

Based on Transcriptome Sequencing: Clinicians Need to Pay Attention to Fever Caused by Common Respiratory Pathogens During the Global Outbreak of COVID-19

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Abstract

Objectives:

During the COVID-19 pandemic, clinicians and public health decision-makers especially focus on fever patients. Other common pathogens that may cause fever are easily overlooked. We aimed to describe the pathogen infection and epidemic trend of non-SARS-CoV-2 occurring in hospitalized patients.

Methods:

An observational cohort study of 733 consecutive patients admitted to Hospital Clinic of the Second Xiangya Hospital for COVID-19. All samples of a pharyngeal swab from patients with fever have been tested for nucleic acid and immune antigens of SARS-CoV-2 and *Influenza A/B virus*. 649 fever patients have been tested for nucleic acid in ten respiratory pathogens. Macrotranscriptome sequencing was performed on 26 samples.

Results:

Of a total of 733 patients with fever, 2.05% patients had confirmed SARS-CoV-2 infections. Fever patients with common respiratory pathogens in fever patients was 8.78%. There is no integration phenomenon between SARS-Cov-2 and the human genome. SARS-CoV-2 positive samples will also be infected with other viruses, especially adenovirus. Macrotranscript analysis showed that there was no significant difference in the species and genus levels of pathogens between Covid-19 patients and other fever patients. The main pathways that affect human metabolism after SARS-Cov-2 infection are the Calvin-Benson-Bassham cycle, pyrimidine deoxyribonucleotides de novo biosynthesis I and D-galactose degradation V.

Conclusions:

Most patients have a fever caused by common respiratory pathogens. Clinicians still need to pay more attention to infections of common respiratory pathogens in addition to SARS-CoV-2. China's public health measures to stop the spread of the epidemic have proven effective.

Highlights

1. We first described the pathogen infection and epidemic trend of non-SARS-CoV-2 occurring in hospitalized patients with COVID-19.
2. Of a total of 733 patients with fever, 15 (2.05%) patients had confirmed SARS-CoV-2 infections. Other respiratory pathogens co-infection at COVID-19 diagnosis was uncommon (1 out of 15, 6.67%).
3. Macrotranscript analysis showed that there was no significant difference in the species and genus levels of pathogens between covid-19 patients and other fever patients.

4. In the face of fever patients, clinicians still need to pay more attention to infections of common respiratory pathogens in addition to SARS-CoV-2.

Background

In December 2019, a series of acute respiratory diseases occurred in Wuhan, Hubei Province, China, now called Coronavirus Disease 2019 (COVID-19)¹. The disease has rapidly spread from Wuhan to other regions. As of February 24, 2021, The number of confirmed COVID-19 cases worldwide is 112,534,427. A new type of coronavirus (SARS-CoV-2) was identified in a bronchial alveolar lavage fluid sample from a patient in Wuhan City and confirmed it as the cause of COVID-19². Now the virus is renamed SARS-CoV-2 by WHO³. Whole-genome sequencing and phylogenetic analysis indicate that SARS-CoV-2 is a unique clade of β -coronal viruses associated with human severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS)². Like other beta coronaviruses, the genome of SARS-CoV-2 has a long ORF1ab polyprotein at the 5' end, followed by four major structural proteins, including the spike surface glycoprotein, small envelope protein, matrix protein, and nucleocapsid protein⁴. SARS-CoV-2 is very similar to bat coronavirus, and bats are presumed to be the main source and bats are presumed to be the main source⁵. Although the origin of SARS-CoV-2 is still under investigation, current evidence suggests that it spread to humans through the spread of wild animals illegally sold in the South China⁶.

Huang et al. first reported 41 COVID-19 cases, most of whom had a history of exposure to the South China Seafood Wholesale Market. The patient's clinical manifestations included fever, unproductive cough, dyspnea, myalgia, fatigue, normal or decreased white blood cell count, and imaging evidence of pneumonia⁷. However, only a small number of articles reported co-infection in patients with COVID-19^{8,9}. Co-infection at COVID-19 diagnosis is uncommon. Few patients developed superinfections during hospitalization. Patients with fever often detect ten pathogens. *Influenza A/B virus* are common influenza virus. They are mainly prone to mutation and the *Influenza A virus* main subtype is called avian influenza. Symptoms after infection are mainly high fever and cough, accompanied by pneumonia and small airway dysfunction¹⁰. The four subtypes of human parainfluenza viruses have different clinical and epidemiological characteristics. The most typical clinical feature of type I and type II is that it causes laryngotracheobronchitis in children, type I is the main cause of this type of laryngotracheobronchitis in children, and type II is the second. Both types I and II can cause other upper and lower respiratory tract diseases. Type III often causes pneumonia and bronchiolitis¹¹. *Human metapneumovirus* (hMPV) is a single-stranded ribonucleic acid virus in the Paramyxoviridae family and was first discovered in the Netherlands in 2001. The virus mainly causes acute respiratory infections in children. Symptoms include fever, cough, shortness of breath, and difficulty breathing. Adults with weak resistance also have a chance to be infected¹². *Human Boca virus* (hBoV) is a genus of the Parvovirinae of the Parvoviridae family. Boca virus usually infects the intestines and respiratory tract. Some viruses may be transmitted through the placenta and cause congenital infection of the fetus. *Human Boca virus* spreads through the air, and it is easy for infants and young children to suffer from diseases such as pneumonia, bronchitis, and bronchopneumonia¹³. Acute infectious diseases caused by *Adenovirus* can easily invade the

