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Brief Communication

Metagenomic analysis of RNA sequencing data reveals SARS-CoV-2-mediated progressive dysbiosis of upper respiratory tract microbiota

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ABSTRACT

COVID-19, an infectious disease caused by a novel coronavirus (SARS-CoV-2) has emerged as global pandemic. Here, we described the changes in microbiota of upper respiratory tract by analyzing the publically available RNA sequencing data of SARS-CoV-2-infected ferrets. The bacterial dysbiosis due to SARS-CoV-2 was largely inversely proportional to the dysbiosis caused by influenza-A virus. The bacterial taxa which are defined as healthy ecostate were significantly reduced during SARS-CoV-2 infection. Altogether, this preliminary study provides a new insight on the possible role of bacterial communities of upper respiratory tract in determining the immunity, susceptibility, and mortality for COVID-19.

During the past few decades, the world has witnessed the change in major causes of human mortality from communicable diseases to non-communicable diseases [1]. But still, the respiratory tract infection due to viruses continues to be one of the top five reasons for mortality [2]. These viruses continuously undergo evolution and transmission between different species probably due to deforestation, habitat destruction, loss of biodiversity and global warming [3,4]. In this 21st century, the important viral epidemics includes severe acute respiratory syndrome (SARS) virus in 2002, influenza epidemic in 2009 and Middle East respiratory syndrome (MERS) virus in 2012 [5]. The whole world is currently (2020–2021) witnessing the epidemic of a novel coronavirus that shares maximum sequence homology with SARS virus and termed as SARS-CoV-2 virus and the disease condition as COVID-19 [6]. The transmission, morbidity and mortality rates of SARS-CoV-2 virus are extremely higher and hence declared as global pandemic by WHO. As the molecular details of this newly evolved virus are not completely understood, different antiviral, antimalarial and antibacterial drugs are being

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repurposed for COVID-19 [6,7]. As a result, the incidence and mortality due to COVID-19 are dramatically increasing worldwide. The major risk factors associated with COVID-19 mortality are ageing, smoking and metabolic complications like hypertension and diabetes [8]. The secondary bacterial infections are the leading cause for mortality in other respiratory viral infections including influenza. The role of secondary bacterial infections on mortality of COVID-19 patients is not completely understood on comparison to other respiratory viral infections [8,9].

The upper respiratory tract is a natural microbial niche in the human body, which is largely comprised of commensal bacterial communities and pathogenic bacteria like *Staphylococcus* and *Streptococcus* [10]. These genera remains dormant during healthy condition but microbial dysbiosis leads to domination of these pathogens causing bacterial pneumonia. The disturbance of upper respiratory tract microbiota by continuous use of antibiotics lead to higher susceptibility for viral infections in humans [11]. A recent study showed the association of variation in nasopharyngeal microbiota with influenza infection and vaccination [12]. A recent study revealed the changes in gut microbiota induced by SARS-CoV-2 is different from influenza patients [13,14]. Though numerous efforts are being executed in understanding the viral genome, vaccine development and host response to SARS-CoV-2, the effect of this viral infection on microbiota of respiratory tract is less unexplored [15,16]. This study is aimed at analyzing the bacterial diversity of upper respiratory tract by mining into the publically available metatranscriptomics data generated from the SARS-CoV-2-infected ferrets.

Methods

The RNA-Seq data from the SARS-CoV-2-infected ferrets were downloaded from NCBI-SRA database (SRP253951) [17]. The downloaded FASTQ files were checked for quality using fastQC program. Subsequently, to determine the microbial taxonomy, the reads were analyzed by using Kaiju tool [18]. The output files from Kaiju were normalized to total read counts and the percentage of abundance of microbial taxa at different levels were calculated. All statistical analyses were performed using the statistical softwares SPSS version 20.0 and GraphPad Prism version 6.01. (The methods are described elaborately in the Supplementary file 1).

Results

Blanco-Melo et al. [16] reported the unique transcriptional signature in cell lines and nasal washes of ferrets infected with SARS-CoV-2 (strain USA–WA1/2020) and influenza A virus (IAV; pH1N1; A/California/04/2009 strain). The nasal washes were collected from the infected and control animals on specific days and bulk RNA sequencing was performed by Blanco-Melo et al. group and the raw data files were deposited in NCBI-SRA (Accession No. SRP253951; The experimental details were described in Fig. 1A & Supplementary Fig. 1). We downloaded the raw FASTQ files and subjected them to bacterial diversity analysis by using Kaiju [17] tools (see supplementary file 1 for methods). Kaiju tool matches sequencing reads to the microbial pangenome comprised of protein sequences from bacteria, virus and archaea and it is more sensitive than 16S rRNA-based taxonomic profiling [17]. All the sequence files had 9–15% of reads mapping to microbial pangenome. The unnormalized read counts matching the microbial pangenome are provided in supplementary file 2 for all samples.

