

Hypoalbuminemia and reduced sputum microbiome diversity associated with antibiotic treatment failure in nursing and healthcare-associated pneumonia

Naoki Hosogaya^{1,2,§,*}, Shoichi Fukui^{2,§}, Takahiro Takazono^{1,3}, Koki Fukushima¹, Ryosuke Morio¹, Satoshi Irifune¹, Shimpei Morimoto², Nana Nakada⁴, Masataka Yoshida¹, Kazuaki Takeda¹, Shotaro Ide¹, Naoki Iwanaga¹, Kazuki Nemoto⁵, Koichi Izumikawa³, Kazuhiro Yatera⁵, Katsunori Yanagihara⁶, Hiroshi Mukae¹

¹Department of Respiratory Medicine, Nagasaki University Hospital, Nagasaki, Japan;

²Clinical Research Center, Nagasaki University Hospital, Nagasaki, Japan;

³Department of Infectious Diseases, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan;

⁴Health Center, Nagasaki University, Nagasaki, Japan;

⁵Department of Respiratory Medicine, University of Occupational and Environmental Health, Japan, Fukuoka, Japan;

⁶Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan.

SUMMARY: Nursing and healthcare-associated pneumonia (NHCAP) pose significant challenges in older populations, yet factors predicting antibiotic treatment failure remain elusive. This exploratory secondary analysis of a multicenter phase IV trial aimed to identify the clinical and microbiome predictors of treatment failure in patients with NHCAP treated with lascefloxacin. Among the 56 evaluable patients (median age 86 years; cured $n = 44$, not cured $n = 12$), paired sputum and tongue samples were analyzed using 16S ribosomal RNA gene clone library sequencing. Alpha diversity was assessed using the Shannon index, Simpson index, observed richness, and Pielou's evenness, whereas beta diversity was calculated using Bray-Curtis dissimilarity and visualized by principal coordinate analysis. Serum albumin was significantly lower in not cured patients (3.0 vs. 3.5 g/dL, $p = 0.0497$) and emerged as the strongest predictor of treatment failure in univariate logistic regression (odds ratio 0.18, 95% confidence interval 0.05–0.73, $p = 0.016$). Sputum Pielou's evenness showed a comparable predictive ability (odds ratio 0.010, $p = 0.047$). The overall microbiome community composition did not differ according to the outcome. Notably, patients with hypoalbuminemia (< 2.85 g/dL) exhibited significantly reduced sputum alpha diversity (Shannon $p = 0.034$, Simpson $p = 0.025$, Pielou's evenness $p = 0.010$). A simple risk stratification combining hypoalbuminemia and denture use identified a high-risk subgroup with markedly elevated treatment failure rates (75.0% vs. 12.5%, $p = 0.001$). These findings suggest an interconnected pathophysiology linking nutritional status and respiratory microbiome stability in patients with NHCAP. Nutritional status and oral health may be modifiable targets for improving treatment outcomes in high-risk patients.

Keywords: oral microbiome, aspiration pneumonia, hypoalbuminemia, alpha diversity, denture, aged

1. Introduction

Nursing and healthcare-associated pneumonia (NHCAP) represent a significant clinical challenge in Japan's super-aged society (1). NHCAP frequently affects older patients with multiple comorbidities and impaired activities of daily living (2), with aspiration reported in approximately 68.6% of cases (3). Although the 30-day mortality rate is approximately 11.9%, host factors including non-ambulatory status, disturbance of consciousness, and hypoalbuminemia significantly worsen prognosis (3).

The oral-lung microbiome axis has emerged as

a key factor in the pathogenesis of pneumonia. The oral cavity serves as the primary source of the lung microbiome through continuous microaspiration, even in healthy individuals (4). Furthermore, the enrichment of the lung microbiome with oral taxa is associated with Th17-type pulmonary inflammation, suggesting that aspirated oral bacteria actively modulate lung immune responses (5). Under pathological conditions, Noguchi *et al.* demonstrated through clone library analysis of bronchoalveolar lavage fluid (BALF) that oral anaerobes were frequently detected in patients with healthcare-associated pneumonia (6). Similarly, 16S rRNA gene

analysis of BALF from Japanese patients with hospital-acquired pneumonia revealed that oral streptococci were the most prevalent organisms (23.1%), followed by *Corynebacterium* spp. (11.6%), *Haemophilus* spp. (6.8%), *S. aureus* (6.8%), and *P. aeruginosa* (5.4%) (7).

Accumulating evidence supports the clinical significance of oral microbiome in regard to predicting pneumonia outcomes. A prospective cohort study of nursing home residents demonstrated that tongue microbiota dominated by *Prevotella* and *Veillonella* were significantly associated with increased pneumonia mortality, with an adjusted hazard ratio of 13.88 for pneumonia-related death (8). Consistent with this finding, a randomized controlled trial in Japanese-assisted living facilities showed that professional oral care reduced pneumonia incidence, fever duration, and mortality (9). However, while these studies have focused on the oral environment, pneumonia development, and mortality, the relationship between the oral microbiome composition and response to antibiotic treatment remains elusive.

A recent multicenter phase IV trial reported an overall clinical efficacy rate of 78.6% for lascufloxacin in patients with NHCAP at test-of-cure (TOC) (10). The present study was a secondary analysis of a trial aimed at identifying microbiome signatures and clinical factors associated with treatment failure. We hypothesized that specific oral bacterial profiles, particularly those associated with poor oral hygiene and denture use, would be enriched in patients in whom antibiotic treatment fails. Our objectives were to: (1) characterize the clinical predictors of treatment failure; (2) identify the bacterial profiles associated with treatment outcomes; and (3) explore the relationship between the oral microbiome and treatment response.

2. Methods

2.1. Study design and data source

This was a secondary analysis of a multicenter, open-label, phase IV clinical trial evaluating lascufloxacin for NHCAP (10). The original trial was conducted at 24 sites in Japan between December 2020 and September 2023. The primary trial enrolled patients with NHCAP, defined according to the Japanese Respiratory Society guidelines (1) who were treated with lascufloxacin 75 mg once daily for 7-10 days. This study conformed to the principles of the Declaration of Helsinki (as revised in 2013). The trial was approved by the Clinical Research Review Board in Nagasaki University (approval number: CRB20-023), and written informed consent was obtained from all participants or their legal representatives. This trial is registered with the Japan Registry of Clinical Trials (jRCTs071200066).

