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Coronavirus Pandemic

Associated factors of respiratory co-infection of COVID-19 and the impact of co-infection on SARS-CoV-2 viral load

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Abstract

Introduction: Emerging evidence indicates that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected individuals are at an increased risk for co-infections. This retrospective study aims to expand the knowledge of associated factors of respiratory co-infection in SARS-CoV-2 positivity.

Methodology: A retrospective study was conducted to recruit fifty-five patients with laboratory-confirmed SARS-CoV-2 positivity. We additionally tested 29 other respiratory pathogens using RT-PCR assay for the same specimens tested for laboratory-confirmed SARS-CoV-2. Both univariate and multivariate analysis were performed to identify independent factors for co-infection. Cox regression was conducted to detect the association between co-infection and viral load after controlling other related factors.

Results: Among all the fifty-five COVID-19 patients, the rate of co-infection with at least one other respiratory pathogen was 76.4% (42/55). The rate of bacterial co-infections was 83.3% (35/42), among which Streptococcus pneumonia was the most common co-infection. Over 70% of neutrophils proportion (OR: 4.563; 95% CI: 1.116-18.648) was an independently associated factor for bacterial co-infection, whereas fever (OR: 4.506; 95% CI: 1.044-19.441) and chest tightness (OR: 0.106; 95% CI: 0.015-0.743) for viral co-infection. The strongest promotion of SARS-CoV-2 viral decreasing load was detected from co-infection of only viruses (HR: 4.039; 95% CI: 1.238-13.177), and the weakest was found from co-infection of only bacteria (HR: 2.909; 95% CI: 1.308-6.472).

Conclusions: Various co-infections variously promote SARS-CoV-2 viral decreasing load. Timely identification of co-infections aggressively contributes to COVID-19 patient management.

Key words: Co-infection; COVID-19; respiratory pathogens; independent factors; negative conversion.

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Introduction

Since a cluster of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China, in December 2019, it has become a global threat and spread rapidly with historic numbers of cases and deaths across more than 200 countries and regions [1,2]. Even though the World Health Organization (WHO) declared that COVID-19 no longer constitutes a public health Emergency of International Concern on May 5, 2023, it continues to have hazardous impacts on human health. From the initial supertransmission and severe lung symptoms to the lung infection symptoms significantly reduced by the more robust transmission of the Omicron variant strain, the novel coronavirus has an ingenious way of avoiding confrontation. Up until this point, the virus has been continuously mutating, causing waves of epidemics [1,3]. Co-infection with other respiratory pathogens such as viruses and bacteria has concerned scientists and clinicians. In a study, 13 pathogens were identified among patients infected with SARS-CoV-2, and 46.6% (89/191) of patients had co-infection with one or more additional pathogens. The viral co-infection rate was 7.3% and the bacterial co-infection rate was 47.1% [4]. More than 50% of COVID-19 patients were co-infected with one or more pathogens [5-6]. However, few studies reported on the factors associated with the presence of a viral or bacterial co-infection and how does it affect the SARS-CoV-2 viral load. The role of co-infection in the impact of COVID-19 infection is not yet clear and we aim to expand the knowledge of distribution and associated factors of co-infection in COVID-19 patients and assess the impact of co-infection on the SARS-CoV-2 viral load.

Methodology

Study area and data collection

All cases with laboratory-confirmed SARS-CoV-2 positivity during the early epidemic (from January 22 to February 14, 2020) in Qingdao were recruited to conduct a retrospective study. All SARS-CoV-2 positive cases with or without clinical symptoms were admitted to the only local designated hospital (Qingdao Chest Hospital). Their swab specimens at admission were firstly used for laboratory confirmation of SARS-CoV-2 infection, and the remaining specimens were stored at -20°C for later retrospective analysis of other respiratory pathogens co-infection. Epidemiological and demographic data were collected from epidemiological investigations. Clinical, laboratory, and therapeutic data were extracted from the Hospital Information System. All COVID-19 patients were diagnosed according to the New Coronavirus Pneumonia Diagnosis and Treatment Plan [7-9]. This study was approved by the Ethics Commission of Qingdao Municipal Center for Disease Control and Prevention (Date: 18 October 2019; Number: QFELL-KY-2019-67).

