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Original Article

Oral Microbial Signatures Predict Susceptibility to SARS-CoV-2 Infection

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ABSTRACT

Growing attention is being directed toward the microbial communities of the oral cavity and digestive tract due to their involvement in numerous systemic health conditions. The mouth acts not only as a host environment for a wide range of potentially pathogenic microorganisms but also as a key access point into the human body. This role is particularly important in the context of the highly transmissible SARS-CoV-2 virus responsible for the current pandemic. Microbial populations in the oral and gastrointestinal systems can influence overall inflammatory status, shape immune function, and reflect the activity of these host responses. The immune response itself contributes to an individual's likelihood of acquiring infections, including SARS-CoV-2. This study aims to explore how specific salivary oral microbiome components may affect susceptibility to SARS-CoV-2 infection. This study included 106 individuals with documented medical and dental histories who agreed to provide saliva samples between January 2017 and December 2019. By March 1, 2021, sixteen of these participants had tested positive for COVID-19 through PCR testing. In this pilot analysis, comparisons of oral bacterial taxa identified through 16S rRNA sequencing showed distinctions in microbiome profiles between those who became COVID-19 positive and those who did not. These bacterial taxa may serve as indicators of heightened vulnerability to SARS-CoV-2 infection among individuals who have not been vaccinated.

Keywords: Oral microbiota, COVID-19 risk, Bacteria, 16S rRNA (16S rDNA), *Neisseria elongata*

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Introduction

The microbial communities within the oral cavity and digestive tract exist in a finely balanced equilibrium, and this microbial stability plays a crucial role in regulating both immune function and inflammatory capacity in the host [1, 2]. The oral and nasal mucosa act as primary portals of entry for numerous respiratory viruses, and the resident microbiome contributes meaningfully to how these viral infections develop and progress [3]. Existing research supports the concept that microbial populations—whether in the gut, oral cavity, or respiratory system—may influence susceptibility and response to viral pathogens such as SARS-CoV-2 [4], which was linked to 5.3 million deaths and 272 million documented infections worldwide by late 2021.

Salivary microorganisms, especially bacteria, help incoming microbes attach to the oral ecosystem and may facilitate their eventual passage into host mucosal surfaces. These salivary microbes may also regulate viral entry into host cells and determine whether viral exposure results in a productive, detectable infection. Additionally, certain oral microbiota can generate compounds that modify epithelial barrier integrity [5]. Numerous investigations have documented alterations in gut, oral, and nasopharyngeal microbial profiles during and after SARS-CoV-2 infection when compared to uninfected controls [6-11], though the underlying mechanisms remain unclear. SARS-CoV-2 significantly affects oral physiology, producing

symptoms such as dysgeusia, xerostomia, loss of taste, and secondary infections, all of which have the potential to shift oral microbial composition [12]. It is therefore reasonable to infer that changes in other oral microbial communities triggered by SARS-CoV-2 may contribute to these clinical manifestations.

Further studies have sought to characterize microbiome changes in the oral and gut environments during active COVID-19 infection and to examine how these changes correlate with disease severity [8, 11, 13-15]. Such research highlights bacterial groups that may increase morbidity in SARS-CoV-2 infection, particularly within the oropharyngeal region [16, 17].

SARS-CoV-2, a betacoronavirus and the causative agent of COVID-19, spreads rapidly through several routes, including oral droplets [18-20]. The oral cavity is considered a likely route of viral entry, and substantial evidence confirms that transmission occurs predominantly via droplets [2]. These droplets typically gain access to the body through the mucosal lining of the nasal cavity, primarily through ACE2- and TMPRSS2-expressing epithelial cells [3]. While much of the research has focused on the nasal–lung pathway of infection [4], entry through the oral cavity is also possible [1, 2]. This is supported by the strong expression of ACE2 and TMPRSS2 receptors in both salivary glands and oral mucosal tissues. However, despite the presence of oral symptoms in many infected individuals, it remains uncertain how frequently the oral cavity serves as the initial site of infection [21].

Once inside the mouth, successful infection requires that the virus survives in saliva or on oral surfaces long enough to attach to the mucosa, penetrate the extracellular matrix through ACE receptor binding, and subsequently replicate at levels sufficient for diagnostic detection. This study therefore examines whether certain salivary microbial taxa may enhance viral adherence and penetration, thereby increasing the likelihood of successful infection.