2.2. Participants

Of the 75 patients who provided written informed consent to participate in the original trial, 56 met the eligibility criteria and had evaluable outcomes at the TOC visit, comprising the full analysis set (FAS) for the secondary analysis. The primary outcome was the treatment response at TOC, classified as 'cured' (complete resolution of signs and symptoms) or 'not cured' (persistent or worsening of clinical status).

2.3. Clinical assessment

The baseline clinical data included demographics, laboratory parameters, oral health status (remaining teeth, denture use), and aspiration risk. Severity was assessed using the A-DROP (Age, Dehydration, Respiratory failure, Orientation disturbance, low blood Pressure) score (11).

2.4. Microbiome sample collection and processing

Paired sputum and tongue dorsum samples were collected at baseline (pretreatment). The sputum samples were collected *via* spontaneous expectoration. Tongue samples were collected by swabbing the dorsal surface of the tongue. The samples were then immediately stored at -80°C until DNA extraction.

2.5. 16S rRNA gene sequencing and clone library analysis

The bacterial flora were analyzed according to the clone library method using amplified fragments of the 16S ribosomal RNA gene, as previously described by Noguchi *et al.* (6). Briefly, DNA was extracted from the specimens by vigorous shaking with sodium dodecyl sulfate (final concentration: 3.0%) and glass beads. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers. The PCR products were cloned using a TOPO TA cloning kit (*Invitrogen*), and colonies were randomly selected from each clone library for sequencing analysis. Sequences were compared with an in-house database containing the 16S rRNA gene sequences of the type strains using a basic local alignment search tool algorithm. This method demonstrated superior detection of oral bacteria, including streptococci and anaerobes, compared to conventional cultivation methods for healthcare-associated pneumonia (6).

2.6. Statistical analysis

Continuous variables were compared using the Wilcoxon rank-sum test and categorical variables were compared using Fisher's exact test. Alpha diversity was assessed using four indices: the Shannon index (12), Simpson index (13), observed species richness, and Pielou's evenness index (14). Beta diversity was calculated using

Bray-Curtis dissimilarity (15), visualized by principal coordinate analysis (PCoA) (16), and statistically evaluated using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations (17).

Given the exploratory nature and limited sample size of this secondary analysis, with only 12 events (not cured) for the minority outcome, the differential abundance of the individual bacterial species was assessed using the Fisher's exact test for detection rates and the Wilcoxon rank-sum test for relative abundance, with *p*-values reported without adjustment for multiple comparisons.

The clinical predictors of treatment failure were evaluated using univariate and multivariable logistic regression analyses. The odds ratios (OR) with 95% confidence intervals (CI) were calculated. The optimal albumin cut-off value was determined using the Youden index (18). Statistical significance was defined as a two-sided *p*-value of < 0.05. All analyses were performed

using the R version 4.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Patient characteristics

The FAS comprised 56 patients (cured: *n* = 44; not cured: *n* = 12). The baseline characteristics are presented in Table 1. The median age was 86 years (IQR: 79.5–89), and 58.9% were male. Denture use was common (67.9%), and the median number of remaining teeth was 10.5 (IQR: 0–21.2). Most patients had moderate-severity pneumonia according to the A-DROP score. Two groups showed comparable age, body weight, C-reactive protein, and pneumonia severity, but serum albumin (cured: 3.5 g/dL [3.1–3.7] vs. not cured: 3.0 g/dL [2.4–3.5], *p* = 0.0497) was significantly different. Denture use tended to be higher in the not cured patients than in the cured patients

Table 1. Baseline characteristics of the study population

Characteristic	Overall (N = 56)	Cured (n = 44)	Not Cured (n = 12)	<i>p</i> -value
Demographics				
Sex, Male/Female	33/23	24/20	9/3	0.322
Age (years)	86.0 (79.5–89.0)	86.0 (79.5–89.0)	85.0 (81.0–89.2)	0.936
Body weight (kg)	46.0 (40.5–49.2)	46.0 (40.4–49.8)	45.8 (40.5–47.9)	0.881
Underlying diseases, <i>n</i> (%)	41 (73.2)	32 (72.7)	9 (75.0)	1.000
NHCAP criteria, <i>n</i> (%)				
Resident in long-term care facility	23 (41.1)	17 (38.6)	6 (50.0)	0.522
Hospitalization within 90 days	19 (33.9)	14 (31.8)	5 (41.7)	0.516
Elderly requiring nursing care	40 (71.4)	31 (70.5)	9 (75.0)	1.000
Continuous endovascular treatment	5 (8.9)	5 (11.4)	0 (0.0)	0.574
Risk factors for resistant bacteria, <i>n</i> (%)				
Any	23 (41.1)	19 (43.2)	4 (33.3)	0.743
Antimicrobial use within 90 days	15 (26.8)	11 (25.0)	4 (33.3)	0.715
Hospitalization within 90 days	22 (39.3)	17 (38.6)	5 (41.7)	1.000
Nursing home/long-term care	10 (17.9)	6 (13.6)	4 (33.3)	0.196
Enteral feeding	0 (0.0)	0 (0.0)	0 (0.0)	–
A-DROP score	2.0 (1.0–2.0)	2.0 (1.0–2.0)	1.5 (1.0–2.0)	0.778
Risk for aspiration (SSA), <i>n</i> (%)				
Concerned	13 (23.2)	10 (22.7)	3 (25.0)	1.000
Not concerned	43 (76.8)	34 (77.3)	9 (75.0)	
Oral status				
Remaining teeth	10.5 (0.0–21.2)	14.0 (0.0–22.5)	3.5 (0.8–16.8)	0.402
Denture use, Yes/No	38/18	27/17	11/1	0.079
Vital signs				
Body temperature (°C)	37.0 (36.6–37.8)	37.0 (36.6–37.7)	37.0 (36.7–37.8)	0.873
Systolic blood pressure (mmHg)	123.0 (109.2–143.5)	123.0 (106.8–145.2)	123.0 (113.8–141.5)	0.952
SpO ₂ (%)	96.0 (93.8–97.0)	96.0 (93.0–97.2)	96.0 (94.8–96.2)	0.984
Laboratory data				
White blood cell count (×10 ³ /μL)	9.4 (7.4–13.0)	10.3 (7.0–13.5)	8.1 (7.6–9.2)	0.353
Neutrophils (%)	80.2 (73.2–84.7)	80.2 (72.3–85.7)	80.6 (78.7–82.3)	0.826
Lymphocytes (%)	11.8 (8.6–15.4)	11.4 (8.3–16.1)	12.2 (10.8–13.7)	0.562
Hemoglobin (g/dL)	11.6 (10.5–12.3)	11.6 (10.5–12.4)	10.6 (10.4–11.8)	0.242
Platelets (×10 ⁴ /μL)	21.2 (16.8–24.9)	20.2 (16.5–24.6)	23.4 (21.1–26.7)	0.062
CRP (mg/dL)	4.5 (2.3–10.3)	3.9 (2.3–9.8)	7.5 (3.9–11.8)	0.161
Albumin (g/dL)	3.5 (3.0–3.7)	3.5 (3.1–3.7)	3.0 (2.4–3.5)	0.0497
Total protein (g/dL)	6.8 (6.1–7.2)	6.8 (6.1–7.2)	6.6 (6.3–7.2)	0.718