mucosa of the respiratory tract and digestive tract, conjunctiva, urinary tract, and lymph nodes. The main manifestations are acute upper respiratory tract infections (acute respiratory infections are caused by *Adenovirus* accounting for 2–4%), followed by eye and gastrointestinal infections. Approximately half of the patients have a recessive infection. Infants and young children are susceptible to *Adenovirus* pneumonia, with severe illness and high mortality¹⁴. Coronavirus is a single-stranded positive-stranded RNA with a diameter of about 100 nanometers, which is irregularly round or quasi-circular. Under the electron microscope, the shape is crown-shaped. It belongs to the coronavirus family. The *human coronavirus* (hCoV) has two antigen types, OC43 and 229E. The incubation period is generally 2 to 5 days. The typical manifestation is the common cold, with a blocked nose, runny nose, sore throat, red throat, and general malaise. Pneumonia may occasionally occur and cause asthma in children. The OC43 strain causes more severe symptoms than 229E¹⁵. *Respiratory syncytial virus* (RSV) is an RNA virus belonging to the Paramyxoviridae family. The disease is spread by air droplets and close contact. Infants and young children have severe symptoms, including high fever, rhinitis, pharyngitis, and laryngitis, and later manifested as bronchiolitis and pneumonia. A small number of sick children may be complicated by otitis media, pleurisy, and myocarditis. After adults and older children are infected, the main manifestations are upper respiratory tract infections¹⁶. The pathological changes of *Mycoplasma pneumoniae* (MP) are mainly interstitial pneumonia, sometimes complicated by bronchial pneumonia, called primary atypical pneumonia. If they have, they are just general respiratory symptoms such as headache, sore throat, fever, and cough, but there are also individual deaths¹⁷. During the COVID-19 pandemic, clinicians and public health decision-makers especially focus on fever patients. Other common pathogens that may cause fever are easily overlooked^{18,19}. Doing so is likely to cause a waste of medical resources, delays in medical treatment, and social panic. The purpose of this case series is to describe the pathogen infection and epidemic trend of non-SARS-CoV-2 occurring in hospitalized patients with COVID-19.

Methods

Sample Collection

This study was approved by the Hospital Ethics Committee, and all study participants were informed. The study population consisted of 733 fever patients at the Second Xiangya Hospital of Central South University, Hunan province, China, between January to February 2020, aged 20 to 70 years. All the patients were diagnosed by two experienced doctors. The samples from the participants were obtained before clinical intensive treatment. Participants who received preoperative radiotherapy or chemotherapy were excluded. Respiratory and stool samples were collected with the consent of all patients. Unless otherwise stated, patients were sampled without gender or age bias. Swabs and stool were stored at -20°C until use.

RNA extraction and qRT-PCR

Whenever a commercial kit is used, the manufacturer's instructions should be followed without modification. Nucleic acids were extracted by SSNP-2000A Automatic Nucleic Acid Extraction Instrument and nucleic acid extraction kits (Sansure biotech, China). 20 µL extracted nucleic acid sample was added into a PCR amplification tube which contains 30 µL of the prepared PCR-mixed solution, and perform fluorescence quantitative PCR detection on a fluorescent PCR instrument (detected through fluorescence quantitative PCR using a fluorescent PCR instrument.) Amplification was performed as follows: 50°C for 30 minutes, 95°C for 60 seconds, and then 40 cycles, including 95°C for 15 seconds, 60°C for 30 seconds.

The primer and probe sequences²⁰:

ORF1b gene

5'-TGGGGYTTTACRGGTAACCT-3' (Forward)

5'-AACRCGCTTAACAAAGCACTC-3' (Reverse)

N gene

5'-TAATCAGACAAGGAACTGATTA-3' (Forward)

5'-CGAAGGTGTGACTTCCATG-3' (Reverse)

Nucleic acids were analysed *Influenza A/B virus* nucleic acids test kits (bioPerfectus, China). 5µL extracted nucleic acid sample was added into a PCR amplification tube which contains 20µL of the prepared PCR-mixed solution and performs fluorescence quantitative PCR detection on a fluorescent PCR instrument. Amplification was performed as follows: 50°C for 30 minutes, 95°C for 5 minutes, and then 40 cycles, including 95°C for 10 seconds, 55°C for 40 seconds. The reference value of the *Influenza A virus* is 34.7(CT). The reference value of the *Influenza B virus* is 34.8(CT).

Colloidal gold immunochromatography

The nurse uses a throat swab to apply on both sides of the back wall of the throat or in the nasal cavity. Take a sufficient amount of specimens and put them in a special disposable specimen collection tube for inspection. The specimens should be sent for inspection immediately after collection or stored at 4°C for no more than 3 days. Otherwise, it should be stored below -20°C, and repeated freezing and thawing should not exceed 3 times. According to the *Influenza A/B virus* antigen detection instructions (Wondfo biotech, China), drop 8 drops of the dilution liquid in the dropper bottle into the plastic tube of the kit, add a sufficient amount of the sample, stir and repeatedly squeeze the tube wall, so that the sample is fully dissolved to the dilution In the liquid. Use a pipette to slowly add 3-4 drops (about 100ul) of the sample solution into the sample hole of the test card, and start recording the time.

Ten respiratory pathogen nucleic acid detection (multiple melting curve method)

The throat swab samples were sucked into the supernatant according to the instructions of the "Nucleic Acid Extraction or Purification Reagents", and the nucleic acid was extracted according to the instructions of the EX-DNA/RNA Virus Nucleic Acid Extraction Kit (Tianlong, China). Configure the RT-PCR system, add 4.4 μ L Res reaction solution, and 0.6 μ L Res enzyme solution to a 1.5mL centrifuge tube in turn. Add 25 μ L of mineral oil after centrifugation. Place the positive control, negative control and samples on the PCR machine for amplification. Amplification was performed as follows: 50°C for 30 minutes, 95°C for 15 minutes, and then 40 cycles, including 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds. After the program is run, the instrument will automatically pay to check the Tm value.

Data collection

We reviewed the care records and laboratory test results of fever patients. Admission data for these patients were from January 26th to February 1st, 2020. The two researchers also independently reviewed data collection forms to confirm repeatedly.

Bioinformatics analysis process

The use of different methodological kits to detect SARS-CoV-2 and other common respiratory viruses on samples of fever patients limits our further understanding of SARS-CoV-2 co-infection. We commissioned Sansure to perform RNA metagenomic sequencing on 26 fever patients' samples (SARS-CoV-2 positive: n=16, SARS-CoV-2 negative: n=10 throat swab: n=22, stool: n=4).

