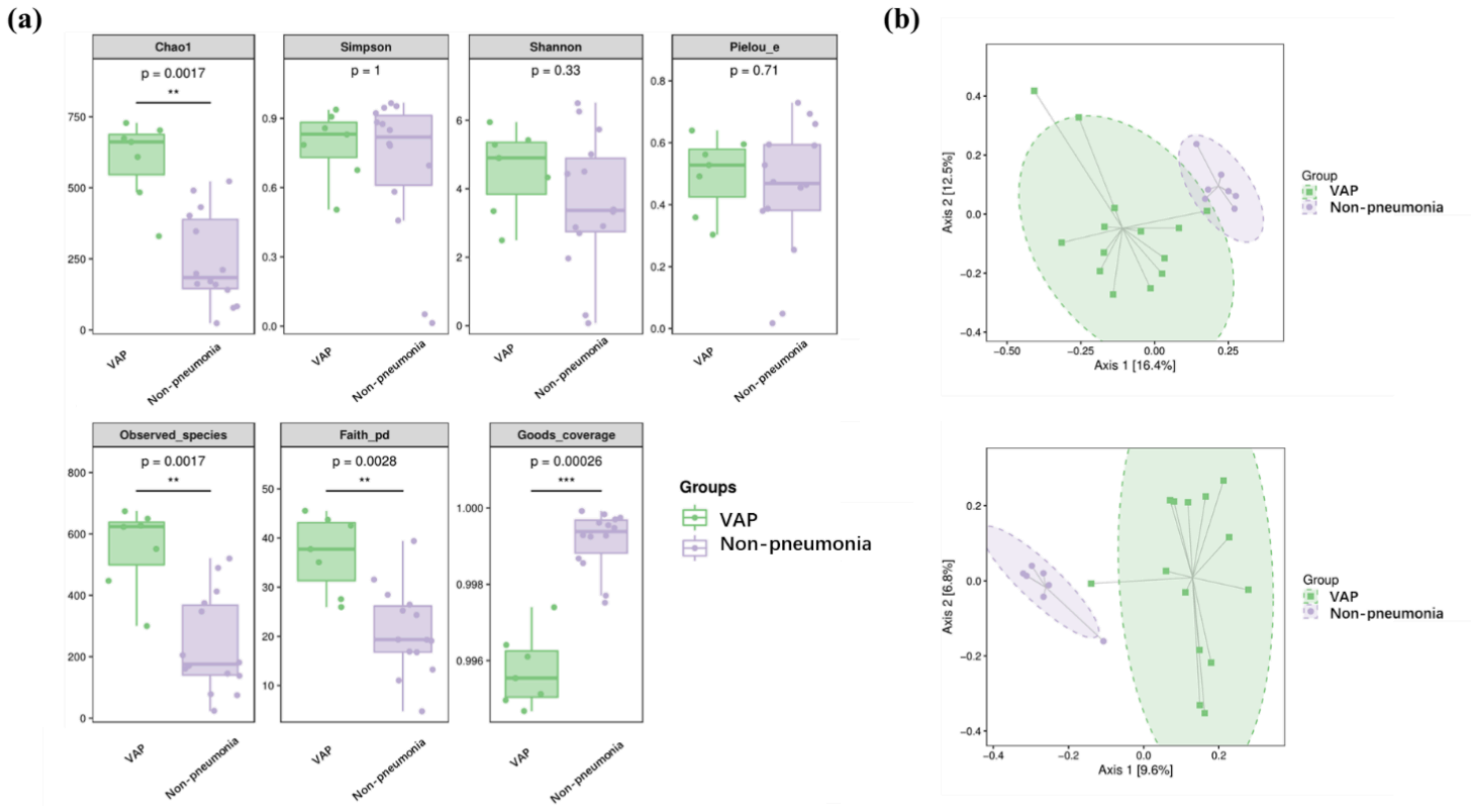


Figure 1

Oral microbial diversity and structure changes between VAP group and non- pneumonia healthy control. Oral microbiota of VAP patients significantly differs from healthy controls. (a)alpha-diversity index in non- pneumonia non-pneumonia and VAP group, including Chao1 index, Simpson index, Shannon diversity index, Pielou's evenness index, observed species richness, Faith's PD index and Goods coverage index, (b)beta-diversity differs between non-pneumonia group and experiment group. Unweighted UniFrac principal coordinate analysis (up panel) and unweighted Jaccard principal coordinate analysis (down panel) showed that oral microbiota of VAP patients differs from non-pneumonia.