Detection of SARS-CoV-2 in respiratory samples

The doctors collected the samples by rubbing 2 of the patient's nostrils and the posterior oropharynx using separate cotton-tipped swabs. The swabs were collected into a single virus collection tube containing a virus preservation solution. Part of the sample was extracted for laboratory testing, and the rest was stored at -20 °C. Tests were carried out in biosafety level 2 facilities, using a commercial Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit (Shanghai BioGerm Medical Technology Co., LTD) in a total reaction volume of 25 μL, targeting SARS-CoV-2 virus frame1ab (ORF). Viral ribonucleic acid (RNA) was extracted from sample material and collected in the elution buffer, and then underwent real-time reverse-transcription-polymerase-chain-reaction (RT-PCR) with SARS-CoV-2-specific primers and probes. Detailed laboratory procedures were referenced and described elsewhere [10,11].

Detection of other respiratory pathogens

The rest of the sample stored at -20 °C was used to test other respiratory pathogens later through RT-PCR via general procedure. 16 bacterial and 13 viral pathogens were retrospectively detected, including *Mycoplasma pneumoniae* (MP), *Moraxella catarrhalis* (MC), *Chlamydophila pneumonia* (CP), *Streptococcus pneumonia* (SP), *Haemophilus influenzae* (HI), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Moraxella catarrhalis* (MA), *Klebsiella pneumoniae* (KP), *Legionella pneumophila* (LP), *Bordetella pertussis* (BP), *Mycobacterium avium* (AV), *Mycobacterium tuberculosis* (TB), *Acinetobacter baumannii* (AB), *Bordetella parapertussis* (BPP), *Stenotrophomonas maltophilia* (SMP), *Serratia marcescens* (SM), influenza A (IVF-A), influenza B (IVF-B), human respiratory syncytial virus (RSV), parainfluenza (PIV, contended 1, 2, 3 and 4 four types), human metapneumovirus (HMPV), coronaviruses 229E, OC43 and NL63 (HCoV-229E, HCoV−OC43 and HCoV-NL63), human bocavirus adenovirus (HBoV), human adenovirus (HAdV), human rhinovirus (HRV), human enterovirus (HEV) and MERS-CoV, were also detected using pathogen specific one-step respiratory viruses multiplex RT-PCR detection Kit (Neuro-Hemin Biotech) and pneumobacter multiplex PCR detection Kit (Neuro-Hemin Biotech). The reliability of detection reagent was verified by a separate fluorescent real-time RT-PCR kit.

Statistical analysis

A retrospective study was conducted to analyze the associated factors of other respiratory co-infection in SARS-CoV-2 positivity and assess the impact of co-infection on SARS-CoV-2 viral load. The considering potential factors included demographics, clinical, laboratory, imaging, and other variables, which were listed in Table 1. Univariate and multivariate analyses were performed in sequence to identify independent factors of co-infection. Mann-Whitney U test, χ^2 test, or Fisher's exact test was first conducted univariate analysis, by comparing differences between groups with and without co-infection of other respiratory pathogens in COVID-19 patients. Next, factors with statistical significance ($p < 0.05$) in the univariate analysis were further analyzed using the Logistic regression model, and the odds ratio (OR) was calculated. Co-infection with bacteria and viruses was separately analyzed.

In addition, we used Cox regression to assess the impact of co-infection on SARS-CoV-2 viral load. Co-infection was introduced into the Cox regression model, which was set as a categorical variable presented by no co-infection (endowed by 0), co-infection of only bacteria (endowed by 1), co-infection of only viruses (endowed by 2), and co-infection of combined bacteria and viruses (endowed by 3), respectively. A previous study suggested that age older than 45 years and chest tightness are independent factors affecting SARS-CoV-2 viral load [10]. Therefore, age older than 45 years and

chest tightness were also introduced to control their impacts.