This retrospective investigation sought to determine whether preexisting salivary bacterial diversity influences susceptibility to SARS-CoV-2 infection. Saliva samples were obtained from established patients with documented pre-pandemic medical and dental histories who were monitored throughout the pandemic. Because adult salivary microbiome composition tends to remain relatively stable over time [22, 23], these samples provided a unique opportunity to analyze oral microbial profiles prior to infection. This enabled assessment of whether specific salivary bacterial taxa are associated with increased vulnerability to clinically evident COVID-19. Given the study's setting and timeframe in central Illinois, the analysis pertains to susceptibility to infection by the alpha, beta, and gamma variants of SARS-CoV-2 (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html).

Materials and Methods

Study population and patient characteristics

This investigation draws on a group of participants originally enrolled in a larger cross-sectional project conducted at the University of Illinois at Chicago College of Dentistry within the General Practice and Prosthodontic clinics from January 8, 2017, through June 21, 2019 [24]. Roughly 272 individuals agreed to join the parent study by signing written informed consent forms, following the requirements of the University of Illinois at Chicago Institutional Review Board 1, which approved the protocol (#2016-0696). All procedures complied with the ethical standards set forth in the Declaration of Helsinki.

Participants were eligible if they were at least 18 years old, had complete medical and dental records—including current medications—had undergone a full periodontal assessment, and had received a clinical and radiographic caries evaluation. Both dentate and edentulous individuals were allowed, as long as they were willing to provide a saliva sample.

Individuals were excluded if they had restored dental implants, wore removable partial dentures, had maxillofacial abnormalities, or had undergone dental scaling within the previous three months. Additional exclusion factors included needing urgent treatment for an acute condition, having fewer than twenty natural teeth (for those who were dentate), recent antibiotic use (within one month), mouthwash use within 12 hours prior to sampling, receiving any SARS-CoV-2 vaccination before March 1, 2021, or lacking confirmed information about their COVID-19 status.

Sample collection

As part of a previous investigation, participants produced stimulated saliva by chewing paraffin for five minutes, during which samples were collected [25]. All individuals had undergone comprehensive dental examinations before participating.

Evaluation of COVID-19 status

Participants were phoned at least three times between April and May 2021; those who could not be reached after multiple attempts were excluded from the study. For those who answered, a standardized telephone questionnaire was administered to collect information on any respiratory illnesses occurring from February 1, 2020, to March 1, 2021, confirmatory PCR test results

for COVID-19, and SARS-CoV-2 vaccination status prior to March 1, 2021.

Characterization of microbial community structure

DNA was extracted from the collected saliva specimens, and the V1–V3 regions of bacterial 16S rRNA genes were amplified using the 27F/534R primer set through a two-step targeted amplicon sequencing protocol, as reported previously [25, 26]. During the second PCR, each sample was uniquely labeled with Fluidigm Access Array barcoded primers at the University of Illinois at Chicago Sequencing Core. Sequencing was then carried out on an Illumina MiSeq platform with the V3 kit, allowing a total of 600 cycles.

For downstream analysis, reverse reads from the FASTQ files were processed using QIIME2 (v2022.2) [27]. Reads with an average quality score below 25 were discarded or trimmed, resulting in sequences truncated to 262 nucleotides. The DADA2 plugin was employed to denoise the data and produce feature tables representing the microbial sequences [28]. Taxonomic identification was performed using the classify-consensus-blast function with the Blast+ consensus classifier, matching sequences against the Human Oral Microbiome Database (v15.22) at 98% identity [29]. Across the 106 saliva samples, the sequencing depth averaged 25,136 reads per sample, ranging from 12,645 to 36,906, for a cumulative total of approximately 2,664,478 reads.

Statistical analysis

Analyses of alpha and beta diversity were conducted using MicrobiomeAnalyst [30]. Alpha diversity metrics included Shannon's Diversity Index, which accounts for both species richness and evenness, and Chao1, which estimates species richness. Differences between groups were evaluated using t-tests within MicrobiomeAnalyst. Beta diversity was assessed and visualized using the Bray–Curtis dissimilarity metric, which is non-phylogenetic.

Differential abundance of taxa between participants who tested positive for COVID-19 and those who remained negative was determined using MaAsLin2 with a zero-inflated negative binomial regression model and CSS normalization [31, 32]. To control for batch effects, the sequence run was included as a random effect in the model. Only taxa with a minimum of two reads were considered, and those present in fewer than 10% of samples were excluded from analysis.

For evaluating demographic and clinical characteristics of the study population, two-tailed Fisher's Exact tests and Student's t-tests were performed using KaleidaGraph (Synergy Software). In MaAsLin2 analyses, q-values were calculated using the Benjamini–Hochberg procedure to control for false discovery.