The overall process is shown below(Fig.1):

1. Perform a new coronavirus fast test (Fastv) on all data to determine whether it is negative or positive. Compare the virus database to find other co-infected viruses. Compare the bacterial database and annotate the species of the bacteria.
2. Compare to the original data (SortmeRNA) ribosomal database. Split the data into other and rRNA parts.
3. Compare the other part of the reads to hg19. Perform differential analysis of human expressed genes.
4. Compare the other part of the reads with SARS-CoV-2 (NC_045512.2). Analyze virus coverage, depth, mutation, and L/S typing.
5. After excluding the human and SARS-CoV-2 sequences from the other part:
 - 1) Assemble of the 8 throat swabs and 6 stool data (Metaspades). And Remove redundant sequences (Cd-hit). Assess the effect of assembly (Quast).
 - 2) Take the above assembly result as the reference sequence, and do quantitative statistics for all samples (Salmon).

3) Analysis of differences between the negative and positive groups.

4) Perform species and function analysis (Humann3).

Statistical analysis

Continuous variables are expressed as median (IQR) and compared using the Mann-Whitney U test. Categorical variables are expressed as numbers (%) and compared by the chi-square test or Fisher's exact test. A two-sided α of less than 0.05 was considered statistically significant.

Result

SARS-CoV-2 and *Influenza A/B virus* infection trends during the epidemic

By Feb 1, 2020, 649 fever patients were doubted as SARS-CoV-2 infection in Hunan. The patients were aged from 2 to 65, and the median age of the patients was 46 years (IQR 29.25-45.5). During the COVID epidemic, all the fever patients admitted to the hospital were tested for the SARS-CoV-2 and Flu A/B nucleic acid, using the kits of *bioPerfectus* and *Sansure biotech* respectively. Among fever patients, 3.96% (29/733) of patients are infected with the *Influenza A virus* and 5.46% (40/733) are infected with the *Influenza B virus*. We found that patients with SARS-CoV-2 do not have co-infections of A and B. The number of newly diagnosed patients with SARS-CoV-2 accounts for only 2.05% of all fever patients (Tab. 1). The number of COVID-19 confirmed cases in these six days (Jan 23th, Jan 26, Jan 28, Jan 29, Jan 31, and Feb 1st) is relatively stable. The number of new patients with fever infected with *Influenza A/B virus* fluctuates greatly and is significantly higher than the number of COVID-19 patients ($P < 0.01$) (Fig.2).

Changes in *Influenza A/B virus* before, during and after the COVID-19 epidemic

Before the epidemic, most patients with fever would only be tested for *Influenza A/B virus* antigen. If the patient has a negative antigen test but is highly suspected of being infected with *Influenza A/B virus* or if an antigen-positive patient wants to seek further diagnosis, the doctor will recommend the patient to take a nucleic acid test. Therefore, the results of the *Influenza A/B virus* tests of patients before the epidemic originated from the antigen gold labeling method. However, during the epidemic, to ensure high detection efficiency and timely treatment of COVID-19 patients, all fever patients in this teaching hospital detected *Influenza A/B virus* and SARS-CoV-2 nucleic acid. The positive rate of each period is shown in Tab.2. The detection rate of *Influenza A/B virus* in fever patients from 2019.8.1 to 8.16 is 8.62% (151/1751) via colloidal gold immunochromatography. The nucleic acid test result is 7.48% (8/107) similar to that of the immunoassay. During the epidemic (2020.1-2020.2), the infection rate of the *Influenza A/B virus* increased to 12.55% (92/733), showing the same trend as the COVID-19. However, After the epidemic, the detection rate of COVID-19 among fever patients in this teaching hospital dropped to zero and the infection rate of the *Influenza A/B virus* became extremely low (0.00%)(Fig.3).

Infections of pathogens in fever patients

We tested ten respiratory pathogen nucleic acid detection for patients. The test results are as follows (Tab.3): The most common pathogen at onset of illness was *Respiratory syncytial virus* (RSV) [16 (2.47%) of 649 patients]. Influenza A(FluA) [9 (1.39%) of 649 patients], Influenza B(FluB) [14 (2.16%) of 649 patients], *M.Pneumonia*(MP) [11 (2.47%) of 649 patients], *Adenovirus*(ADV) [10 (1.54%) of 649 patients], *Parainfluenza virus I*(PIV1) [0 (0.00%) of 649 patients], *Parainfluenza virus II*(PIV2) [1 (0.15%) of 649 patients], *Parainfluenza virus III*(PIV3) [0 (0.00%) of 649 patients], *Coronavirus virus 229E* (HCoV-229E) [5 (0.77%) of 649 patients], *Human metapneumovirus* (HPMV) [13 (2.00%) of 649 patients], *Human Coronavirus virus OC43* (HCoV-OC43) [1 (0.15%) of 649 patients], *Human Boca virus* (HBoV) [0 (0.00%) of 649 patients]. The proportion of patients with SARS-CoV-2 among fever patients is [15/733 (2.05%)]. The other febrile pathogens rate of fever patients is higher than patients with SARS-CoV-2 ($P < 0.01$) (Fig. 4).

Basic statistics of bioinformatics analysis data

Quickly check the SARS-CoV-2 virus. The mean and depth of SARS-CoV-2 coverage of positive samples were 98.03% (44.80%-99.87%) and 12.9X (2.8X-167.8X). The statistics are as follows (Tab. 4):

Analysis of variation and intra-host variation

Use samtools mpileup and in-house scripts to analyze SARS-CoV-2 mutations. Extract all mutation sites with a frequency above 90% and the site depth $\geq 10X$. A total of 18 loci were detected from 9 samples. 209001889R and 209001909R share 2 identical mutations (27925, 28377). 209001897R and 209001903R share the C28854T mutation (Tab.5).

Extract all the sites with a frequency of more than 5% and the genotype depth of the mutation $> 5X$, and remove the sites with a mutation frequency of more than 95%. A total of 42 Intra-host mutations (intra-host mutations) were obtained, which were sporadic. The same mutation was not found in any two samples. For details, please see " Supplementary Table 1".