Continuous and categorical variables were presented as median (interquartile range, IQR) and n (%), respectively. A *p* value less than 0.05 (two-tailed) was considered statistically significant. Analyses were performed using SPSS software (version 22.0) and R software (version 3.6.3).

Results

Characteristics of COVID-19 patients

A total of 55 patients hospitalized with COVID-19 were included in this study, with a median age of patients was 45 years (IQR: 32-58). Among confirmed cases, 31 were female, which accounted for 56.4%, followed by 24 male cases for 43.6%.

The median duration from disease onset to hospital admission was 2 (IQR: 1-5) days, with a median of 19 days from illness onset to hospital discharge (IQR: 16-25.5). As shown in Table 1, 80% (44/55) of the COVID-19 patients were non-severe (mild to moderate cases),

Table 1. Results from univariate analysis of co-infection with other respiratory pathogens.

Variable	Total (n = 55)	Bacterial Co-infection (n = 35)	*p* value	Virus Co-infection (n = 13)	*p* value
Demographics					
Sex					
Male	24 (43.6)	19 (54.3)	0.035*	3 (23.1)	0.087
Female	31 (56.4)	16 (45.7)		10 (76.9)	
Age	45 (32-58)	40 (30-56)	0.132	50 (39-75)	0.125
Clinical characteristics					
Disease severity					
Non-severe	44 (80.0)	28 (80.0)	0.909	10 (76.9)	0.751
Severe	11 (20.0)	7 (20.0)		3 (23.1)	
Fever					
No	13 (23.6)	7 (20.0)	0.401	6 (46.2)	0.029*
Yes	42 (76.4)	28 (80.0)		7 (53.8)	
Non-productive cough					
No	34 (61.8)	20 (57.1)		10 (76.9)	0.200
Yes	21 (38.2)	15 (42.9)	0.345	3 (23.1)	
Sputum production					
No	46 (83.6)	30 (85.7)	0.582	10 (76.9)	0.454
Yes	9 (16.4)	5 (14.3)		3 (23.1)	
Headache					
No	47 (85.5)	30 (85.7)		9 (69.2)	0.053
Yes	8 (14.5)	5 (14.3)	0.942	4 (30.8)	
Chest tightness					
No	49 (89.1)	34 (97.1)	0.033*	10 (76.9)	0.045*
Yes	6 (10.9)	1 (2.9)		3 (23.1)	
Laboratory findings					
Leucocyte count					
$< 4 \times 10^9$/L	5 (9.1)	5 (14.3)	0.195	1 (14.2)	0.971
$4\text{-}10 \times 10^9$/L	49 (89.1)	29 (82.9)		12 (92.3)	
$\geq 10 \times 10^9$/L	1 (1.8)	1 (2.8)		0	
Neutrophil percentage					
< 50%	6 (10.9)	4 (11.4)	0.049*	2 (15.4)	0.565
50%-70%	34 (61.8)	25 (71.4)		8 (61.5)	
≥ 70%	15 (27.3)	6 (17.2)		3 (23.1)	
Lymphocyte percentage					
< 20%	16 (29.1)	7 (20.0)	0.084	3 (23.1)	0.566
20%-40%	32 (58.2)	23 (65.7)		8 (61.5)	
≥ 40%	7 (12.7)	5 (14.3)		2 (15.4)	
Imaging					
CT imaging					
Normal	11 (20)	8 (22.9)	0.483	3 (23.1)	0.751
Abnormal	44 (80)	27 (77.1)		10 (76.9)	
Involved lung field					
Unilateral	14 (25.5)	10 (37.0)	0.499	3 (30.0)	0.942
Bilateral	30 (54.5)	17 (63.0)		7(70.0)	
Radiological characteristics					
Non-High -density shadow	17 (38.6)	13 (48.1)	0.124	2 (20.0)	0.271
High-density shadow	27 (61.4)	14 (51.9)		8 (80.0)	
Other					
Comorbidities					
No	42 (76.4)	29 (82.9)	0.134	9 (69.2)	0.488
Yes	13 (23.6)	6 (17.1)		4 (30.7)	
Ct-values of ORF					
< 30	38 (69.1)	23 (65.7)	0.473	11 (84.6)	0.166
≥ 30	17 (30.9)	12 (34.3)		2 (15.4)	
Days of negative conversion	13 IQR (10-18)	13 IQR (10-15)	0.269	10 IQR (7.5-16.5)	0.336
Days from illness onset to hospital discharge	19 IQR (16-25.5)	18.5 IQR (15-23.5)	0.123	17.5 IQR (14.5-24.5)	0.412

*: $p < 0.05$; IQR: interquartile range; ORF: SARS-CoV-2 virus frame1ab; Severe COVID-19 as defined by National Institutes of Health: available from: https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum.