Results and Discussion

Participants and population description

In April 2021, attempts were made to reach approximately 272 participants who had previously provided saliva samples between November 2017 and December 2019. Of these, 166 individuals were excluded either because they could not be contacted or because they were unsure of their COVID-19 infection status. The remaining 106 participants had confirmed COVID-19 status as of March 1, 2021. Within this cohort, 16 participants had previously tested positive for COVID-19 via PCR and were assigned to the positive group, while the remaining 90 participants, who reported no respiratory illness from February 1, 2020, to March 1, 2021, and never received a positive PCR result, comprised the negative group.

The study examined differences in the salivary microbiome between these two groups. **Table 1** provides an overview of participant characteristics at the time of sample collection, including age, dental health measures, tobacco use, and the number of prescription medications. Both groups were generally similar, although tobacco use tended to be more common among individuals in the COVID-19 positive group.

Table 1. Demographics of the study population.

Characteristic	COVID-19 Negative (n=90)	COVID-19 Positive (n=16)	p-value*
Gender			0.493
Male	36 (40.0%)	7 (43.8%)	
Female	54 (60.0%)	9 (56.3%)	
Tobacco Use			0.126
Yes	14 (15.6%)	5 (31.3%)	
No	76 (84.4%)	11 (68.8%)	
Periodontal Disease			0.604
Yes	40 (44.4%)	5 (31.3%)	
No	50 (55.6%)	10 (62.5%)	
Dentate Status			0.312
Dentate (has teeth)	80 (88.9%)	14 (87.5%)	
Edentulous (no teeth)	10 (11.1%)	2 (12.5%)	
Age (years)	52.3 ± 15.7	47.2 ± 13.3	0.23
Number of Medications	3.27 ± 4.62	1.73 ± 3.17	0.22
Number of Carious Lesions	4.50 ± 7.88	4.13 ± 6.45	0.86

[a]Statistical significance for gender, periodontal health, tobacco use, and dentate status was evaluated using Fisher's exact test.
Statistical significance for age, number of medications, and caries was assessed using Student's t-test.

Microbial diversity analysis and differentially abundant taxa

Analysis of alpha diversity revealed no detectable differences between the COVID-19 positive and negative groups **(Figure 1)**. Similarly, overall beta diversity comparisons did not indicate significant variation between the groups **(Figure 2)**. Such findings are consistent with saliva samples, which represent a composite of microbial taxa originating from multiple oral niches.

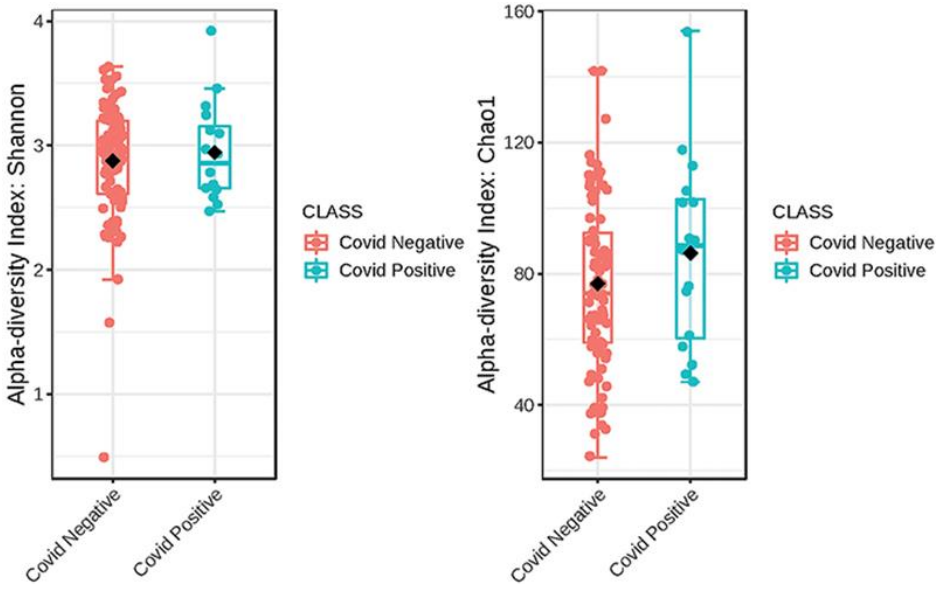

Figure 1. Comparison of salivary microbiome diversity between participants who reported COVID-19 infection and those who did not. Shannon Diversity index analysis revealed no significant difference in species richness between the groups ($t = -0.616$, $p = 0.543$). Likewise, Chao1 index comparisons confirmed similar levels of microbial richness ($t = -1.19$, $p = 0.2510$).