Values are presented as median (IQR), *n* (%), or *n/n*. The Fisher's exact test was used to analyze categorical variables. The Wilcoxon rank-sum test was used to analyze continuous variables. Abbreviations: IQR, interquartile range; NHCAP, nursing and healthcare-associated pneumonia; SSA, Standardized Swallowing Assessment; CRP, C-reactive protein.

(91.7% vs. 61.4%, $p = 0.079$).

3.2. Alpha diversity in microbiome and treatment outcome

The overall alpha diversity of the sputum and tongue microbiomes did not differ significantly between the two groups. For sputum samples, Pielou's evenness showed a trend toward lower values in not cured patients ($p = 0.086$), whereas other indices showed no significant associations (Shannon $p = 0.348$, Simpson $p = 0.213$, observed richness $p = 0.787$; Figure 1A). For the tongue samples, no indices showed any significant differences between the two groups (Shannon $p = 0.686$, Simpson $p = 0.745$, observed richness $p = 0.575$, Pielou's evenness $p = 0.836$; Figure 1B).

3.3. Correlation between sputum and tongue alpha diversity

The correlation between sputum and tongue diversity indices was found to be moderate to strong (Figure 2). Shannon index showed significant correlation ($\rho = 0.420$, $p = 0.002$), as did Simpson index ($\rho = 0.339$, $p = 0.012$)

and observed richness ($\rho = 0.450$, $p < 0.001$). Pielou's evenness showed a weaker, non-significant correlation ($\rho = 0.250$, $p = 0.069$).

3.4. Beta diversity

Hierarchical clustering heat maps of the bacterial species demonstrated substantial inter-individual variation without clear clustering by outcome (Figure 3A). Beta diversity analysis by PCoA and PERMANOVA showed no significant differences in the community composition between the outcome groups for sputum ($R^2 = 0.0127$, $p = 0.863$) or tongue samples ($R^2 = 0.0147$, $p = 0.700$) (Figure 3B). Paired analysis of sputum versus tongue samples revealed significant differences in the community composition between the sample types (PERMANOVA $R^2 = 0.036$, $p = 0.001$; Figure 3C).

3.5. Exploratory analysis of individual bacterial species

In the exploratory differential abundance analysis, three sputum bacterial species showed significantly different detection rates between the outcome groups (Fisher's exact test, $p < 0.05$, uncorrected; Table 2): *Veillonella*

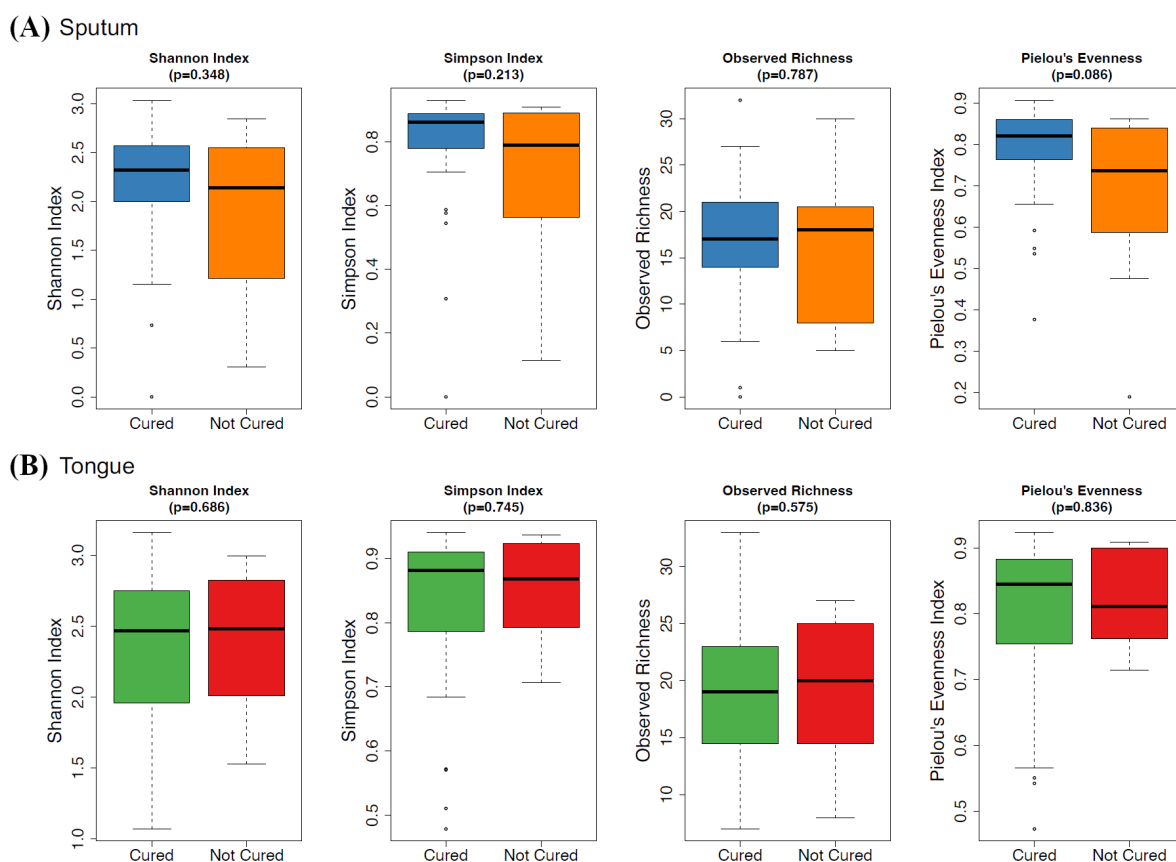


Figure 1. Alpha diversity of sputum and tongue microbiomes by treatment outcome. (A) Sputum microbiome alpha diversity indices comparing the cured ($n = 44$) and not cured ($n = 12$) patients. Shannon index ($p = 0.348$), Simpson index ($p = 0.213$), observed species richness ($p = 0.787$), and Pielou's evenness ($p = 0.086$) showed no significant differences, although evenness showed a trend toward lower values in not cured patients. (B) Tongue microbiome alpha diversity indices comparing cured and not cured patients. No significant differences were observed for Shannon index ($p = 0.686$), Simpson index ($p = 0.745$), observed richness ($p = 0.575$), or Pielou's evenness ($p = 0.836$). Box plots display median with interquartile range; whiskers extend to $1.5 \times$ IQR. P-values were calculated using Wilcoxon rank-sum test.



