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Comparison of microbial composition and diversity in the upper respiratory tract between SARS-CoV-2 and influenza virus infections

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Dear Editor,

Coronavirus disease 2019 (COVID-19) is still the most emerging infectious disease plaguing human society, and secondary bacterial infection is one of the major causes of its progression to severe illness and death. In this study, pharyngeal swabs were collected from patients with COVID-19 and influenza, and the microbiome landscape of the upper respiratory tract (URT) of both patients was compared, revealing disease-specific associated microorganisms. This will provide targeting for early treatment of the corresponding disease in the context of the highly overlapped characteristics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus infection. In addition, a number of studies have confirmed that patients with COVID-19 are at much higher risk of developing complications such as bacterial or fungal infections than patients with influenza. It is important to study the dynamics of the microbiota of the URT (the primary site of pathogen invasion), this will provide early warning of potential secondary bacterial infections, further contributing to the timely implementation of clinical interventions.

COVID-19 has been ravaging human society for about two years, and there is still no specific treatment. The pathogen, SARS-CoV-2, has a high degree of overlap with influenza viruses in terms of the mode of transmission and infection characteristics, both of which are transmitted through contact with respiratory droplets from patients, with infection manifesting as fever, dry cough, sore throat, and in severe cases developing into life-threatening respiratory disease and lung damage (Liu et al., 2020; Solomon et al., 2020). Several studies have shown that secondary bacterial infections are an important cause of mortality and morbidity associated with SARS-CoV-2 and influenza viruses (Ramos-Sevillano et al., 2019; Zhou et al., 2020); however, little is known about their relationship with respiratory microbes. The lack of research on oropharyngeal microorganisms, which are important in maintaining the health of the human body (Bowen et al., 2018), makes it difficult to keep track of the dynamics of pathogenic bacteria and to provide early warning of potential bacterial infections.

Here, we performed a metagenomic analysis of oropharyngeal swab samples from influenza and COVID-19

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patients, with the aim of uncovering the characteristic microbiome and potential pathogens in the URT of patients at the beginning of the COVID-19 outbreak. Samples for this study were obtained from 101 donors in Wuhan, including 31 COVID-19 patients, 39 influenza patients, and 31 healthy donors (Table S1 in Supporting Information). The study protocol was reviewed and approved by the ethics committee of Union Hospital of Tongji Medical College (2019S940). Patient's swab samples were heat inactivated at 56°C and RNA was extracted and purified using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The cDNA products obtained after reverse transcription were further subjected to library construction using the VAHTS Universal Plus DNA Library Prep Kit (Vazyme Biotech Co., Ltd., Nanjing, China), and sequencing data of approximately 5 to 11 Gb (2×150 bp) per sample were produced on the MGI-Seq 2000 platform.

Although infected with SARS-CoV-2 or influenza virus, the top 20 genera with the highest abundance in oropharyngeal samples were mostly colonized conditional pathogens, which were revealed by classification of the clean data based on Kraken2 analysis (Table S2 and Figure S1 in Supporting Information). Furthermore, microbial structural differences were observed among individual donors and between the different groups (Figure 1A). We calculated the alpha and beta diversity among each group. Alpha diversity analysis revealed that there was no significant difference in the number of microbial genera between COVID-19, influenza, and healthy groups (Chao1 and observed OTU index) (Figure 1B), but the within-sample diversity was significantly reduced in the COVID-19 and influenza groups, especially in COVID-19 patients (Shannon and Simpson index) (Figure 1B). In the β-diversity analysis, influenza patients showed a higher similarity in microbial composition to healthy controls, whereas COVID-19 patients had significant community differences and 670 unique genera, the majority of which were low in abundance (Figure 1C and D). These results suggest that SARS-CoV-2 infection causes changes in the population structure of URT microorganisms that are different from those caused by influenza virus.

The analysis of the abundance of major microorganisms between different groups showed that there were apparent differences, *Prevotella*, *Veillonella* accounting for more than 50% of the composition in COVID-19 patients and less than 25% in healthy controls (Figure 1E). The differences were observed in more detail on the relative abundance. In patients with COVID-19 and influenza, increased abundance of both *Veillonella* and *Pseudomonas* and decreased abundance of *Treponema* were found, when compared with healthy controls, showing commonality in the viral infection group (Figure 1F; Figure S2 in Supporting Information). Additionally, an increase in *Capnocytophaga* and a decrease in *Lautropia* and *Schaalia* were observed in influenza patients. In COVID-19 patients, a higher abundance of *Prevotella* and a lower abundance of *Streptococcus*, *Haemophilus*, and *Fusobacterium* were recorded (Figure 1F; Figure S2 in Supporting Information). Particularly, *Neisseria*, which is identified as a marker genus for influenza infection based on the linear discriminant analysis (LDA) score (Figure 1G), showed an opposite trend in the two groups of patients, with a significant decrease in the COVID-19 group and an increase in the influenza group (Figure 1F). The results indicated that the relative abundance of specific bacteria in the URT of COVID-19 patients showed significant differences when compared with the influenza and healthy groups. Considering that many of these identified microorganisms are common drug-resistant bacteria, we performed an antibiotic resistance analysis. The results showed that multiple antibiotics, such as ampicillin, gentamicin and kanamycin, were commonly resistant in the population, and more detectable chloramphenicol, streptomycin, and sulfisoxazole resistant genotypes were observed in both the COVID-19 and influenza groups than in the healthy group (Figure 1H; Table S3 in Supporting Information). These results suggest that viral infection may lead to the emergence of more resistant bacteria.