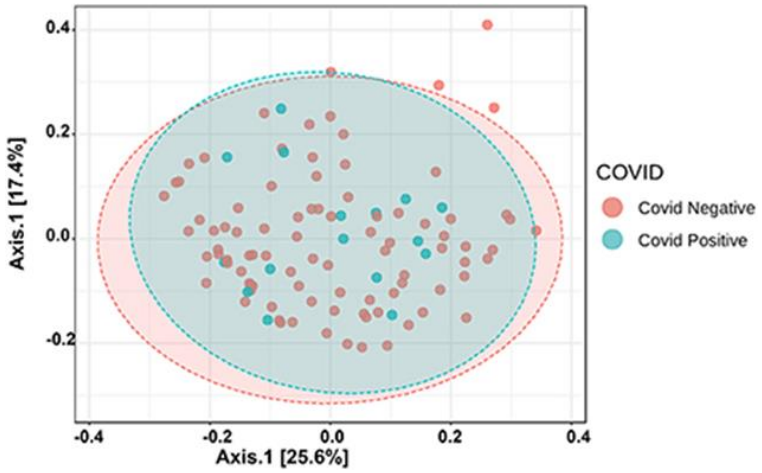

Figure 2. Principal coordinates analysis (PCoA) depicting the salivary microbiome profiles of participants with and without COVID-19, based on Bray–Curtis distance metrics.

Analysis of the salivary microbiome revealed only modest differences between participants who tested COVID-19 positive and those who did not. MaAsLin2 analysis **(Table 2)** identified several bacterial taxa—including Schaalia cardiffensis, Bergeyella (genus), Bacteroidetes_[G-5] s__bacterium_HMT_511, Neisseria elongata, and Prevotella dentalis—that showed variation in abundance between the groups at a false discovery rate (FDR) below 0.1 [31, 32]. Multivariate modeling, which accounted for age, tobacco use, and medication count, is illustrated in **Figure 3**.

Table 2. Identification of salivary bacterial taxa with differential abundance in participants who subsequently tested positive for COVID-19, as determined using MaAsLin2.

Rank / Taxon	Coefficient (β)	Std. Error	P-value	FDR-adjusted q-value	Direction in COVID-19+
g__Bacteroidetes (G.5) s__bacterium HMT_511	1.59	0.389	4.27×10^{-5}	0.0070	Higher
g__Schaalia s__cardiffensis	1.23	0.321	1.31×10^{-4}	0.0108	Higher
g__Neisseria s__elongata	–1.05	0.296	3.69×10^{-4}	0.0203	Lower
g__Bergeyella (unclassified species)	0.94	0.278	7.52×10^{-4}	0.0310	Higher
g__Prevotella s__dentalis	1.52	0.479	1.52×10^{-3}	0.0503	Higher

[a]Q or FDR values less than 0.1 (1.00E-01) are regarded as statistically significant. The coef represents the model coefficient (i.e., the estimated effect size), stderr is the standard error of that coefficient, and qval is the adjusted p-value obtained through the Benjamini-Hochberg false discovery rate (FDR) correction.