Extracting the genotypes at positions 8782 and 28144 in all samples, 5 samples can distinguish the L/S type, including 4 S type and 1 L type (Tab.6).

SARS-Cov-2 and human genome integration analysis

For the sample "209001902R" with the highest virus depth, the reads that were not completely aligned to humans and that were not completely aligned to SARS-Cov-2 were re-analyzed, and no reads that reliably supported fusion were found (Supplementary Table 2).

Analysis of mixed infection with other pathogens

4(25%) positive samples were combined with Human adenovirus infection. 209001894R was Human respiratory syncytial virus B infection (Supplementary Table 3).

Metatranscriptome analysis

The data of the 8 throat swabs and 6 stool samples were spaded and de-redundant (cd-hit) to obtain the gene set. And use QAST to evaluate the assembly effect. Compare the reference gene set (throat swabs and stool separately) to all samples, and calculate the expression level. Use "edgeR" to do DEGs analysis on the negative and positive groups. The results are shown in the form " Supplementary Table 4". The volcano plot (ggplot2) of the differential expression of transcripts of swab and stool in negative and positive samples is shown in the Figure 5.

Metaphlan was used to analyze the origin of mRNA sequence species. Calculate the Shannon, Simpson, and inverse Simpson indexes (R package vegan) at the species and genus levels. Calculate t-test, Wilcox test value (python3 scipy). Calculate the kind of horizontal unifracs distance (R package rbiom). Draw PCoA diagrams (ape, ggplot2). See Supplementary Table 4 form "Diversity". The following Fig.6 is a PCoA diagram made by unifracs distance. Humann3 is used to make functional annotations on the part of the macro transcript data, and the difference between the positive and negative two groups is calculated. The pathways with Wilcox-test p-value<0.05, t-test p-value<0.05 and Equal_Vari_P>0.05 (levene test for homogeneity of variance) were screened out (Tab.7 and Supplementary Table 5).

For the rRNA part, use Kraken2 to do taxonomy on the data, and calculate the Shannon, Simpson, and inverse Simpson indices. There was no significant difference in species and genus levels (Wilcox-test, p=0.1824, p=0.1654).

Discussion

During the epidemic, although we must pay attention to the spread of SARS-CoV-2 and medical control, appropriate infection control measures must be also implemented to prevent and control possible infections of other respiratory pathogens. Clinicians should not ignore that common respiratory pathogens cause fever or even co-infection with SARS-CoV-2²¹. Therefore, this study aims to better understand the fever symptoms of patients caused by common respiratory pathogens during the COVID-19 epidemic.

The co-infection of *Influenza A/B virus* at COVID-19 diagnosis is uncommon. Most patients have a fever caused by common respiratory pathogens such as *Influenza A virus* (FluA), *Influenza B virus* (FluB), *M.Pneumonia*(MP), *Adenovirus* (ADV), *Respiratory syncytial virus* (RSV), *Parainfluenza virus I* (PIV1), *Parainfluenza virus II* (PIV2), *Parainfluenza virus III* (PIV3), *Coronavirus virus 229E* (HCoV-229E), *Human metapneumovirus* (HMPV), *Human Coronavirus virus OC43* (HCoV-OC43), *Human Boca virus* (HBoV). These findings are quite different when compared with those of other viral pandemics. As it relates to hospitalised patients with COVID-19, such findings could prove essential in defining the role of empiric antimicrobial therapy or stewardship strategies. At present, most doctors for patients with confirmed SARS-CoV-2 will use broad-spectrum antibacterial and antiviral treatment. 99 COVID-19 patients in Wuhan, China reported that 71%, 76%, and 15% used antibiotics, antiviral agents, and antifungal agents²². For confirmed patients, clinicians can give patients empirical broad-spectrum antibacterial treatment according to relevant guidelines²³. But for patients with undiagnosed fever to avoid the abuse

of antibiotics, we should use drugs carefully. According to the data shown in our research, infectious doctors still have to pay great attention to the infection of common respiratory pathogens in patients with fever, and can give patients ordinary anti-cold drugs.

In order to further explore the impact of SARS-CoV-2 infection on the host and co-infection with other respiratory pathogens. We conducted a rapid test for the new coronavirus (fastv) on 26 fever samples. Comparing virus library and bacterial library for species annotation. We found that the mutation after SARS-CoV-2 infection was sporadic, and the same mutation was not found in any two samples. Due to the defects of sample quality, only 5 samples can distinguish L/S type, of which 4 are S type and 1 is L type. This part of the data indicates that the SARS-CoV-2 detected in patients with covid-19 in low-risk areas in China has a small trend of variation and most of them are low-virulence S-types^{24,25}. And the study found that there is no integration phenomenon between SARS-Cov-2 and the human genome. The study found that SARS-CoV-2 positive samples will also be infected with other viruses, especially adenovirus. Therefore, it is necessary to use antiviral preventive drugs for fever patients during the epidemic²⁶. Macrotranscript analysis showed that there was no significant difference in the species and genus levels of pathogens between covid-19 patients and other fever patients. This finding proves that SARS-Cov-2 infection does not cause significant changes in the human microbial population. In addition, the main pathways that affect human metabolism after SARS-Cov-2 infection are the Calvin-Benson-Bassham cycle, pyrimidine deoxyribonucleotides de novo biosynthesis I and D-galactose degradation V (Leloir pathway) and other common aerobic respiration and nucleic acid metabolism pathways. This also further confirmed the positive regulation of SARS-Cov-2 on human oxidative stress and inflammatory storm. Clinicians need to pay attention to the co-infection of covid-19 patients with pathogens, but also to pay attention to the patient's blood gas, electrolyte levels, and peripheral blood immune factor levels²⁷.