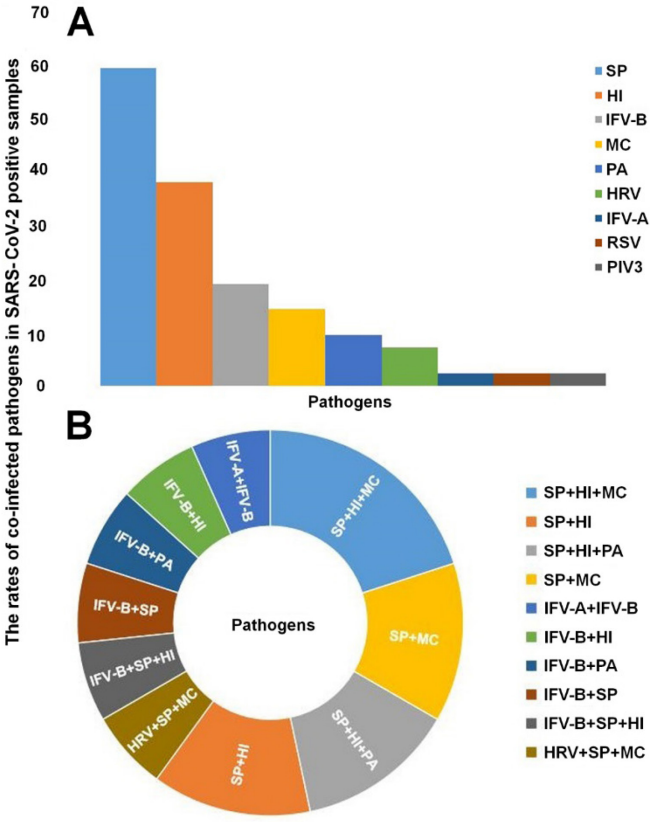
and 20% (11/55) were severe cases. The most common symptoms at the onset of the disease were fever (76.4%) and non-productive cough (38.2%). Thirteen patients had underlying diseases, cardiovascular and cerebrovascular disease, endocrine disease, gastrointestinal disease, respiratory disease, and neurological disorder.

On admission, five patients had leucopenia (leucocyte count < 4×10^9/L). Fifteen patients had an increased proportion of neutrophils (> 70%), and seven patients showed elevated lymphocyte percentage (> 40%). Abnormalities in chest computed tomograms (CT) were detected in 44 patients (80%). The most common pattern of CT change was high-density shadow (61.4%). All patients received standard care including supportive and antiviral treatment according to the latest clinical guidelines [7-9]. More detailed information is reported in Table 1.

Co-infection in COVID-19 patients

Among all the 55 COVID-19 patients, 42 cases (76.4%) had RT-PCR positive detection against at least one of other respiratory pathogens, including bacterial co-infection (35/55, 63.6%) and viral co-infection (13/55, 23.6%). As shown in Figure 1, four types of bacteria and five types of viruses were detected in COVID-19 patients. Among co-infected cases, 83.3% (35/42) were detected with bacterial pathogens, which were as follows: SP (25/42, 59.5%), HI (16/42, 38.1%), MC (6/42, 14.3%), and PA (4/42, 9.5%). Moreover, 31.0% (13/42) of co-infected patients were detected with viral pathogens, which included IFV-B (8/42, 19.0%), HRV (3/42, 7.1%), IFV-A (1/42, 2.4%), RSV (1/42, 2.4%) and PIV3 (1/42, 2.4%). In addition, 64.3% (27/42) of co-infected cases were detected with a single pathogen, followed by 35.7% (15/42) with more than one pathogen. The common combination of co-infection (more than one other pathogen) was SP + HI + MC (3/42, 7.1%), SP + HI (2/42, 4.8%), SP + HI + PA (2/42, 4.8%), and SP + MC (2/42, 4.8%). More information regarding the distribution of co-infection of other respiratory pathogens in COVID-19 patients is shown in Figure 1.