The changes in microbiota of upper respiratory tract after seven days was prominent during IAV than SARS-CoV-2 infection [Fig. 1B]. This was in correlation with the host transcriptomics response [16], where the elicitation of cytokines and other immune responses were higher in IAV treatment and muted during SARS-CoV-2 treatment. As previously reported [11,12], we observed proliferation of betaproteobacteria particularly *Pseudomonadales* during IAV infection. Though no significant changes were observed in most bacterial phyla during SARS-CoV-2 infection, significant reduction in *Bacteroides*, *Chlamydiae* and *Actinobacteria* was noted [Fig. 1B]. The composition of bacterial orders exhibited an inverse correlation between IAV and SAR-CoV-2 infection [Fig. 1C and D]. Among the proteobacterial orders, the level of only *Burkholderiales* were significantly increased during SARS-CoV-2 infection [Fig. 1C].

While other bacterial orders like *Lactobacillales*, *Chlamydiales* and *Flavobacteriales* were significantly reduced during SAR-CoV-2 [Fig. 1D]. Subsequently, analyses were performed to understand the progressive changes in the level of bacterial orders after SARS-CoV-2 infection from day 1 to day 14. On contrast to blooming of *Pseudomonadales* during IAV infection, a significant fall in *Pseudomonadales* was observed after seven days of SARS-CoV-2 infection [Fig. 1D]. The other bacterial orders that significantly reduced as the days of infection increased included *Lactobacillales*, *Flavobacteriales*, *Bacillales*, *Clamydiales*, *Corynebacteriales*, *Chromatiales*. The reduced orders constituted largely the commensal bacteria including *Corynebacterium* and *Lactobacillus*. It is important to note that *Bacillales* that includes bacterial genera *Streptococcus* and *Staphylococcus* were decreased during SARS-CoV-2 but in contrast were increased during IAV infection leading to bacterial pneumonia [9–13].

Discussion

A drastic fall in the level of commensal bacteria during SARS-CoV-2 indicates the loss of beneficial bacteria that coordinate lung–gut axis and play a major role in pulmonary health and diseases. We have reported the changes in microbiota as the days of infection increases. The viral load in nasal washes showed a progressive depletion from day 7 onwards and reached zero on day 14 [17] but still the nasal microbiota disturbed persists. This indicates that the microbial dysbiosis caused by viral infection may continue to play a key role during post-COVID recovery on the pulmonary health.

It is important to note that the abundant bacterial genera, which are defined as healthy ecostate is lost during both viral infections [Fig. 1]. The bacteria comprising the healthy ecostate plays a major role in boosting the immunity and also