Conclusion

During the COVID-19 epidemic, due to the strengthening of the Chinese government, medical institutions, and public protection measures, the number of patients with *Influenza A/B virus* during the same period has been greatly reduced. In the face of fever patients, clinicians still need to pay more attention to infections of common respiratory pathogens in addition to SARS-CoV-2. China's public health measures to stop the spread of the epidemic have proven effective.

List Of Abbreviation

COVID-19	Corona Virus Disease 2019
SARS-CoV-2	<i>Severe acute respiratory syndrome coronavirus 2</i>
MERS	the Middle East respiratory syndrome
hMPV	<i>Human metapneumovirus</i>
hBoV	<i>Human Boca virus</i>
hCoV	<i>Human coronavirus</i>
RSV	<i>Respiratory syncytial virus</i>
MP	<i>Mycoplasma pneumoniae</i>
PCR	Polymerase Chain Reaction
FluA	Influenza A
FluB	Influenza B
MP	<i>M.Pneumonia</i>
ADV	<i>Adenovirus</i>
PIV	<i>Parainfluenza virus</i>
HCoV-229E	<i>Coronavirus virus 229E</i>
HCoV-OC43	<i>Human Coronavirus virus OC43</i>

Declarations

Ethical Approval and Consent to participate

Not Applicable

Consent for publication

All authors agree to publish.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

All the authors have no conflict of interest.

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Authors' contributions

All authors have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; and been involved in drafting the manuscript or revising it critically for important intellectual content, and participated sufficiently in the work to take public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work.

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Tables

Due to technical limitations, table 1 to 7 xlsx are only available as a download in the Supplemental Files section.