Figure 2. Correlation between sputum and tongue microbiome alpha diversity. Scatter plots showing paired correlations between the sputum (x-axis) and tongue (y-axis) alpha diversity indices for 56 patients. Shannon index ($\rho = 0.420$, $p = 0.002$), Simpson index ($\rho = 0.339$, $p = 0.012$), and observed species richness ($\rho = 0.450$, $p < 0.001$) showed significant positive correlations. Pielou's evenness showed a weaker, non-significant correlation ($\rho = 0.250$, $p = 0.069$). Spearman's rank correlation coefficients (ρ) and p -values are displayed for each comparison.

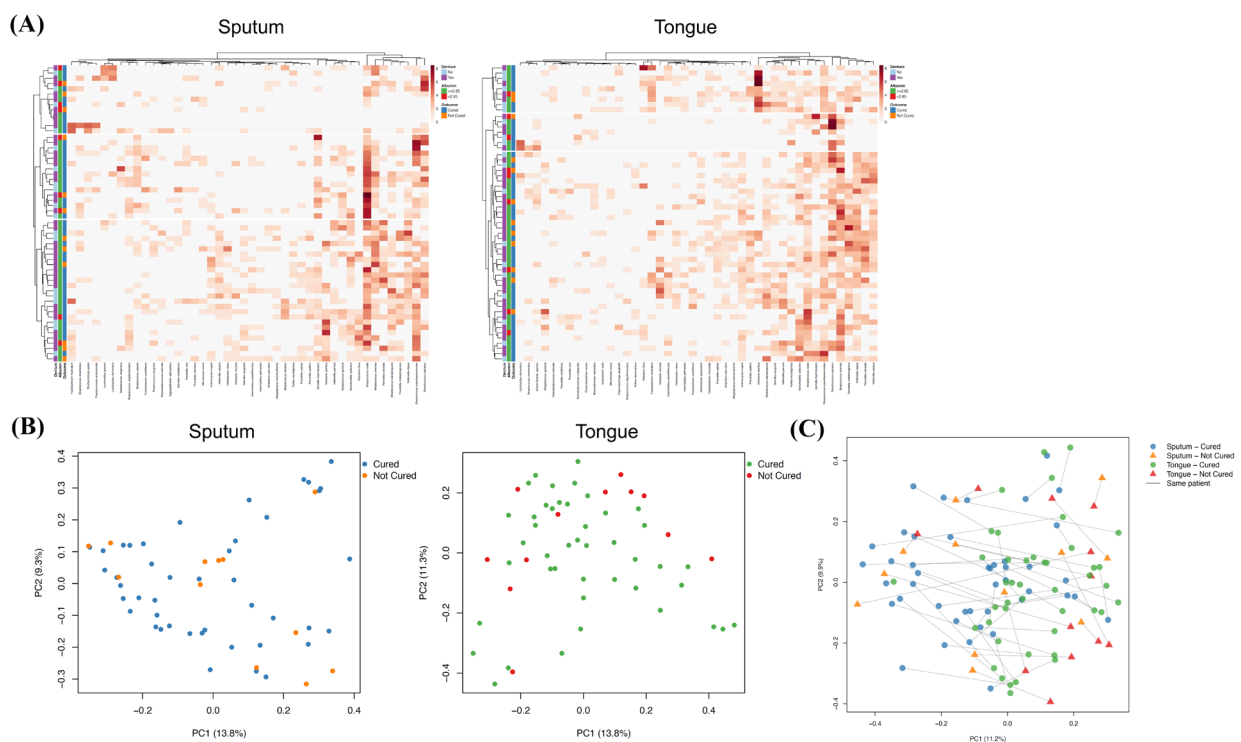


Figure 3. Beta diversity analysis of sputum and tongue microbiome. (A) Hierarchical clustering heatmaps of the bacterial species composition in the sputum (left) and tongue (right) samples. Color intensity represents square-root transformed relative abundance (%). Clinical annotations include treatment outcome (Cured/Not Cured), serum albumin status (≥ 2.85 or < 2.85 g/dL), and denture use. Bacterial species detected in fewer than 10% of patients (≤ 5 patients) were excluded from the heatmap visualization. (B) Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity for the sputum (left) and tongue (right) samples, with points colored by treatment outcome. No significant differences in community composition were observed between outcome groups for sputum ($R^2 = 0.0127$, $p = 0.863$) or tongue samples ($R^2 = 0.0147$, $p = 0.700$). (C) Paired PCoA comparing sputum and tongue samples from the same patients. Lines connect paired samples from the same individual. Community composition was significantly different between sample types (PERMANOVA $R^2 = 0.036$, $p = 0.001$).

rodentium (cured 0% vs. not cured 16.7%, $p = 0.043$), *Lautropia mirabilis* (0% vs. 16.7%, $p = 0.043$), and *Campylobacter showae* (0% vs. 16.7%, $p = 0.043$). All three species were detected exclusively in not cured patients and were absent in cured patients. However, these species were detected in only two of the 12 not cured patients (16.7%). *Actinomyces viscosus* showed a similar trend (4.5% vs. 25%, $p = 0.060$). In tongue samples, *Actinomyces graevenitzi* was significantly more frequent in not cured patients (18.2% vs. 58.3%,

$p = 0.010$), and *Corynebacterium matruchotii* was exclusively detected in not cured patients (0% vs. 16.7%, $p = 0.043$).