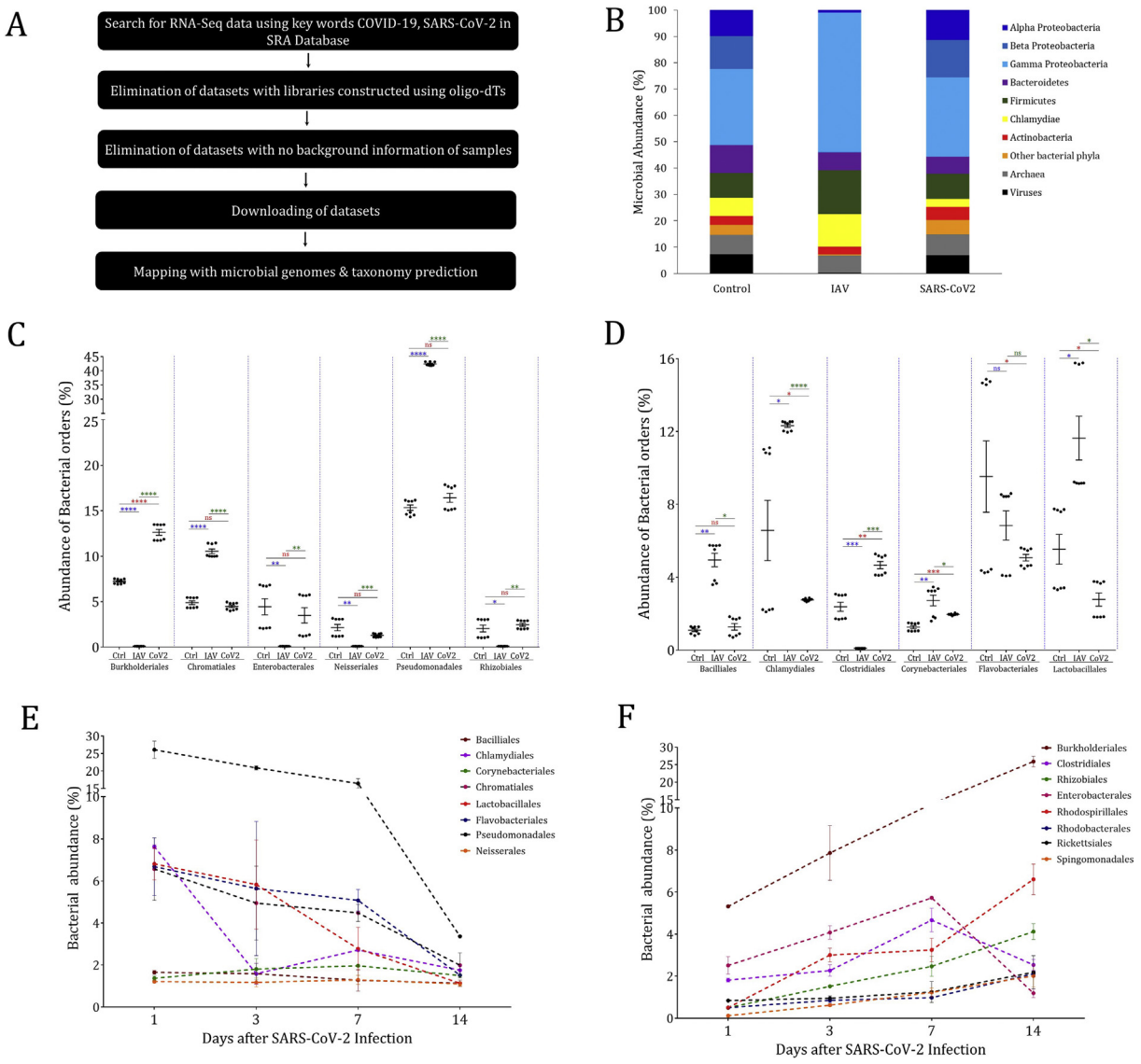

Fig. 1 **Effect of SARS-CoV-2 and influenza A virus infection on the upper respiratory tract microbiota of ferrets. (A)** Workflow of selection of datasets and analysis **(B)** Abundance of microbial groups and bacterial phyla in the nasal washes on day seven post-infection. **(C)** Abundance of bacterial orders belonging to proteobacterial phylum on day seven post-infection. **(D)** Abundance of bacterial orders belonging to other phyla on day seven post-infection. **(E)**. Bacterial orders displaying inverse correlation with the days of SARS-CoV-2 post-infection. **(F)**. Bacterial orders displaying direct correlation with the days of SARS-CoV-2 post-infection. Horizontal lines represent (C–F) represent mean; error bars represent standard deviation. Asterisks (C&D) represent statistical significance by one-way ANOVA with Tukey post-hoc analysis. ****$p < 0.001$ ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns - no significance. The blue, red and green asterisks (C& D) represent comparison between Ctrl vs IFA, Ctrl vs CoV2 and IFA vs CoV2 respectively. The experiments were performed with 8 replicates. Abbreviations used: Ctrl: Control; IAV: Influenza A virus; CoV2: SARS-CoV-2 virus.

produces anti-pathogenic metabolites and prevents the disease incidence and mortality during viral infections [19,20]. In an ecological study among forest frogs (*Rana temporaria*), a community of frogs with a specific skin microbiome developed resistance to viral infections during an epidemic [21]. Thus the commensal bacteria seem to play a vital role in defense against viral infections but the molecular mechanisms are not understood.

This study has some limitations including the non-availability of health status and clinical outcomes of ferrets infected with SARS-CoV-2 Altogether, despite of these limitations this preliminary bioinformatics investigation showed the changes in microbiota of upper respiratory tract during SARS-CoV-2 infection. The microbiota changes are inversely proportional to the changes induced by IAV but loss of healthy ecostate is common during both infections. At present, studies are initiated to investigate the microbiota of COVID-19 patients at different stages to explore their role in immunity, susceptibility to infection, response to repurposed drugs and mortality. Based on this knowledge, novel therapeutics for

COVID-19 can be framed by targeting the abundant bacteria *via* specific antibiotics or bacteriophages and recovery of healthy ecostate *via* nasal administration of probiotics formulations made of commensal and beneficial bacteria.

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2021.02.008.

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