URT microbes are important in maintaining healthy homeostasis in the URT and organism as a whole (Bowen et al., 2018). Our results uncovered the microbiome features in the URT of patients with COVID-19 and influenza. We observed that microorganisms with reduced abundance in viral infection patients were mostly the marker microbiota in healthy populations (Figure 1G); their decline may lead to an increase in opportunistic pathogens. Once epithelial integrity is impaired by viral infection, these pathogens may trigger bacterial infections (Bao et al., 2020; Hall et al., 2017). In fact, bacteria co-infections are common in cases of COVID-19 and influenza (Klein et al., 2016; Lansbury et al., 2020). We also confirmed the close association of SARS-CoV-2 and influenza virus infection with the increased abundance of *Prevotella*/*Veillonella*/*Pseudomonas* and *Neisseria*/*Capnocytophaga*, respectively. Their population dynamics should be considered with additional attention and consideration during treatment. Notably, the blind use of antibiotics is undesirable (Blair et al., 2015), as more antibiotic resistant genes have been detected in both COVID-19 and influenza patients' sample than in healthy control samples (Figure 1H; Table S3 in Supporting Information), which makes the treatment of severe infections challenging. Overall, this study revealed that both SARS-CoV-2 and influenza virus infections lead to structural imbalances in the oropharyngeal microbiome. It should be noted that this study did have limitations, such as the lack of clinical information details on patients. Insufficient clinical background prevented us from excluding the effects of age, gender, and treatment on the oropharyngeal microbiota. In the future, it is necessary to