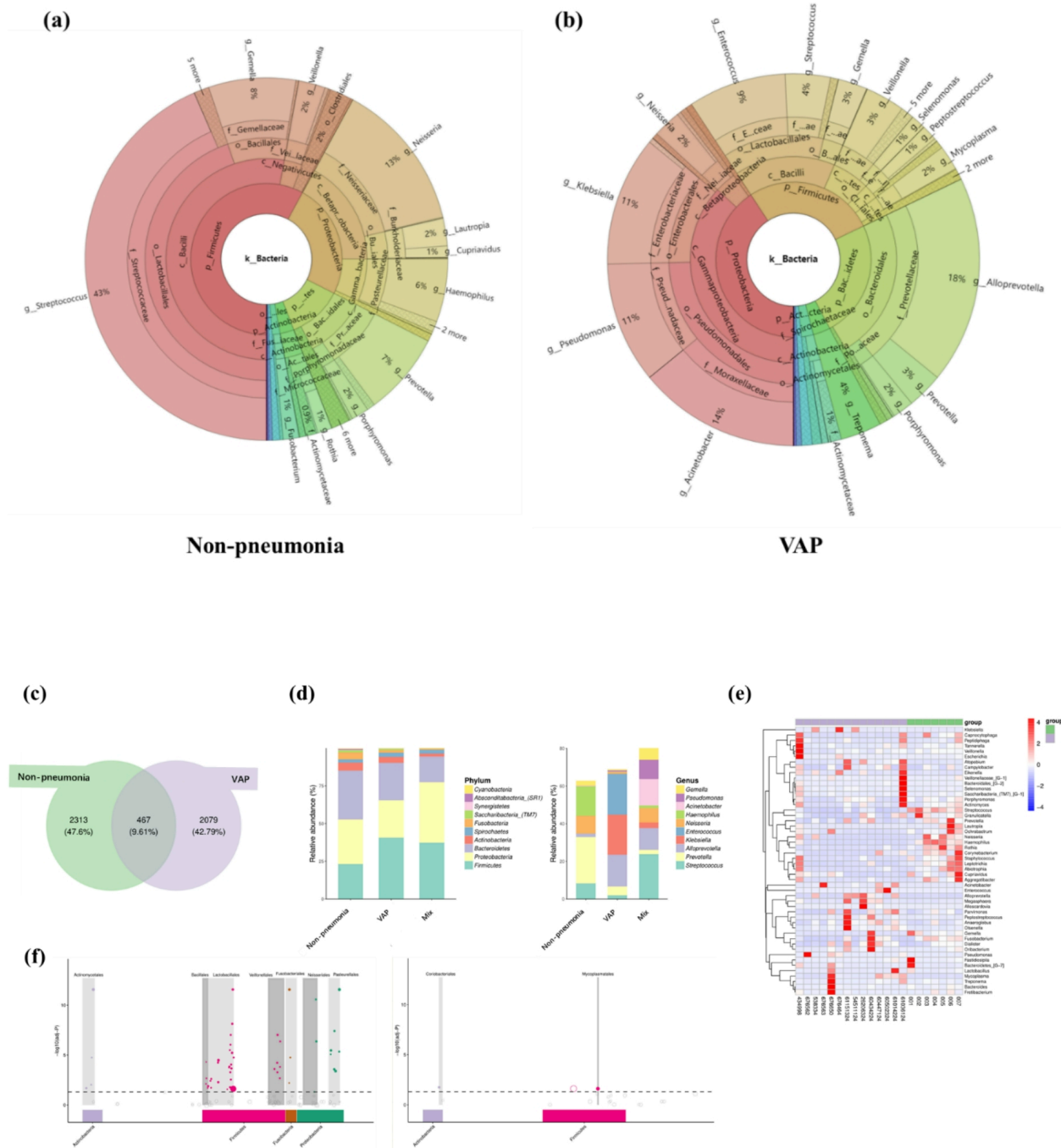


Figure 2

Phylogenetic alteration of the oral microbiota

Figure 2(a)-(f) shows of oral microbiota phylogenetic alterations between VAP patients and healthy controls. (a) Krona plot of healthy oral microbiome (non-pneumonia healthy control), (b) Krona plot of VAP patient's oral microbiome (VAP group), (c)Venn diagram shows unique and shared ASV/OTUs between VAP patients and non-pneumonia control, (d)relative sequence abundance percentage of 10

compositional differences in oral microbiota at different timepoint after oral care.