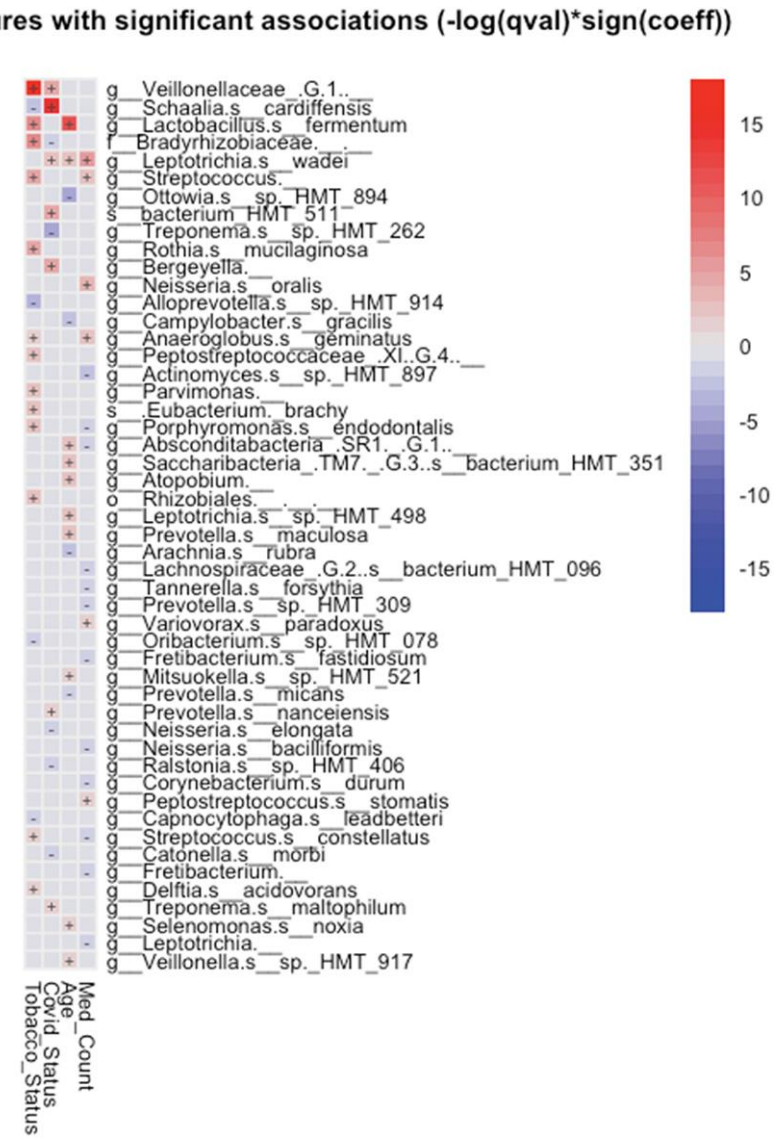

Figure 3. Statistically significant associations (FDR < 0.25) between bacterial taxa and clinical variables, as identified by MaAsLin2 multivariable analysis. Every reported link (e.g., future COVID-19 diagnosis and salivary microbiota) was adjusted for all other covariates, including the participant's smoking status, age, and the total number of prescribed medications.

A limited subset of oral bacterial taxa was found to differ in abundance in participants who later tested positive for COVID-19. Among these, Prevotella and Neisseria are particularly notable due to previous associations with COVID-19. Multivariable analysis **(Figure 3)** indicated that variations in these taxa may be partially influenced by differences in age, tobacco use, and medication counts across the studied groups.

One Prevotella species was observed at higher abundance in saliva samples from individuals who subsequently became SARS-CoV-2 positive **(Table 2)**. Prior studies have reported enrichment of Prevotella pallens in saliva from patients with active COVID-19, whereas taxa such as Rothia mucilaginosa and certain Streptococcus species were decreased [13]. Another study comparing patients with active COVID-19 to healthy controls found elevated levels of Haemophilus parainfluenzae, Veillonella infantium, Soonwooa purpurea, Prevotella salivae, Prevotella jejuni, and Capnocytophaga gingivalis in the oral mucosa, alongside reductions in other taxa [7]. Conversely, a separate study did not observe increased Prevotella at the genus level in COVID-19 saliva samples [33]. Enrichment of Prevotella genes in lung samples from COVID-19 patients and computational predictions of potential roles for Prevotella-encoded proteins in host-virus interactions suggest that elevated Prevotella may contribute directly to viral infection and disease progression in some individuals [34]. Differences in 16S rRNA regions analyzed across studies complicate direct comparisons; nonetheless, the increased abundance of the single Prevotella species in individuals who became COVID-19 positive **(Figure 3; Table 2)** provides tentative support for this hypothesis.

Low abundance of Neisseria has been proposed to contribute to heightened inflammation in COVID-19 patients [35]. Several studies have reported reduced Neisseria levels in infected individuals, with Iebba *et al*. noting particularly lower levels of Neisseria elongata in oral rinses from COVID-19 patients at FDR <0.11 [7, 8, 15]. Consistent with these findings, Neisseria elongata was present at lower levels in participants who later contracted COVID-19 **(Table 2)**, suggesting that reduced abundance of this species may precede infection.

Although the taxa differing between the groups could serve as potential markers of susceptibility to COVID-19, the underlying mechanisms remain unclear. Variations in viral susceptibility may reflect host genetics, immune status, inflammatory responses, or microbial interactions [36]. At the start of this study, it was uncertain whether the oral cavity serves as a primary site of SARS-CoV-2 infection, and this remains unresolved [37, 38]. Therefore, the observed differences in salivary microbiota among COVID-19 positive participants could reflect lifestyle factors influencing systemic susceptibility. This concept aligns with previous evidence linking oral microbiome variations to disease risk elsewhere in the body [39].

This study has several limitations. It is a retrospective, observational, single-center study, and minor differences in oral microbiota may result from multiple indirect factors. The small number of COVID-positive participants limits the statistical power [39, 40]. Additionally, a few individuals in the COVID-negative group could have experienced unrecognized infections [41], though the large negative cohort minimizes the potential impact of this issue. Salivary analysis employed V2–V3 16S rRNA sequencing, capturing a broad but incomplete representation of oral bacteria [42]. Other limitations include: (1) absence of functional gene analysis, (2) collection of saliva samples in some cases more than a year before the pandemic, (3) lack of precise dates for COVID-19 positivity, and (4) no consideration of dental restorations such as crowns, though patients with implants were excluded. A prospective study with more frequent saliva collection could address several of these constraints.

