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Epidemiological and co-infection characteristics of common human coronaviruses in Shanghai, 2015–2020: a retrospective observational study

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ABSTRACT

The coronavirus disease (COVID-19) pandemic is a major challenge worldwide. However, the epidemic potential of common human coronaviruses (HCoVs) remains unclear. This study aimed to determine the epidemiological and co-infection characteristics of common HCoVs in individuals with influenza-like illness (ILI) and severe acute respiratory infection (SARI). This retrospective, observational, multicentre study used data collected from patients admitted to nine sentinel hospitals with ILI and SARI from January 2015 through December 2020 in Shanghai, China. We prospectively tested patients for a total of 22 respiratory pathogens using multi-real-time polymerase chain reaction. Of the 4541 patients tested, 40.37% (1833/4541) tested positive for respiratory pathogens and 3.59% (163/4541) tested positive for common HCoVs. HCoV infection was more common in the non-endemic season for respiratory pathogens (odds ratio: 2.33, 95% confidence interval: 1.64–3.31). HCoV-OC43 (41.72%, 68/163) was the most common type of HCoV detected. The co-infection rate was 31.29% (51/163) among 163 HCoV-positive cases, with HCoV-229E (53.13%, 17/32), the HCoV type that was most frequently associated with co-infection. Respiratory pathogens responsible for co-infections with HCoVs included parainfluenza virus, rhinovirus/enterovirus, influenza A virus, and adenovirus. Furthermore, we identified one patient co-infected with HCoV-OC43 and HCoV-NL63/HKU1. The prevalence of common HCoVs remains low in ILI/SARI cases, in Shanghai. However, the seasonal pattern of HCoVs may be opposite to that of other respiratory pathogens. Moreover, HCoVs are likely to co-exist with specific respiratory pathogens. The potential role of co-infections with HCoVs and other pathogenic microorganisms in infection and pathogenesis of ILI and SARI warrants further study.

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KEYWORDS Human coronavirus; SARS-COV-2; co-infection; respiratory pathogen; HCoV-OC43; HCoV-229E

Introduction

A total of seven human coronaviruses (HCoVs) have been identified, namely, HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first four HCoVs are common HCoVs that have long co-existed with humans and have low pathogenicity, tending to not to cause severe disease [1–4]. Previous studies have found that common HCoVs are widespread globally and are more prevalent during the winter months [5–7]. Approximately 30–50% of common HCoV infections are co-infections with other respiratory pathogens [8–10]. Co-infection may be a risk factor for more severe disease, and higher mortality [11].

As of 18 June 2021, the World Health Organization (WHO) has reported a cumulative total number of 177,108,695 confirmed cases of coronavirus disease (COVID-19) worldwide. SARS-CoV-2 is an emerging human coronavirus with many similarities to common HCoVs [12]. SARS-CoV-2 infection may cause severe illness; however, a substantial proportion of SARS-CoV-2 infections are mild and asymptomatic, with approximately 40% of infections being completely asymptomatic and some infections are not diagnosed due to the limited severity of the symptoms

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[13]. Some studies suggest that after a long period of epidemic spread, SARS-CoV-2 will eventually enter seasonal circulation, as human immunity improves and the virus evolves [14–18].

Although antibodies to HCoV-OC43 cross-react with SARS-CoV-2, the presence of cross-reacting antibodies has not been demonstrated to be associated with the prevention of COVID-19 [19], and one study found a worse clinical outcome in COVID-19 patients with previous humoral immunity to α-coronaviruses [20]. There is thus the potential for HCoV infection status to influence the epidemiological and clinical characteristics of COVID-19, especially during the post-pandemic period when SARS-CoV-2 transforms into a common seasonal respiratory virus.

Therefore, the aim of this study was to characterise the epidemiology and co-infection features of common HCoVs. We performed a retrospective, observational, and multicentre study of patients admitted to hospitals with acute respiratory infections in Shanghai from January 2015 through December 2020. The findings of the study would help to gain a better understanding of HCoV infections and provide a basis for further studies on the association between HCoVs and SARS-CoV-2 infection epidemic characteristics. The results will also assist in the development of control and prevention strategies of these coronavirus infections.