3.6. Clinical predictors of treatment outcome

Receiver operating characteristic (ROC) analysis revealed that serum albumin level had a moderate predictive ability for treatment failure (area under the curve [AUC] = 0.687; Figure 4A). Youden index

Table 2. Significant bacteria associated with treatment outcome (uncorrected $p < 0.05$)

Bacteria	Cured detected, n (%)	Not Cured detected, n (%)	p value for Fisher's exact test	Not Cured median proportion (IQR)	Cured median detected proportion (%), IQR	Not Cured median detected proportion (%), IQR	p value for Wilcoxon test
Sputum							
<i>Veillonella rodentium</i>	0 (0)	2 (16.7)	0.043	0 (0-0)	NA (NA)	1.26 (NA)	0.007
<i>Lautropia mirabilis</i>	0 (0)	2 (16.7)	0.043	0 (0-0)	NA (NA)	1.35 (NA)	0.007
<i>Campylobacter showae</i>	0 (0)	2 (16.7)	0.043	0 (0-0)	NA (NA)	1.49 (NA)	0.007
<i>Actinomyces viscosus</i>	2 (4.5)	3 (25)	0.060	0 (0-0.35)	1.39 (NA)	1.52 (1.46-2.01)	0.025
Tongue							
<i>Actinomyces graevenitzi</i>	8 (18.2)	7 (58.3)	0.010	1.35 (0-2.41)	2.33 (1.17-2.50)	2.35 (1.61-3.59)	0.004
<i>Corynebacterium matruchotii</i>	0 (0)	2 (16.7)	0.043	0 (0-0)	NA (NA)	5.91 (NA)	0.007

IQR: interquartile range; NA: not applicable (insufficient samples for calculation).

optimization identified 2.85 g/dL as an optimal cutoff (Figure 4B).

3.7. Clinical-microbiome correlations

In the sputum samples, patients with low albumin (< 2.85 g/dL) showed significantly lower alpha diversity compared to those with higher albumin (Figure 5A): Shannon index ($p = 0.034$), Simpson index ($p = 0.025$), and Pielou's evenness ($p = 0.010$). In contrast, denture use was not significantly associated with any sputum alpha diversity measure (Figure 5B). Tongue microbiome diversity did not differ significantly according to the albumin status (Figure 5C) or denture use (Figure 5D).

3.8. Logistic regression for treatment failure

In univariate logistic regression (Table 3), serum albumin was significantly associated with treatment failure (OR = 0.18, 95% CI: 0.05–0.73, $p = 0.016$), as was Pielou's evenness index in sputum (OR = 0.010, 95% CI: < 0.01 –0.95, $p = 0.047$). Denture use showed a trend to be associated with treatment failure (OR = 6.93, 95% CI: 0.82–58.58, $p = 0.076$).

Given that our study included only 12 not cured cases (the minority outcome), we included two explanatory variables in the multivariable model (events per variable [EPV] = 6), which is supported by Vittinghoff and McCulloch, who demonstrated that problems are uncommon with 5–9 EPV in their simulation study (19). In a two-variable model including both predictors, neither achieved statistical significance (albumin: OR = 0.26, 95% CI: 0.06–1.13, $p = 0.072$; Pielou: OR = 0.050, 95% CI: < 0.01 –7.68, $p = 0.246$).

3.9. Risk stratification

The patients were stratified into two risk groups based on a combination of albumin < 2.85 g/dL and denture use (Figure 6). Patients with both risk factors (High Risk group, $n = 8$) showed a markedly elevated treatment failure rate (75.0%, 6/8) when compared to Others (12.5%, 6/48; Fisher's exact test, $p = 0.001$).

4. Discussion

In this exploratory secondary analysis of the lascefloxacin phase IV trial, we identified serum albumin level as the strongest predictor of treatment failure in patients with NHCAP. Sputum microbiome evenness also showed predictive potential, whereas the overall microbiome community composition did not differ between outcome groups. Notably, patients with low albumin levels had significantly reduced sputum diversity. A simple risk stratification, combining low albumin levels and denture use, identified a high-risk subgroup with markedly elevated failure rates.

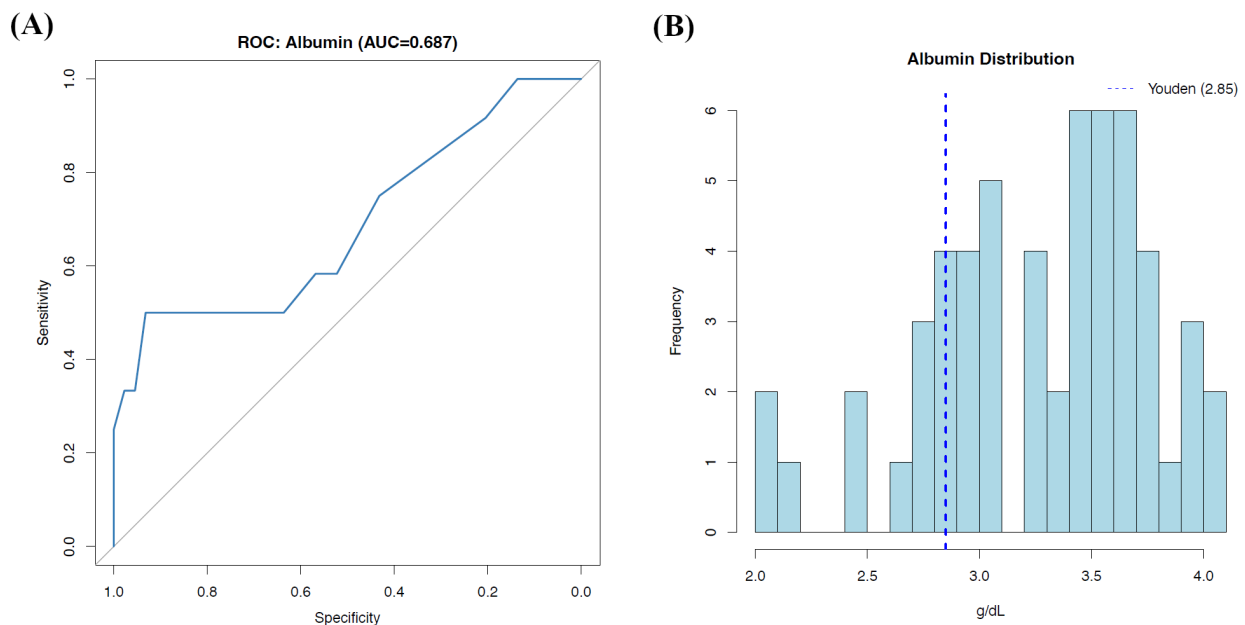


Figure 4. Serum albumin as a predictor of treatment failure. (A) Receiver operating characteristic (ROC) curve for serum albumin predicting treatment failure. Area under the curve (AUC) = 0.687. (B) Histogram showing the distribution of the baseline serum albumin levels in the study population. The vertical dashed line indicates the optimal cutoff (2.85 g/dL) determined by Youden index.