Given the March 1, 2021 cutoff for determining prior infection and the U.S. study location, the findings may primarily pertain to the alpha and beta SARS-CoV-2 variants [43]. Furthermore, salivary characteristics such as viscosity, pH, and levels of immunoglobulins or inflammatory cytokines were not assessed, although these factors may influence the oral microbiome's ability to prevent microbial adherence and viral penetration [44].

Conclusion

The presence of these bacterial taxa in saliva may be linked to a higher susceptibility to early SARS-CoV-2 infection among unvaccinated individuals. This study provides a foundation for further investigations into the complex oral microbiome and its potential role in either protecting against or promoting infection by respiratory viruses.

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Conflict of Interest: None

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Ethics Statement: The studies involving human participants were reviewed and approved by UIC IRB. The patients/participants provided their written informed consent to participate in this study.

References

1. Herrera D, Serrano J, Roldán S. Sanz, M. Is the oral cavity relevant in SARS-CoV-2 pandemic? Clin Oral Investig. (2020) 24:2925–30. 10.1007/s00784-020-03413-2 [DOI] [PMC free article] [PubMed] [Google Scholar]
2. Huang N, Pérez P, Kato T, Mikami Y, Okuda K, Gilmore RC, et al. SARS-CoV-2 infection of the oral cavity and saliva. Nat Med. (2021) 27:892–903. 10.1038/s41591-021-01296-8 [DOI] [PMC free article] [PubMed] [Google Scholar]
3. World Health Organization . Modes of Transmission of Virus Causing COVID-19: Implications for IPC Precaution Recommendations (2020). Available online at: https://www.who.int/docs/defaultsource/coronaviruse/20200329-scientific-brief1-final-corrected2-revised-final.pdf
4. Tada A, Senpuku H. The impact of oral health on respiratory viral infection. Dent J. (2021) 9:4–43. 10.3390/dj9040043 [DOI] [PMC free article] [PubMed] [Google Scholar]
5. Lin D, Yang L, Wen L, Lu H, Chen Q, Wang Z. Crosstalk between the oral microbiota, mucosal immunity, and the epithelial barrier regulates oral mucosal disease pathogenesis. Mucosal Immunol. (2021) 14:1247–58. 10.1038/s41385-021-00413-7 [DOI] [PubMed] [Google Scholar]
6. Gupta A, Karyakarte R, Joshi S, Das R, Jani K, Shouche Y, et al. Nasopharyngeal microbiome reveals the prevalence of opportunistic pathogens in SARS-CoV-2 infected individuals and their association with host types. Microbes Infect. (2022) 24:104880. 10.1016/j.micinf.2021.104880 [DOI] [PMC free article] [PubMed] [Google Scholar]
7. Iebba V, Zanotta N, Campisciano G, Zerbato V, Di Bella S, Cason C, et al. Profiling of oral microbiota and cytokines in COVID-19 patients. Front Microbiol. (2021) 12:671813. 10.3389/fmicb.2021.671813 [DOI] [PMC free article] [PubMed] [Google Scholar]
8. Ma S, Zhang F, Zhou F, Li H, Ge W, Gan R, et al. Metagenomic analysis reveals oropharyngeal microbiota alterations in patients with COVID-19. Signal Transduct Target Ther. (2021) 6:191. 10.1038/s41392-021-00614-3 [DOI] [PMC free article] [PubMed] [Google Scholar]
9. Sarkar A, Harty S, Moeller AH, Klein SL, Erdman SE, Friston KJ, et al. The gut microbiome as a biomarker of differential susceptibility to SARS-CoV-2. Trends Mol Med. (2021) 27:1115–34. 10.1016/j.molmed.2021.09.009 [DOI] [PMC free article] [PubMed] [Google Scholar]
10. Wang H, Wang H, Sun Y, Ren Z, Zhu W, Li A, et al. Potential associations between microbiome and COVID-19. Front Med (Lausanne). (2021) 8:785496. 10.3389/fmed.2021.785496 [DOI] [PMC free article] [PubMed] [Google Scholar]
11. Yamamoto S, Saito M, Tamura A, Prawisuda D, Mizutani T, Yotsuyanagi H. The human microbiome and COVID-19: A systematic review. PLoS ONE. (2021) 16:e0253293. 10.1371/journal.pone.0253293 [DOI] [PMC free article] [PubMed] [Google Scholar]
12. Cuevas-Gonzalez MV, Espinosa-Cristóbal LF, Donohue-Cornejo A, Tovar-Carrillo KL, Saucedo-Acuña RA, García-Calderón AG, et al. COVID-19 and its manifestations in the oral cavity: a systematic review. Medicine (Baltimore). (2021) 100:e28327. 10.1097/MD.0000000000028327 [DOI] [PMC free article] [PubMed] [Google Scholar]
13. Miller EH, Annavajhala MK, Chong AM, Park H, Nobel YR, Soroush A, et al. Oral microbiome alterations and SARS-CoV-2 saliva viral load in patients with COVID-19. Microbiol Spectr. (2021) 9:e0005521. 10.1128/Spectrum.00055-21 [DOI] [PMC free article] [PubMed] [Google Scholar]
14. Soffritti I, D'Accolti M, Fabbri C, Passaro A, Manfredini R, Zuliani G, et al. Oral Microbiome dysbiosis is associated with symptoms severity and local immune/inflammatory response in COVID-19 patients: a cross-sectional study. Front Microbiol. (2021) 12:687513. 10.3389/fmicb.2021.687513 [DOI] [PMC free article] [PubMed] [Google Scholar]
15. Wu Y, Cheng X, Jiang G, Tang H, Ming S, Tang L, et al. Altered oral and gut microbiota and its association with SARS-CoV-2 viral load in COVID-19 patients during hospitalization. NPJ Biofilms Microbiomes. (2021) 7:61. 10.1038/s41522-021-00232-5 [DOI] [PMC free article] [PubMed] [Google Scholar]
16. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. (2020) 81:266–75. 10.1016/j.jinf.2020.05.046 [DOI] [PMC free article] [PubMed] [Google Scholar]
17. Zhang H, Zhang Y, Wu J, Li Y, Zhou X, Li X, et al. Risks and features of secondary infections in severe and critical ill COVID-19 patients. Emerg Microbes Infect. (2020) 9:1958–64. 10.1080/22221751.2020.1812437 [DOI] [PMC free article] [PubMed] [Google Scholar]