Figure 1. The rates of co-infected pathogens in SARS-CoV-2 positive samples.



A: single virus classification; **B:** combined viruses classification.

Independent factors of co-infection

Table 1 summarizes the results of the univariate analysis. We evaluated the effect of each factor on the co-infection of other respiratory pathogens among COVID-19 patients by the Mann-Whitney U test, χ^2 test, or Fisher's exact test. Results from the multivariate Logistic regression model are shown in Table 2. To the bacterial co-infection, sex, chest tightness, and neutrophil percentage were significantly related to co-infection of bacterial pathogens. Results from multivariate logistic regression introduced the three above factors and revealed that over 70% of neutrophils proportion (OR: 4.563; 95% CI: 1.116-18.648) were independent factors.

Moreover, according to the results of univariate analysis, fever, and chest tightness were significantly related to co-infection of viral pathogens, and after introducing two of them into the Logistic regression model, fever (OR: 4.506; 95% CI: 1.044-19.441) and chest tightness (OR: 0.106; 95% CI: 0.015-0.743) were independent factors.

Association between co-infection and SARS-CoV-2 viral loading

All patients achieved SARS-CoV-2 turning negative, with the median duration of complete turning negative being 13 days (IQR: 10-18). Moreover, it was 13 days (IQR: 10-15) in patients with bacterial co-infection, as well as 10 days (IQR: 7.5-16.5) in patients with viral co-infection. Results from the multivariate Cox regression model revealed that after controlling

Table 2. Independent factors of specific co-infection in COVID-19 patients.

Factors	OR	95% CI	*p* value
Bacterial co-infection			
Sex			
Female	Reference		
Male	0.344	0.094-1.252	0.105
Chest tightness			
No	Reference		
Yes	6.172	0.834-45.676	0.075
Neutrophil percentage			
< 50%	Reference		
50%-70%	2.300	0.292-18.099	0.550
> 70%	4.563	1.116-18.648	0.035*
Viral co-infection			
Fever			
No	Reference		
Yes	4.506	1.044-19.441	0.044*
Chest tightness			
No	Reference		
Yes	0.106	0.015-0.743	0.024*

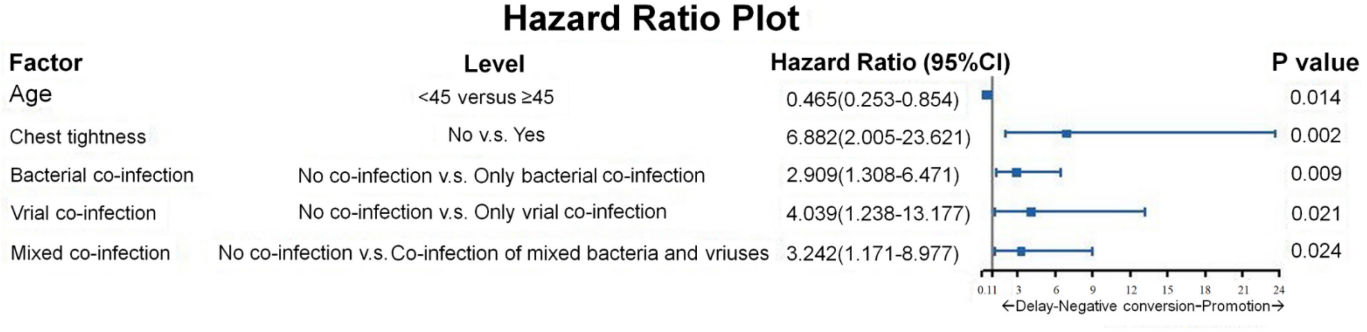
*: *p* value < 0.05; Bacterial co-infection: co-infection of only bacteria and mixed bacteria and viruses; Viral co-infection: co-infection of only viruses and mixed bacteria and viruses.