Figures

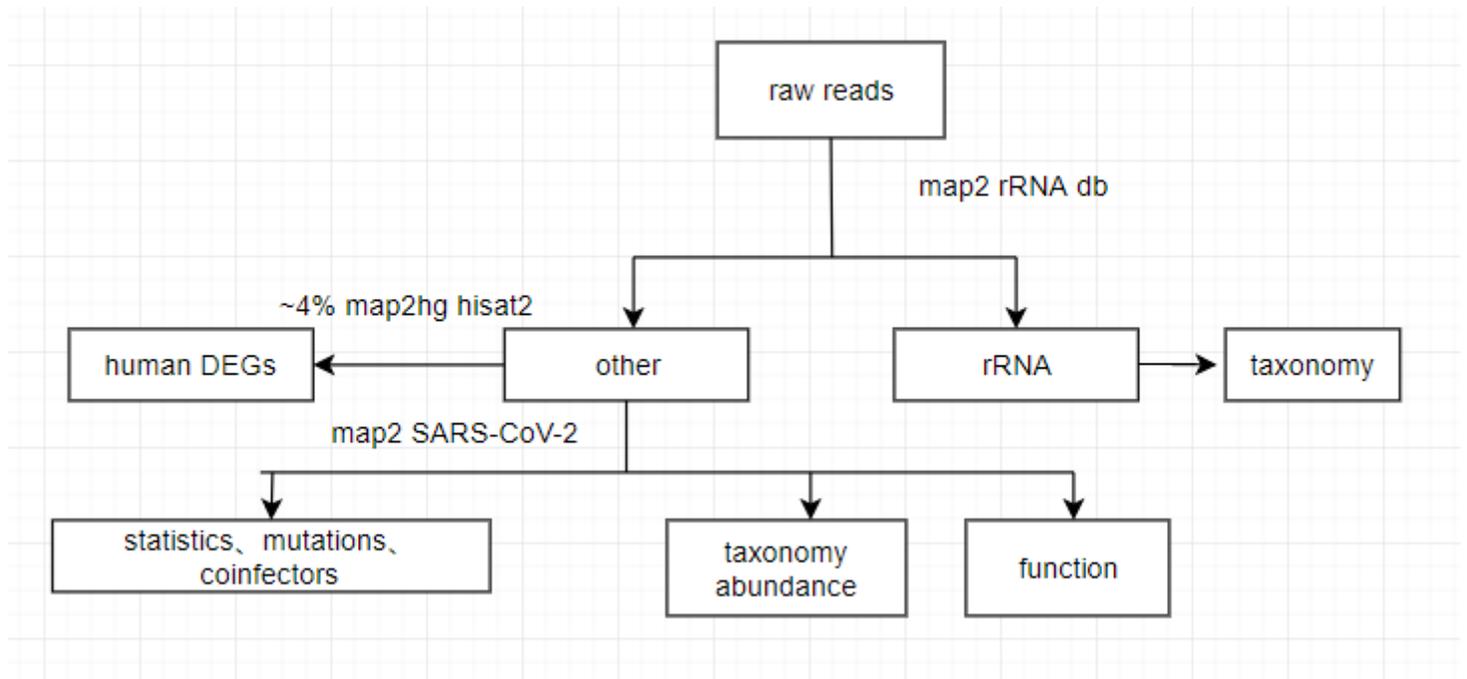


Figure 1

Bioinformatics analysis process

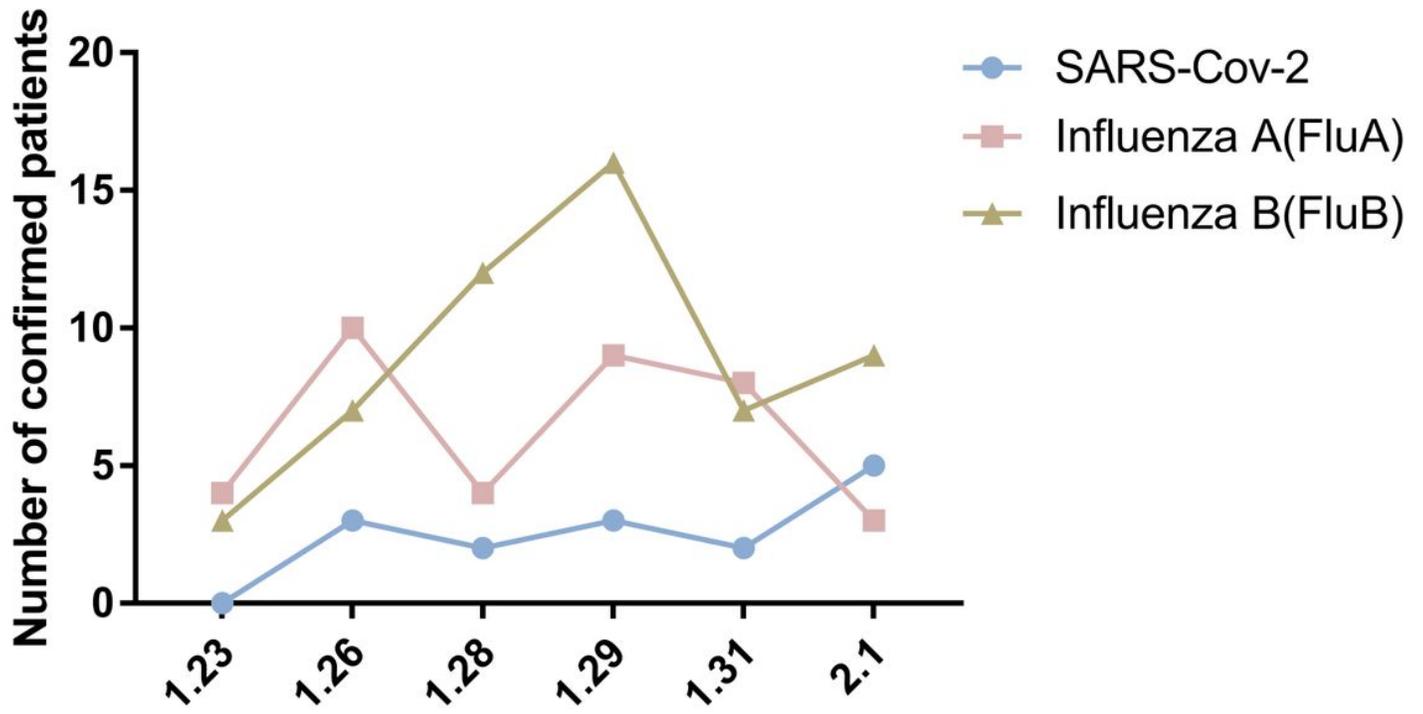


Figure 2

SARS-CoV-2 and Influenza A/B virus infection trends during the epidemic.

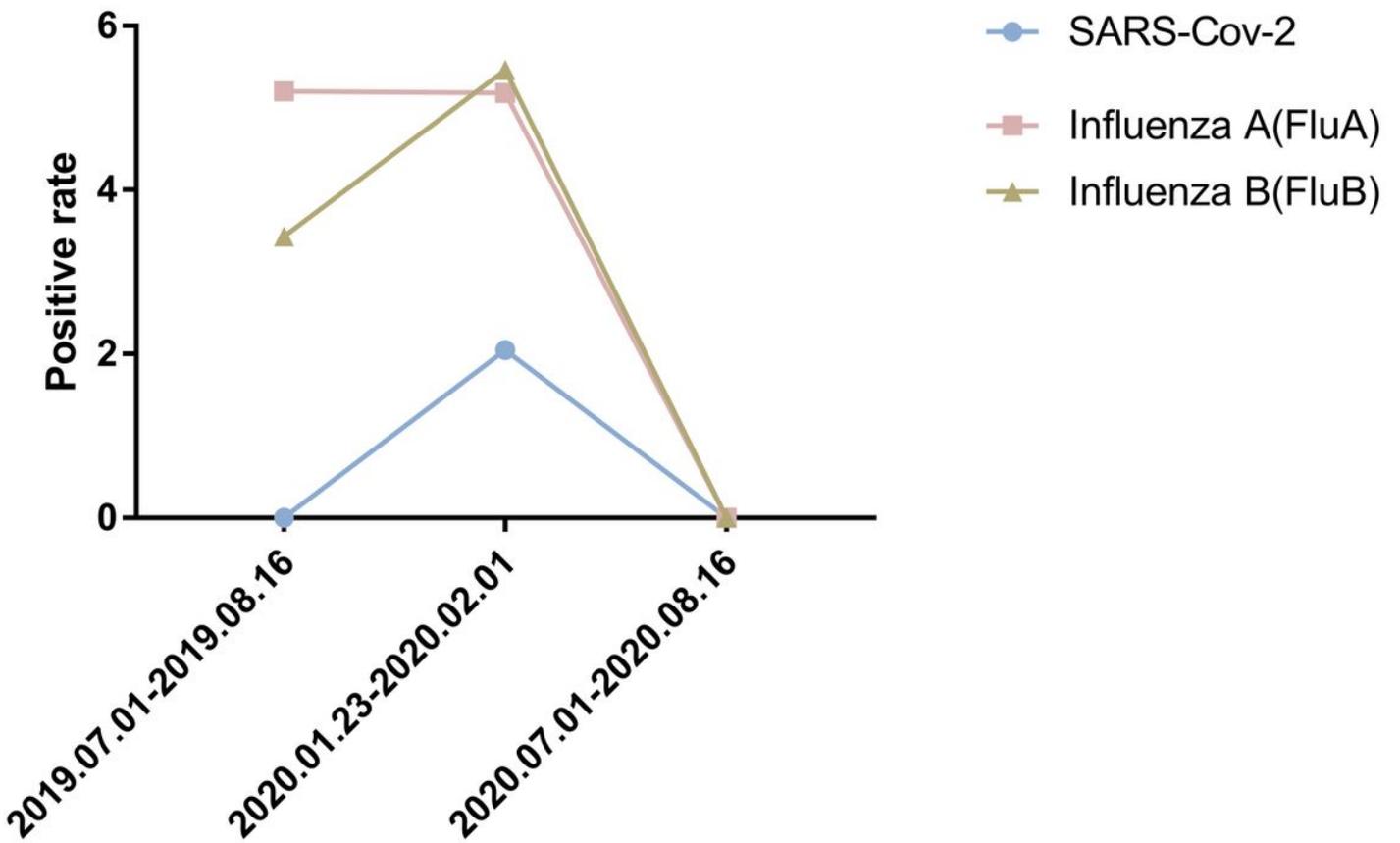


Figure 3

Changes in Influenza A/B virus before, during, and after the COVID-19 epidemic. 2020.1.23-2.1: Jan 23th, Jan 26, Jan 28, Jan 29, Jan 31, and Feb 1st.