18. Bao L, Zhang C, Dong J, Zhao L, Li Y, Sun J. Oral microbiome and SARS-CoV-2: beware of lung co-infection. Front Microbiol. (2020) 11:1840. 10.3389/fmicb.2020.01840 [DOI] [PMC free article] [PubMed] [Google Scholar]
19. Netz RR, Eaton WA. Physics of virus transmission by speaking droplets. Proc Natl Acad Sci U S A. (2020) 117:25209–11. 10.1073/pnas.2011889117 [DOI] [PMC free article] [PubMed] [Google Scholar]
20. Stadnytskyi V, Anfinrud P, Bax A. Breathing, speaking, coughing or sneezing: what drives transmission of SARS-CoV-2? J Intern Med. (2021) 290:1010–27. 10.1111/joim.13326 [DOI] [PMC free article] [PubMed] [Google Scholar]
21. Brandão TB, Gueiros LA, Melo TS, Prado-Ribeiro AC, Nesrallah A, Prado GVB, et al. Oral lesions in patients with SARS-CoV-2 infection: could the oral cavity be a target organ? Oral Surg Oral Med Oral Pathol Oral Radiol. (2021) 131:e45–51. 10.1016/j.oooo.2020.07.014 [DOI] [PMC free article] [PubMed] [Google Scholar]
22. Cameron SJ, Huws SA, Hegarty MJ, Smith DP, Mur LA. The human salivary microbiome exhibits temporal stability in bacterial diversity. FEMS Microbiol Ecol. (2015) 91:fiv091. 10.1093/femsec/fiv091 [DOI] [PubMed] [Google Scholar]
23. Yamanaka W, Takeshita T, Shibata Y, Matsuo K, Eshima N, Yokoyama T, et al. Compositional stability of a salivary bacterial population against supragingival microbiota shift following periodontal therapy. PLoS ONE. (2012) 7:e42806. 10.1371/journal.pone.0042806 [DOI] [PMC free article] [PubMed] [Google Scholar]
24. Schwartz JL, Peña N, Kawar N, Zhang A, Callahan N, Robles SJ, et al. Old age and other factors associated with salivary microbiome variation. BMC Oral Health. (2021) 21:490. 10.1186/s12903-021-01828-1 [DOI] [PMC free article] [PubMed] [Google Scholar]
25. Adami GR, Ang MJ, Kim EM. Comparison of microbiome in stimulated saliva in edentulous and dentate subjects. Methods Mol Biol. (2021) 2327:69–86. 10.1007/978-1-0716-1518-8_5 [DOI] [PubMed] [Google Scholar]
26. Naqib A, Poggi S, Wang W, Hyde M, Kunstman K, Green SJ. Making and Sequencing Heavily Multiplexed, High-Throughput 16S Ribosomal RNA Gene Amplicon Libraries Using a Flexible, Two-Stage PCR Protocol. Methods Mol Biol. (2018) 1783:149–69. 10.1007/978-1-4939-7834-2_7 [DOI] [PubMed] [Google Scholar]
27. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. (2019) 37:852–7. 10.1038/s41587-019-0209-9 [DOI] [PMC free article] [PubMed] [Google Scholar]
28. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. (2016) 13:581–3. 10.1038/nmeth.3869 [DOI] [PMC free article] [PubMed] [Google Scholar]
29. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, et al. The human oral microbiome. J Bacteriol. (2010) 192:5002–17. 10.1128/JB.00542-10 [DOI] [PMC free article] [PubMed] [Google Scholar]
30. Chong J, Liu P, Zhou G, Xia J. Using microbiomeanalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat Protoc. (2020) 15:799–821. 10.1038/s41596-019-0264-1 [DOI] [PubMed] [Google Scholar]
31. Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol. (2021) 17:e1009442. 10.1371/journal.pcbi.1009442 [DOI] [PMC free article] [PubMed] [Google Scholar]
32. Zhang X, Mallick H, Yi N. Zero-inflated negative binomial regression for differential abundance testing in microbiome studies. J Bioinf Genom. (2016) 2, 2–4. 10.18454/jbg.2016.2.2.129040451 [DOI] [Google Scholar]
33. Rafiqul Islam SM, Foysal MJ, Hoque MN, Mehedi HMH, Rob MA, Salauddin A, et al. Dysbiosis of Oral and Gut Microbiomes in SARS-CoV-2 Infected Patients in Bangladesh: Elucidating the Role of Opportunistic Gut Microbes. Front Med (Lausanne). (2022) 9:821777. 10.3389/fmed.2022.821777 [DOI] [PMC free article] [PubMed] [Google Scholar]
34. Khan AA, Khan Z. COVID-2019-associated overexpressed Prevotella proteins mediated host-pathogen interactions and their role in coronavirus outbreak. Bioinformatics. (2020) 36:4065–9. 10.1093/bioinformatics/btaa285 [DOI] [PMC free article] [PubMed] [Google Scholar]
35. Demirci M. Could Neisseria in oral microbiota modulate the inflammatory response of COVID-19? Oral Dis. (2021). 10.1111/odi.14082. [Epub ahead of print]. [DOI] [PMC free article] [PubMed] [Google Scholar]