Serum albumin level emerged as the most robust predictor of treatment failure in univariate analysis. This finding is consistent with a previous large-scale study on community-acquired pneumonia that demonstrated that serum albumin levels at admission were independently associated with ICU admission and 30-day mortality (20). Similarly, hypoalbuminemia has been consistently associated with poor prognosis in patients with NHCAP in Japan (3).

Sputum Pielou's evenness, a measure of how evenly species are distributed within a community, showed predictive potential comparable to that of albumin, with lower evenness associated with treatment failure. This finding suggests that disruption of the microbiome balance, characterized by the dominance of a few taxa over others, may be more relevant to treatment outcomes than overall species richness. Indeed, different alpha diversity metrics capture distinct aspects of community structure, and evenness indices may reveal dysbiotic states that are not detected by richness alone (21).

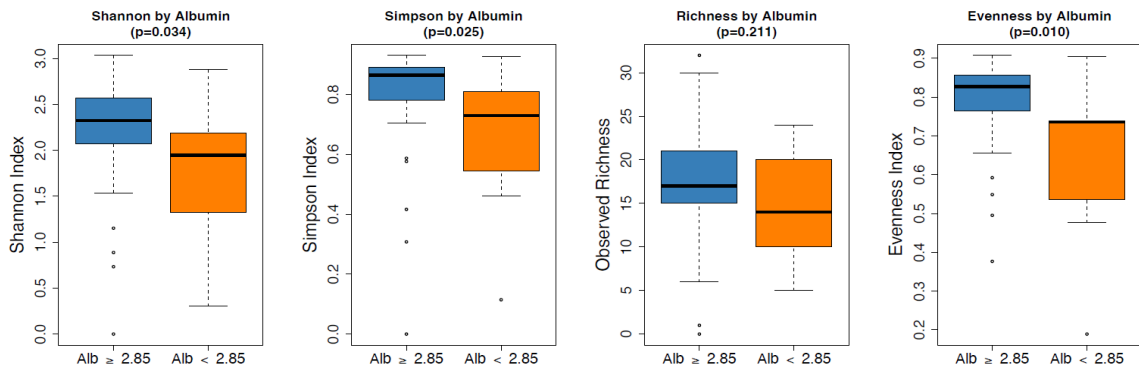
The relationship between albumin and alpha diversity suggests a shared underlying pathophysiology of NHCAP. Patients with low albumin (<2.85 g/dL) showed significantly reduced sputum alpha diversity across multiple indices (Shannon $p = 0.034$, Simpson $p = 0.025$, Pielou's evenness $p = 0.010$). Several causal relationships may explain this association: (1) malnutrition may impair immune function and mucosal integrity, promoting oral dysbiosis that extends to the lower respiratory tract; (2) reduced oral microbiome diversity may reflect impaired oral function, affecting nutrient intake; (3) a common upstream factor, such as frailty or systemic inflammation, may affect both; and (4) bidirectional effects may create

a vicious cycle of malnutrition, dysbiosis, and impaired host defense. This study precludes the determination of a causal direction, but the observed association suggests that nutritional status may be a key determinant of respiratory microbiome stability in aged patients with pneumonia.

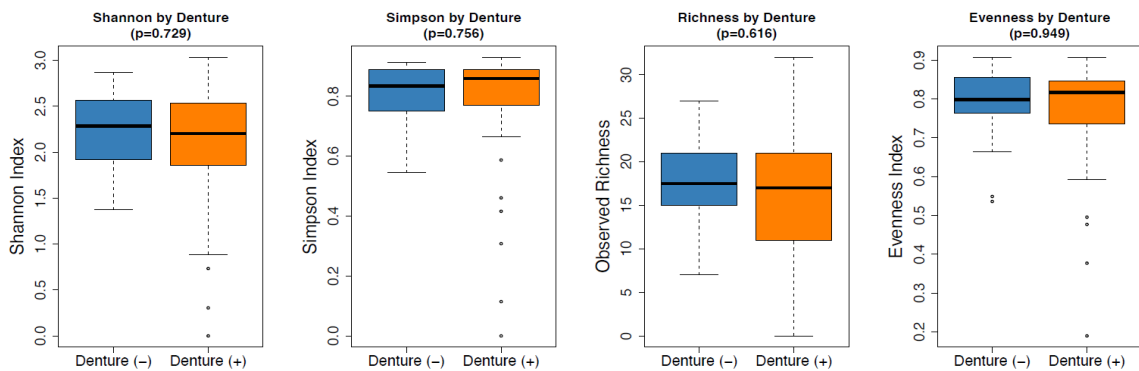
Bacterial species showing exploratory enrichment in patients with treatment failure share the biological characteristics relevant to oral dysbiosis. In sputum, *Veillonella rodentium*, *Lautropia mirabilis*, and *Campylobacter showae* were exclusively detected in not cured patients. *Veillonella* species were highly abundant in both supra- and sub-gingival biofilms (22). *Lautropia mirabilis* is a Gram-negative motile coccus originally isolated from the gingival margin and dental plaque of humans; its capacity for polysaccharide production suggests its possible participation in dental plaque formation (23). *Campylobacter showae* has also been isolated from periodontitis sites (24). However, the detection rate of these bacteria was found to be only 16.7% (2 of 12 not cured patients), severely limiting the reliability of these associations. These findings should therefore be regarded as preliminary exploratory observations, and the potential link between these specific bacterial species and treatment failure requires validation in larger and adequately powered studies.