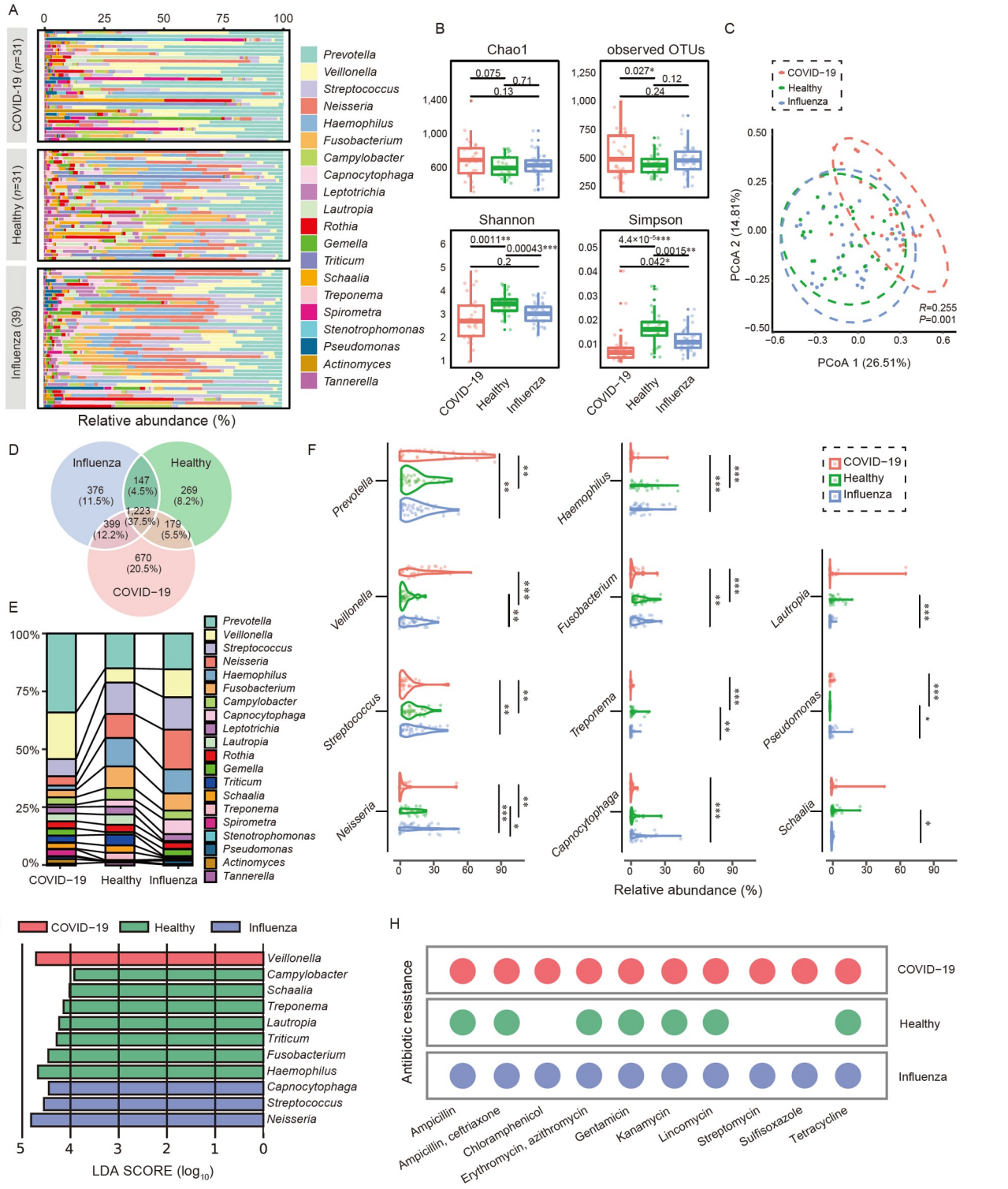

Figure 1 Comparison of microbial composition and diversity in the upper respiratory tract of COVID-19 patients, influenza patients, and healthy controls. Statistical analyses were performed based on the genus level. A, The microbial composition of 101 individual donors. Only the top 20 microbial genera with the highest abundance are shown. B, The α-diversity of COVID-19 patients, healthy controls and influenza patients was calculated according to the genus-level Chao index, observed OTUs, Shannon index, and Simpson index. Paired *t*-tests were used to measure the differences between groups. C, β-diversity of COVID-19 patients, healthy controls, and influenza patients. Differences between groups were tested by PERMANOVA. D, Common and specific microorganisms in the oropharynx of COVID-19 patients, healthy controls, and influenza patients. E, The major microbial composition of the three clinical subgroups. F, Microorganisms with significant differences in abundance among the 3 clinical subgroups were detected by the paired *t*-test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. G, LEfSe identifies oral microbial markers in COVID-19 patients, healthy controls, and influenza patients. H, Antibiotic resistance detected in the 3 groups.

acquire more metagenomic data from the URT samples of individuals with viral infection, and conduct a detailed multi-factor analysis, which can help us gain a deeper understanding of the correlation between clinical manifestations and URT microbial composition and diversity.

Compliance and ethics *The authors declare that they have no conflict of interest. The bioproject number for the raw sequencing data reported in this paper is PRJCA006528 (available at http://bigd.big.ac.cn).*

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References

Bao, L., Zhang, C., Dong, J., Zhao, L., Li, Y., and Sun, J. (2020). Oral microbiome and SARS-CoV-2: beware of lung co-infection. Front Microbiol 11, 1840.

Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., and Piddock, L.J. V. (2015). Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13, 42–51.

Bowen, W.H., Burne, R.A., Wu, H., and Koo, H. (2018). Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. Trends Microbiol 26, 229–242.

Hall, M.W., Singh, N., Ng, K.F., Lam, D.K., Goldberg, M.B., Tenenbaum, H.C., Neufeld, J.D., G. Beiko, R., and Senadheera, D.B. (2017). Inter-personal diversity and temporal dynamics of dental, tongue, and salivary microbiota in the healthy oral cavity. NPJ Biofilms Microbiomes 3, 2.

Klein, E.Y., Monteforte, B., Gupta, A., Jiang, W., May, L., Hsieh, Y.H., and Dugas, A. (2016). The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. Influenza Other Respir Viruses 10, 394–403.

Lansbury, L., Lim, B., Baskaran, V., and Lim, W.S. (2020). Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect 81, 266–275.

Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., Wang, Z., Li, J., Li, J., Feng, C., et al. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci 63, 364–374.

Ramos-Sevillano, E., Wade, W.G., Mann, A., Gilbert, A., Lambkin-Williams, R., Killingley, B., Nguyen-Van-Tam, J.S., and Tang, C.M. (2019). The effect of influenza virus on the human oropharyngeal microbiome. Clin Infect Dis 68, 1993–2002.

Solomon, D.A., Sherman, A.C., and Kanjilal, S. (2020). Influenza in the COVID-19 Era. JAMA 324, 1342–1343.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395, 1054–1062.

SUPPORTING INFORMATION

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