36. Uchida H, Ovitt CE. Novel impacts of saliva with regard to oral health. J Prosthet Dent. (2022) 127:383–91. 10.1016/j.prosdent.2021.05.009 [DOI] [PMC free article] [PubMed] [Google Scholar]
37. Casillas Santana MA, Dipp Velázquez FA, Sámano Valencia C, Martínez Zumarán A, Zavala Alonso NV, Martínez Rider R, et al. Saliva: what dental practitioners should know about the role of this biofluid in the transmission and diagnostic of SARS-CoV-2. Medicina (Kaunas). (2021) 57. 10.3390/medicina57040349 [DOI] [PMC free article] [PubMed] [Google Scholar]
38. Sakaguchi W, Kubota N, Shimizu T, Saruta J, Fuchida S, Kawata A, et al. Existence of SARS-CoV-2 Entry Molecules in the Oral Cavity. Int J Mol Sci. (2020) 21. 10.3390/ijms2117600 [DOI] [PMC free article] [PubMed] [Google Scholar]
39. Belstrøm D. The salivary microbiota in health and disease. J Oral Microbiol. (2020) 12:1723975. 10.1080/20002297.2020.1723975 [DOI] [PMC free article] [PubMed] [Google Scholar]
40. Wade WG. The oral microbiome in health and disease. Pharmacol Res. (2013) 69:137–43. [DOI] [PubMed] [Google Scholar]
41. Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect. (2021) 54:12–6. 10.1016/j.jmii.2020.05.001 [DOI] [PMC free article] [PubMed] [Google Scholar]
42. Lazarevic V, Whiteson K, Gaïa N, Gizard Y, Hernandez D, Farinelli L, et al. Analysis of the salivary microbiome using cultureindependent techniques. J Clin Bioinforma. (2012) 2:4. 10.1186/2043-9113-2-4 [DOI] [PMC free article] [PubMed] [Google Scholar]
43. SARS-CoV-2 Variant Classifications and Definitions . Centers for Disease Control and Prevention (2021). [Google Scholar]
44. Wade WG. Resilience of the oral microbiome. Periodontol 2000. (2021) 86:113–22. 10.1111/prd.12365 [DOI] [PubMed] [Google Scholar]