Denture use showed a trend to be associated with treatment failure (91.7% vs. 61.4%, $p = 0.079$). Denture biofilms harbor complex microbial communities that may increase the aspiration risk (25), and poor oral hygiene in the aged population is associated with respiratory pathogen colonization (26). Interestingly, denture use was not significantly associated with sputum microbiome

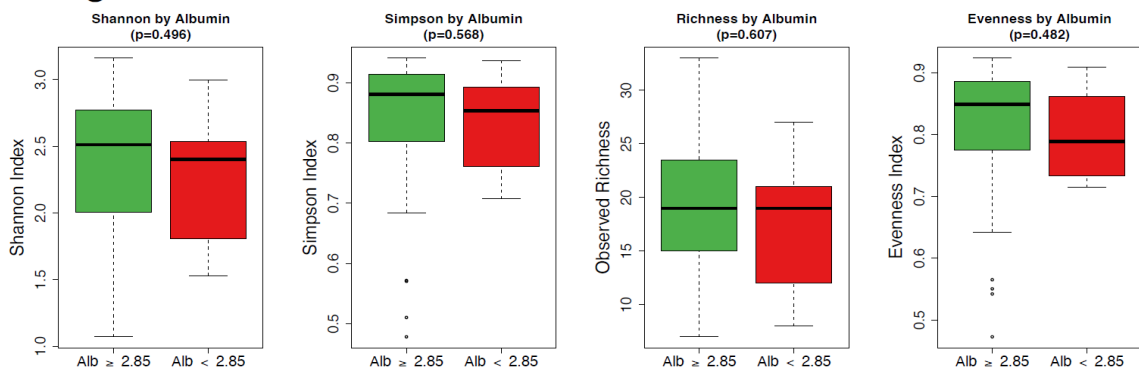
(A) Sputum



(B)



(C) Tongue



(D)

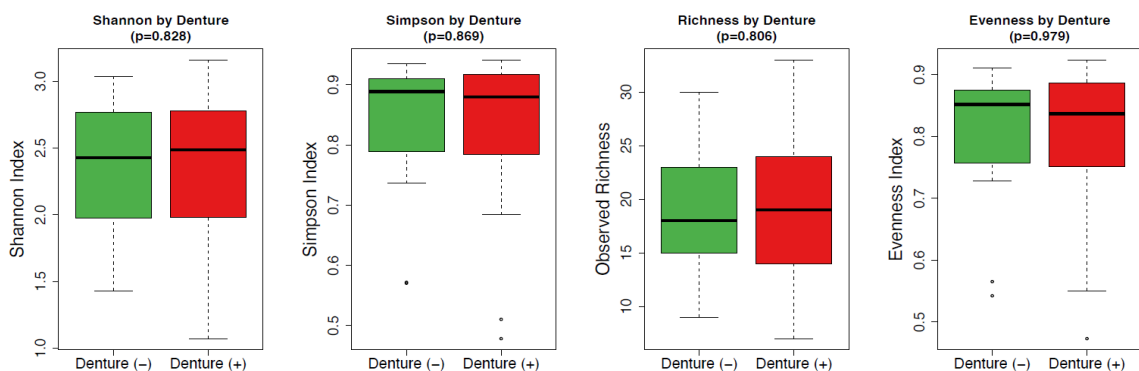


Figure 5. Alpha diversity of sputum and tongue microbiome stratified by albumin status and denture use. (A) Sputum microbiome alpha diversity by albumin status. Patients with low albumin (< 2.85 g/dL) showed significantly lower Shannon index ($p = 0.034$), Simpson index ($p = 0.025$), and Pielou's evenness ($p = 0.010$) compared to those with albumin ≥ 2.85 g/dL. (B) Sputum microbiome alpha diversity by denture use. No significant associations were observed. (C) Tongue microbiome alpha diversity by albumin status. No significant differences were detected. (D) Tongue microbiome alpha diversity by denture use. No significant associations were observed. P-values were calculated using Wilcoxon rank-sum test.

Table 3. Logistic regression analysis for treatment failure

Variable	Unadjusted			Adjusted		
	OR	95% CI	P	OR	95% CI	P
Serum albumin, g/dL	0.18	0.05–0.73	0.016	0.26	0.06–1.13	0.072
Pielou's evenness (sputum)	0.010	< 0.01–0.95	0.047	0.050	< 0.01–7.68	0.246
Denture use	6.93	0.82–58.58	0.076			
Simpson index (sputum)	0.11	0.01–2.10	0.141			
Shannon index (sputum)	0.54	0.21–1.37	0.192			
Sex, male	2.50	0.60–10.50	0.211			
CRP, mg/dL	1.05	0.95–1.16	0.346			

Abbreviations: OR, odds ratio; CI, confidence interval; CRP, C-reactive protein. Adjusted model includes serum albumin and Pielou's evenness.

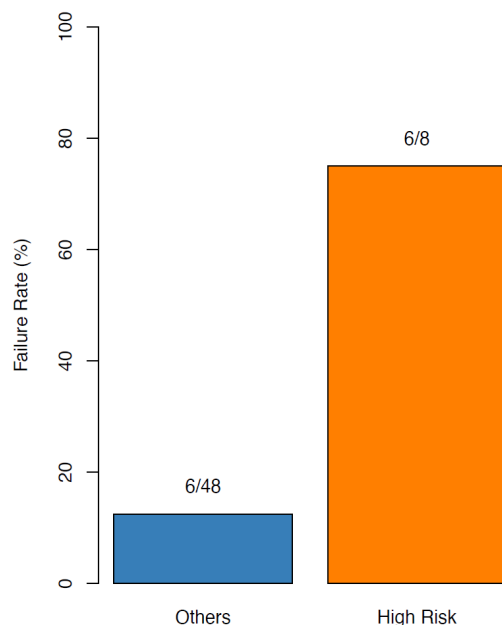


Figure 6. Treatment failure rates by risk stratification. Patients with both risk factors (albumin < 2.85 g/dL and denture use; High Risk group, $n = 8$) showed significantly higher treatment failure rate (75.0%, 6/8) when compared to Others (12.5%, 6/48; Fisher's exact test $p = 0.001$).

diversity, suggesting that the effect of dentures may operate through mechanisms other than community composition, such as increased bacterial load or impaired mucociliary clearance. The combination of denture use and low albumin levels, reflecting both increased bacterial exposure and impaired host defense, created a high-risk phenotype with a significantly elevated treatment failure rate (75.0% vs. 12.5%, $p = 0.001$), providing a simple and clinically applicable risk stratification tool.