independent factors reported in the previous study, such as age older than 45 years and chest tightness, co-infection of other respiratory pathogens was also significantly associated with SARS-CoV-2 viral loading [10]. Co-infection of only bacteria, only viruses and combined them were all able to promote to decrease SARS-CoV-2 viral load, but the promotion was various in terms of different types of co-infection. As shown in Figure 2, the strongest promotion for turning viral negative was detected with co-infection of only viruses (HR: 4.039; 95% CI: 1.238-13.177), and the weakest was found for co-infection of only bacteria (HR: 2.909; 95% CI: 1.308-6.471). Interestingly, the promotion in co-infection of combined bacteria and viruses was between co-infection of only bacteria and only viruses, and its HR was 3.242 with a 95% CI ranging from 1.171 to 8.977.

Discussion

Owing to the important implication of respiratory co-infection for COVID-19 management, we found a large proportion of co-infection with other respiratory pathogens among COVID-19 patients during the early epidemic in Qingdao, China. Meanwhile, we determined independent factors associated with co-infection by univariate and multivariate analysis. Besides, decreasing SARS-CoV-2 viral load was considered to evaluate the impact of co-infection on COVID-19 patients. Our findings suggested the distribution of co-infection in COVID-19 and provided evidence that various co-infection variously affect SARS-CoV-2 negative turning. A reported rate of COVID-19 co-infection with 39 pathogen detection was 94.2% (virus 31.5%, bacteria 91.8%) from Zhu [12], as well as other reported rates of co-infected pathogens from 13.5% to 20.7% [13,14]. In our study, the co-infection rate of COVID-19 patients was 76.4% (virus 23.6%, bacteria 63.6%), which was close to these studies. To further verify whether the high rate of COVID-19 co-infection is related to SARS-CoV-2 infection, we collected pneumonia cases in fever clinics considered as suspected cases of COVID-19, including 178 febrile outpatients with pneumonia who were admitted to the local hospitals in Qingdao at the same time. As shown in Figure 3, the common pathogens in COVID-19 patients and pneumonia cases were almost

Figure 2. Results of association between co-infection and negative conversion from Cox regression.

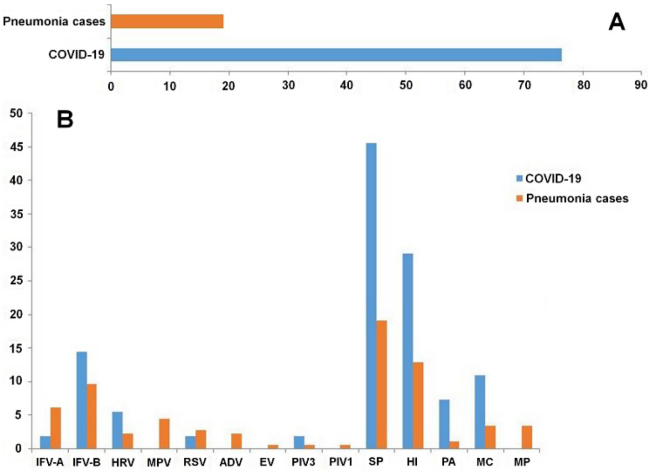


the same, including SP, HI, MC, and IFV-B, IFV-A. However, there was a significant difference in rates of co-infection between COVID-19 patients and pneumonia cases ($p < 0.05$), and the rate of co-infection in COVID-19 patients was four times of the co-infection rate of pneumonia cases (19.1%).