(a)-(h) shows the effect of oral care on oral microbiota in 8 VAP patients. Oral swabs took at 0, 4, 8, 12, 16, 20 and 24h, (i) principal component analysis of oral microbiota composition at different timepoints. (j)weighted and (k)unweighted uniFracprincipal coordinates analysis of microbial compositional changes at different timepoints.

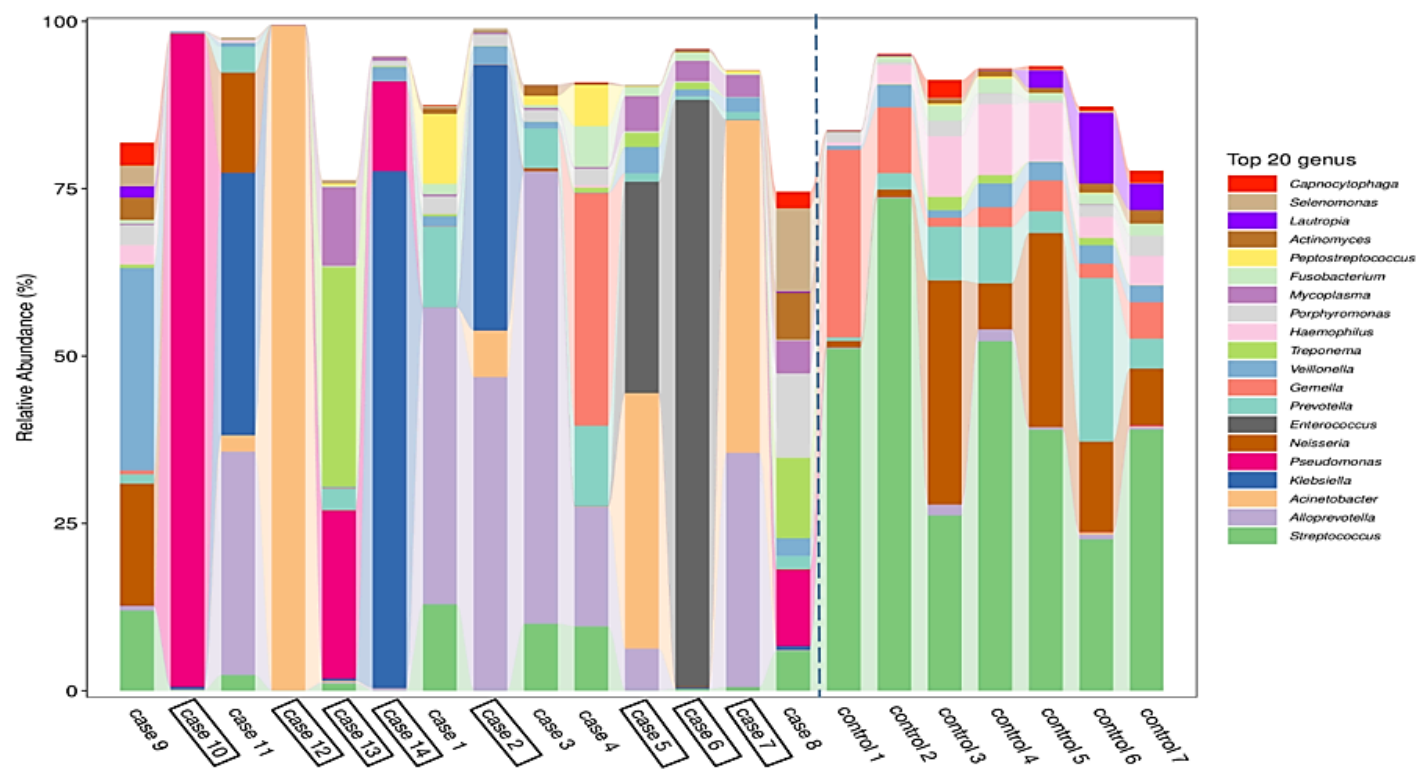
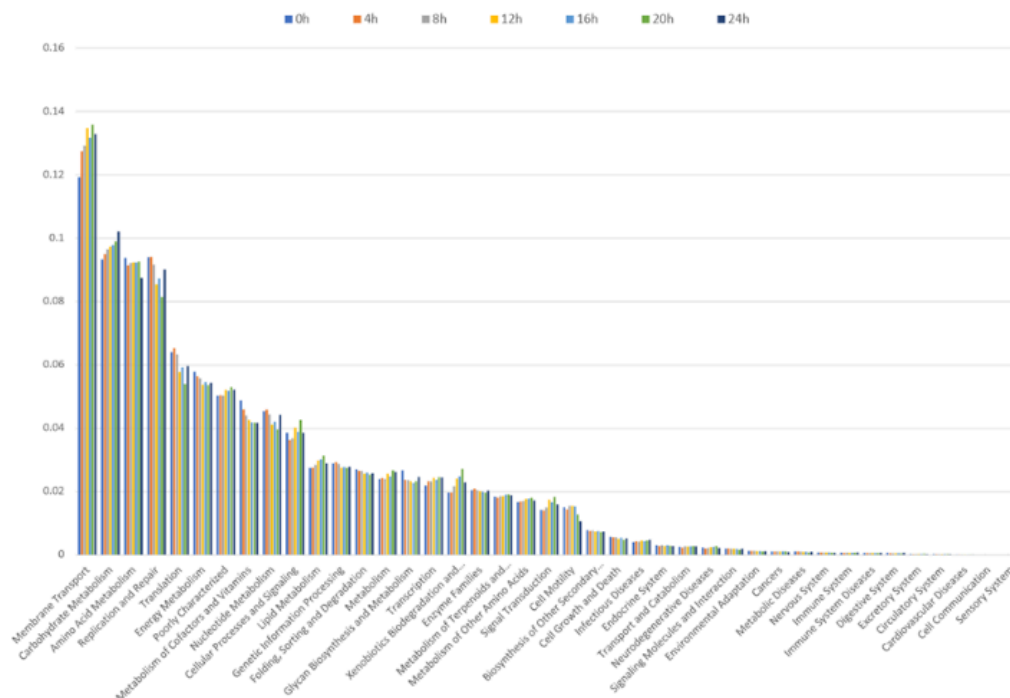