This study has several limitations that warrant consideration. First, this was a secondary analysis of a single-arm trial without a control group, which limited the causal inference. Second, the sample size was modest ($n = 56$, with only 12 events for the minority outcome), limiting the statistical power for multivariable analysis; the multivariable logistic regression model with two variables was highly unstable, and when both albumin and Pielou's evenness were included in the

model, neither reached significance ($p = 0.072$ and $p = 0.246$, respectively). This loss of significance in the multivariable model, despite significance in the univariate analyses, reflects insufficient statistical power to determine whether the two factors act independently or are interrelated. The findings from this study should be regarded as hypothesis-generating rather than confirmatory. Third, the sputum may not accurately represent the microbiome of the lower respiratory tract. Fourth, the 16S rRNA gene clone library method used in this study has an inherently low throughput, with approximately 100 clones sequenced per sample. This technique can detect the most dominant bacterial species but may overlook low-abundance bacteria that could have important biological functions. Consequently, the microbiome profiles described here represent only the most prevalent taxa and the true diversity of the respiratory and oral microbiomes may be underestimated. Fifth, the cross-sectional design precluded the determination of the causal direction between albumin and microbiome diversity.

In conclusion, the serum albumin level was the strongest predictor of NHCAP treatment failure, with sputum microbiome evenness showing comparable predictive ability. This suggests an interconnected pathophysiology linking the nutritional status and respiratory microbiome stability. A simple risk stratification combining low albumin (< 2.85 g/dL) and denture use identified patients at markedly elevated risk. These findings suggest that nutritional optimization and oral health interventions may improve the outcomes of patients with high-risk of NHCAP. Larger longitudinal prospective studies are needed to validate these findings and clarify causal relationships.

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References

- Kohno S, Imamura Y, Shindo Y, Seki M, Ishida T, Teramoto S, Kadota J, Tomono K, Watanabe A. Clinical Practice Guidelines for Nursing- and Healthcare-associated Pneumonia (NHCAP) [Complete translation]. *Respir Investig.* 2013; 51:103-126.
- Mukae H, Iwanaga N, Horita N, *et al.* The JRS guideline for the management of pneumonia in adults 2024. *Respir Investig.* 2025; 63:811-828.
- Imamura Y, Miyazaki T, Watanabe A, Tsukada H, Nagai H, Hasegawa Y, Tomono K, Ito I, Teramoto S, Ishida T, Kadota J, Kohno S, Mukae H. Prospective multicenter survey for Nursing and Healthcare-associated Pneumonia in Japan. *J Infect Chemother.* 2022; 28:1125-1130.
- Bassis CM, Erb-Downward JR, Dickson RP, Freeman CM, Schmidt TM, Young VB, Beck JM, Curtis JL, Huffnagle GB. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. *mBio.* 2015; 6:1-10.
- Segal LN, Clemente JC, Tsay JCJ, *et al.* Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat Microbiol.* 2016; 1:16031.
- Noguchi S, Mukae H, Kawanami T, Yamasaki K, Fukuda K, Akata K, Ishimoto H, Taniguchi H, Yatera K. Bacteriological Assessment of Healthcare-Associated Pneumonia Using a Clone Library Analysis. *PLoS ONE.* 2015; 10:e0124697.
- Nemoto K, Yatera K, Akata K, Ikegami H, Yamasaki K, Hata R, Naito K, Noguchi S, Kawanami T, Fukuda K, Mukae H. Comparative study of bacterial flora in bronchoalveolar lavage fluid of pneumonia patients based on their pneumonia subtypes and comorbidities using 16S ribosomal RNA gene analysis. *J Infect Chemother.* 2022; 28:1402-1409.
- Kageyama S, Takeshita T, Furuta M. Relationships of variations in the tongue microbiota and pneumonia mortality in nursing home residents. *J Gerontol A Biol Sci Med Sci.* 2018; 73:1097-1102.
- Yoneyama T, Yoshida M, Ohru T, *et al.* Oral care reduces pneumonia in older patients in nursing homes. *J Am Geriatr Soc.* 2002; 50:430-433.
- Takazono T, Hosogaya N, Fukushima K, *et al.* Efficacy and safety of lascefloxacin for nursing- and healthcare-associated pneumonia: A single-arm, open-label clinical trial. *J Infect Chemother.* 2024; 30:597-602.
- Miyashita N, Matsushima T, Oka M; Japanese Respiratory Society. The JRS guidelines for the management of community-acquired pneumonia in adults: an update and new recommendations. *Intern Med.* 2006; 45:419-428.
- Shannon CE. A mathematical theory of communication. *Bell Syst Tech J.* 1948; 27:379-423.
- Simpson EH. Measurement of diversity. *Nature.* 1949; 163:688.
- Pielou EC. The measurement of diversity in different types of biological collections. *J Theor Biol.* 1966; 13:131-144.
- Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. *Ecological monographs.* 1957; 27:326-349.
- Gower JC. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika.* 1966; 53:325-338.
- Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 2001; 26:32-46.
- Youden WJ. Index for rating diagnostic tests. *Cancer.* 1950; 3:32-35.
- Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and cox regression. *Am J Epidemiol.* 2007; 165:710-718.
- Viasus D, Garcia-Vidal C, Simonetti A, Manresa F, Dorca J, Gudiol F, Carratalà J. Prognostic value of serum albumin levels in hospitalized adults with community-acquired pneumonia. *J Infect.* 2013; 66:415-423.
- Cassol I, Ibañez M, Bustamante JP. Key features and guidelines for the application of microbial alpha diversity metrics. *Sci Rep.* 2025; 15:622.
- Zhou P, Manoel D, Belibasakis GN, Kotsakis GA. Veillonellae: Beyond Bridging Species in Oral Biofilm Ecology. *Front Oral Health.* 2021; 2:774115.
- Gerner-Smidt P, Keiser-Nielsen H, Dorsch M, Stackebrandt E, Ursing J, Blom J, Christensen AC, Christensen JJ, Frederiksen W, Hoffmann S, Holten-Andersen W, Ying YT. *Lautropia mirabilis* gen. nov., sp. nov., a Gram-negative motile coccus with unusual morphology isolated from the human mouth. *Microbiology.* 1994; 140:1787-1797.
- Macuch PJ, Tanner AC. *Campylobacter* species in health, gingivitis, and periodontitis. *J Dent Res.* 2000; 79:785-792.
- O'Donnell L, Smith K, Williams C. Dentures are a reservoir for respiratory pathogens. *J Prosthodont.* 2016; 25:99-104.
- Khadka S, Khan S, King A, Goldberg LR, Crocombe L, Bettiol S. Poor oral hygiene, oral microorganisms and aspiration pneumonia risk in older people in residential aged care: a systematic review. *Age Ageing.* 2021; 50:81-87.

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§These authors contributed equally to this work.

*Address correspondence to:

Naoki Hosogaya, Clinical Research Center, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.

E-mail: nhosogaya@nagasaki-u.ac.jp

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