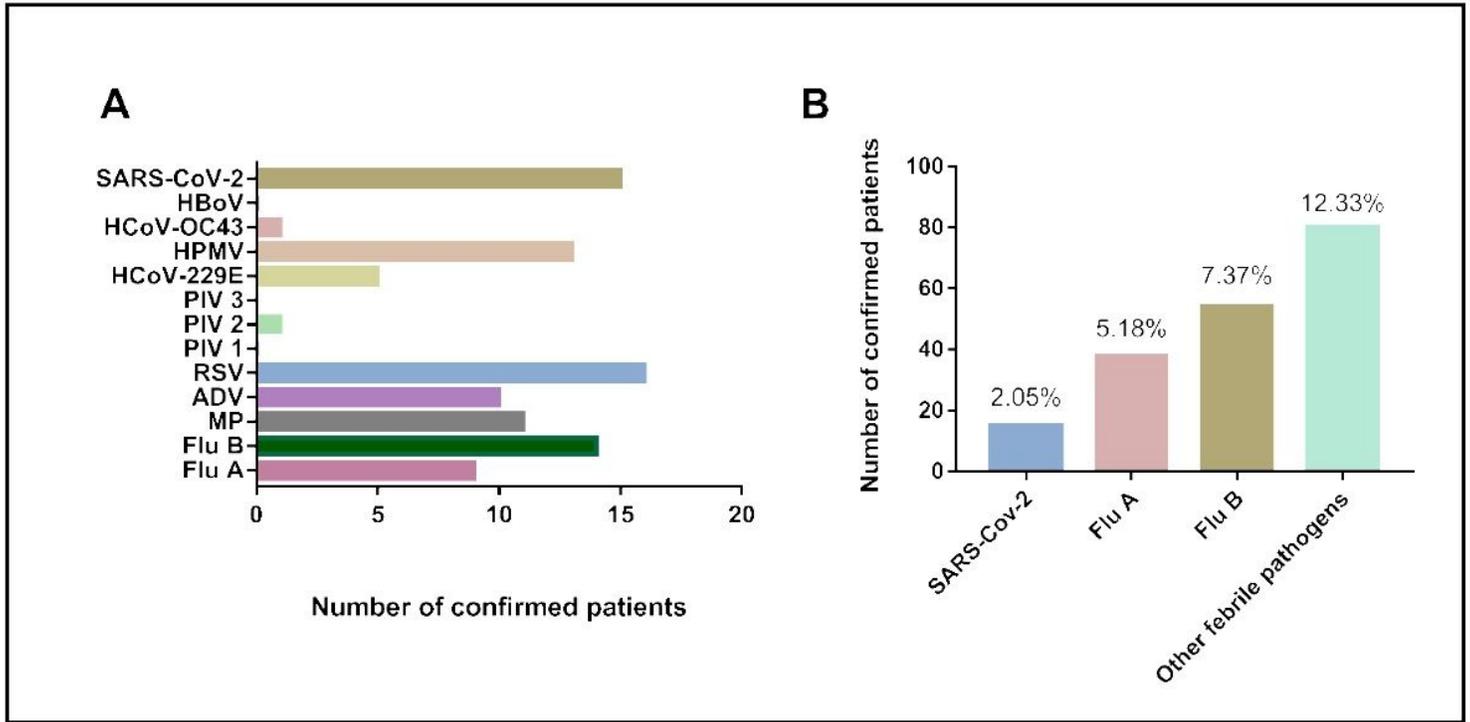


Figure 4

Infections of other pathogens in fever patients without SARS-CoV-2.