Figure 4

Top 20 bacterial genera in the 24-hour oral swab samples

most abundant 20 genera in oral swab samples. Control 1- 7 belongs to non- pneumonia control, case 1- 14 columns belong to VAP patients. Case 2, 5, 6, 7, 10, 12, 13, 14were dead cases.

(a)



(b)

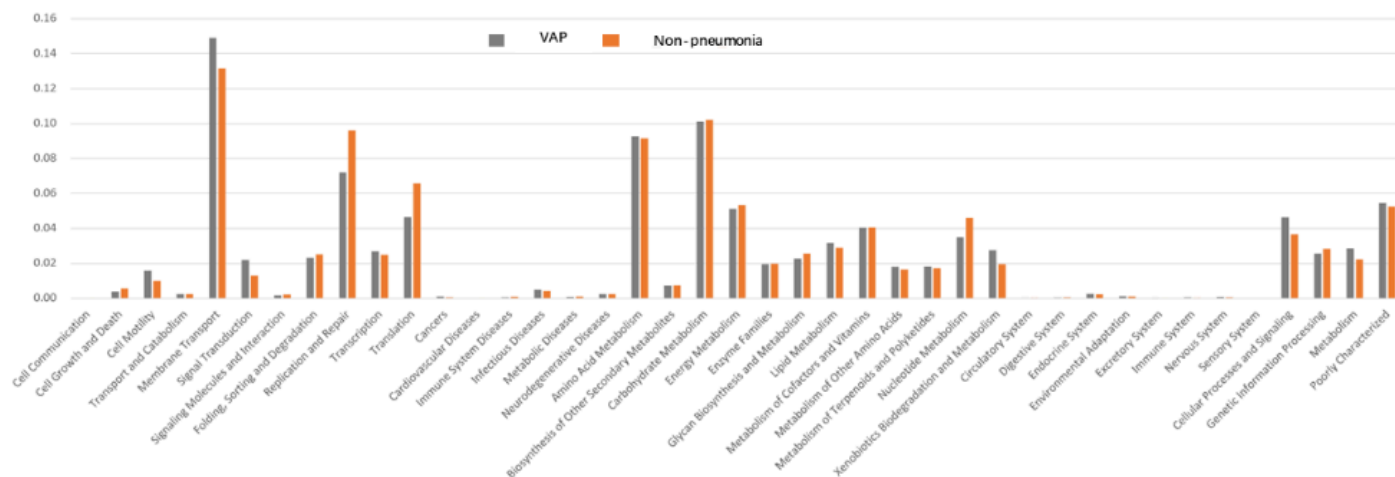


Figure 5

KEGG functional metabolic analysis

Figure 5(a)-(b) shows the KEGG functional metabolic analysis result. (a) time-related changes in community functional metabolism after oral care, (b) KEGG annotation result of VAP and non-pneumonia healthy control, no significant changes observed.

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