To our knowledge, this has been the first study focused on independent factors associated with SARS-CoV-2 co-infection. Based on previous findings that co-infection was not associated with disease severity, we further analyzed in terms of separately bacteria and viruses to determine characteristics of co-infection in COVID-19 patients. Among all co-infected patients, 83.3% had bacterial co-infection, which was more than twice the viral co-infection (31.0%). For co-infection of bacteria, the most common bacterial pathogens were SP and HI. Results from the multivariate Logistic model revealed that over 70% of neutrophils proportion was an independent factor of co-infection of bacteria, which positively associated with bacterial co-infection. Moreover, for co-infection of viruses, the most common viral pathogen is INF-B. After multivariate Logistic regression analysis, fever and chest tightness were independent factors of co-infection of viruses. Fever was positively associated with the co-infection of viruses, whereas chest tightness was negatively associated. These findings suggest the need to conduct comprehensive microbiologic surveys and clinical evaluation for other respiratory pathogens in COVID-19 patients, and clinicians should pay more attention to the co-infection for confirmed SARS-CoV-2 cases, which have great implications for COVID-19 treatment. Additionally, these independent factors may help clinicians identify keys for co-infection prevention in COVID-19 patients.

At present, there have been limited studies reporting the impact of co-infection on COVID-19 patients. Our findings have demonstrated that co-infection could impact SARS-CoV-2 viral load, suggesting that various co-infection promoted various shedding patterns of SARS-CoV-2. Compared with COVID-19 patients without co-infection, patients with co-infection could promote the duration of SARS-CoV-2 RNA shedding, and the effect of promotion varies from different types of co-infection pathogens. Results from multivariate Cox regression revealed that among all types of co-infection, the strongest promotion was detected with co-infection of only viruses, and the weakest was found for co-infection of only bacteria. Interestingly, the promotion of combined bacteria and virus co-infection was between co-infection of only bacteria and only viruses. However, there is no clear explanation for these

Figure 3. The comparison of co-infection between COVID-19 and pneumonia cases.



A: overall rate of co-infection; **B:** rate of infection for common respiratory pathogens.

findings. One of the potential explanations may be attributed to combination therapy. Although there has been no treatment guideline for co-infection in COVID-19, and the recommendations from different organizations are also inconsistent, combination therapy with non-anti-SARS-CoV-2 agents in co-infected COVID-19 patients has been seriously considered. In China, antibiotic therapy was recommended under different situations for COVID-19 patients in whom co-bacterial infection cannot be ruled out. Empirical antibiotics, such as amoxicillin, azithromycin, or fluoroquinolones, was recommended for mild cases, but broad-spectrum antibiotic covering all possible pathogens was suggested for severe cases [15]. Based on the limited data of the present work, it remains unclear which antimicrobial agents should be empirically prescribed in patients with COVID-19. In addition, an antimicrobial stewardship program should be implemented to prevent the rising rates of antimicrobial resistance that could be caused by an increase in inappropriate antibiotic use for viral pneumonia [16]. Besides combination therapy, another potential explanation may be attributed to the more antagonistic effect of bacteria for SARS-CoV-2 than it of other viruses. Our findings suggest that there may be an interaction between viral or bacterial replication and amplification in COVID-19 co-infection. As of now, there has been no evidence to explain this phenomenon. Wilks *et al* proposed that the defense system of the host, as a supraorganism, contained commensal bacteria and an immune system against bacterial and viral pathogens [17]. Several researchers supported the view that the microbiota could inhibit viral replication, and affect

virally induced pathogenesis [18-20]. Moreover, viruses in multiple infections can interact with each other in different ways, with different results such as antagonism [21]. These views may be useful in explaining our findings.

Notably, there are some limitations of this study. A small COVID-19 case was evaluated in comparison with other studies. The sample size was a little small, which affected the reproducibility of the results. Additionally, the potential factors were only considered in limited aspects. Our study was unable to confirm the co-infection derived from hospital or community-acquired. In our study, we try to identify the interaction between SARS-CoV-2 and other respiratory pathogens. The timeline of viral load of SARS-CoV-2 and other respiratory pathogens during the disease period may be also an effective variable for better understanding the interaction with co-infected pathogens. Due to the lack of continuous Ct values of all pathogens, we are unable to analyze this aspect, but future studies should pay more attention to doing this work.

Conclusions

Various co-infections variously promote SARS-CoV-2 viral decreasing loading. Timely identification of co-infections aggressively contributes to COVID-19 patient management.

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