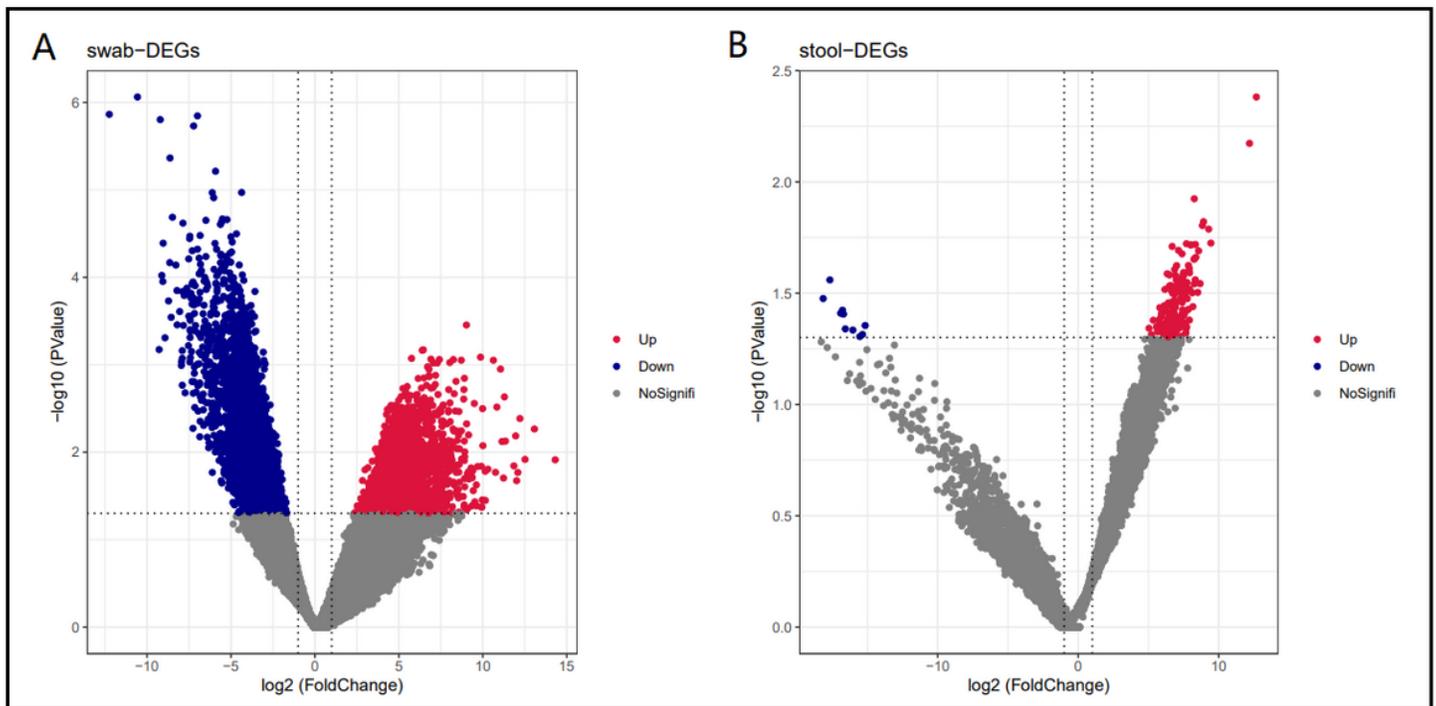


Figure 5

(A) The volcano plot of the differential expression of transcripts of swab in negative and positive samples. (B) The volcano plot of the differential expression of transcripts of stool in negative and positive samples. For differential transcripts, please see the form "throat swab-DEGs" and "stool-DEGs" in Supplementary Table 4.

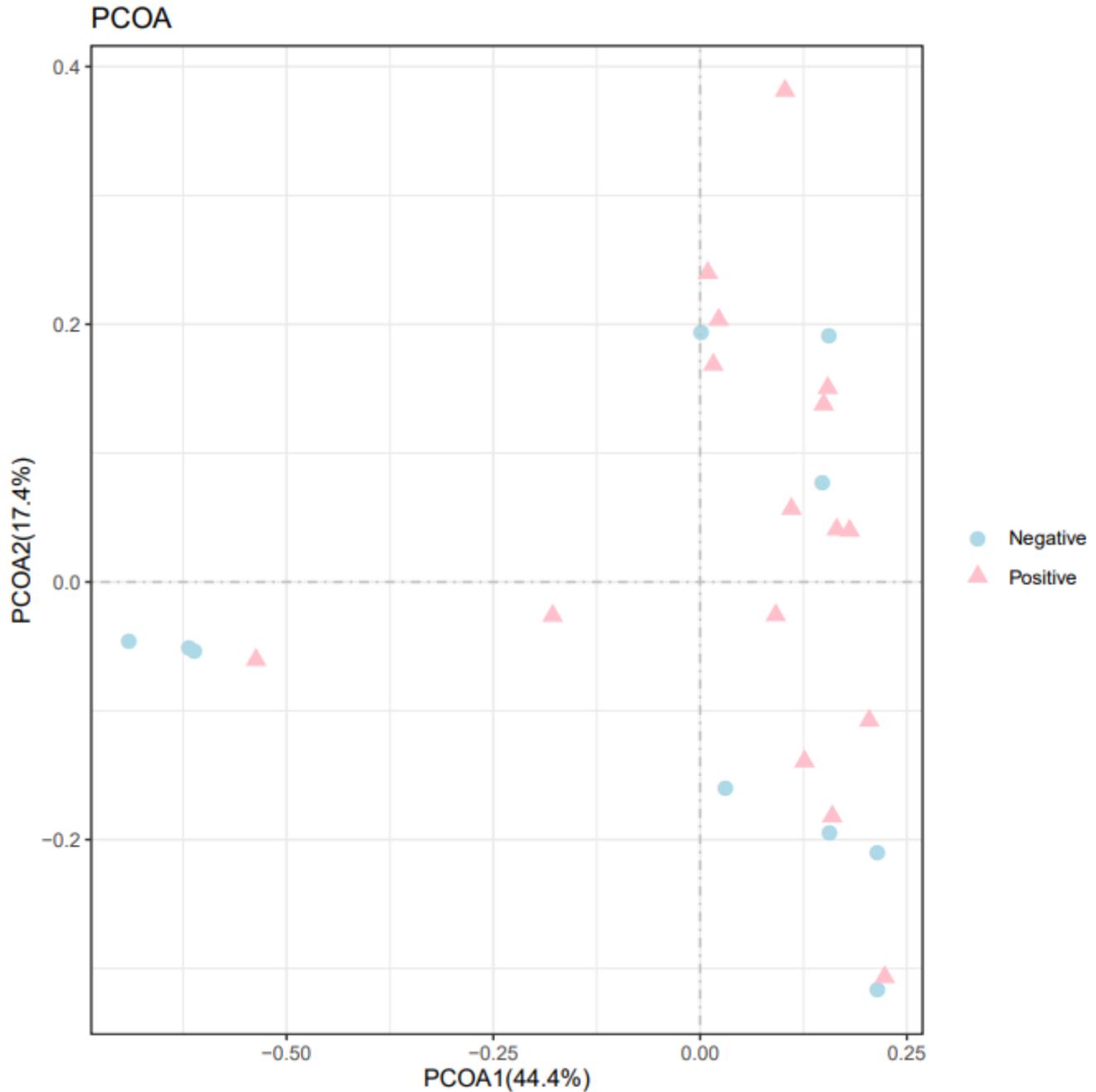


Figure 6

PCoA diagram made by unifrac distance

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.xlsx](#)
- [SupplementaryTable2.xlsx](#)
- [SupplementaryTable3.xlsx](#)
- [SupplementaryTable4.xlsx](#)
- [SupplementaryTable5.xlsx](#)
- [Tab.1.xlsx](#)
- [Tab.2.xlsx](#)
- [Tab.3.xlsx](#)
- [Tab.4.xlsx](#)
- [Tab.5.xlsx](#)
- [Tab.6.xlsx](#)
- [Tab.7.xlsx](#)