Materials and methods

Ethical approval

This study was based on the data obtained from the Comprehensive Surveillance of Acute Respiratory Infections in Shanghai implemented by the Shanghai Municipal Centre for Disease Control and Prevention. The data analysis and study protocols of the project (including "Analysis of the results of the Comprehensive Surveillance of Acute Respiratory Infections in Shanghai" and "Study on the pathogenic characteristics and clinical outcome of severe acute respiratory infections in children and adults") were reviewed and approved by the Ethics Review Committee of the Shanghai Municipal Centre for Disease Control and Prevention (IRB numbers: 2017-31 and 2021-70). Requirement for informed consent was waived by the IRB because the data were kept confidential, de-identified, and were included as part of the normal diagnostic procedures.

Case definition

Outpatients and inpatients with acute respiratory infections of all age groups were included in this study. Patients who met the WHO definitions of influenza-like illness (ILI) and severe acute respiratory infection (SARI) [21] were eligible for inclusion. ILI was defined as "an acute respiratory tract infection with fever (measured temperature ≥38°C) with cough or sore throat," and SARI was defined as "a case of acute respiratory infection with fever (measured temperature or previous temperature ≥38°C) with cough that required hospitalisation within 10 days of onset." All eligible patients were sampled systematically, and patients without specimens, or with missing personal information and clinical records were excluded.

Study design

The present study, a retrospective observational multicentre study, was conducted at the Shanghai Municipal Centre for Disease Control and Prevention from 2015 through 2020.

Patients were included in this study from a total of nine sentinel hospitals across urban and rural areas in Shanghai. The sentinel hospitals included Renji East Hospital in Pudong New Area (tertiary hospital), Xuhui Central Hospital in Xuhui District (tertiary hospital), Yangpu Central Hospital in Yangpu District (tertiary hospital), Tongren Hospital in Changning District (tertiary hospital), Integrated Hospital of Traditional Chinese and Western Medicine in Baoshan District (tertiary hospital), Luwan Branch of Ruijin Hospital in Huangpu District (secondary hospital), Liqun Hospital (secondary hospital) and Taopu Community Health Centre (primary hospital) in Putuo District, and Sanxing Community Health Centre in Chongming District (primary hospital). Healthcare workers at the sentinel hospitals recorded the basic information, clinical characteristics, and medication status of the included cases sampled systematically from Jan 2015 to Dec 2020. The specimens were collected at the same time as the clinical diagnostic examinations were being performed after patients were identified as eligible for acute respiratory pathogen surveillance (one nasopharyngeal swab and other upper respiratory specimens from outpatients; one nasopharyngeal swab, or one sputum, and other lower respiratory specimens from inpatients), to carry out further examinations.

Pathogen examination

The collected specimens were stored at 4°C and then examined using a real-time reverse transcription polymerase chain reaction (RT–PCR) kit (ResipiFinder 2SMART, PathoFinder, Netherlands) for influenza A virus (IAV, including subtype H1N1 and H3N2), influenza B virus (IBV, including subtype Yamagata and Victoria), parainfluenza virus (PIV, including subtypes 1–4), respiratory adenovirus (AdV), respiratory syncytial virus (RSV, including subtypes A and B),

rhinovirus/enterovirus (EV/RV), coronavirus (CoV, including types HCoV-OC43, HCoV-NL63, HCoV-HKU1, and HCoV-229E), human metapneumovirus (HMPV), bocavirus (BOCV), *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP), *Legionella pneumophila* (LP), and *Bordetella pertussis* (BP), within 24 h. If not examined within 48 h, the specimens were stored at −70°C. The kit was unable to differentiate between HCoV-NL63 and HCoV-HKU1, thus we combined the results as HCoV-NL63/HKU1.

Image processing

The number of respiratory pathogens and common HCoVs by year, and by month for all years combined were plotted using Excel (Microsoft Office, Redmond, WA, USA). The number of cases was used as a weight to plot a chord diagram to show the interrelationship of co-infection between HCoVs and other pathogens using the chord diagram package of Python. A heatmap was plotted using HemI 1.0.3.7 (Heatmap Illustrator) [22] with the number of cases as the weight.

Statistical analysis

Age was described using median and interquartile range. The study patients included all age groups, but fewer were aged ≤18 years. Considering the age distribution of the patients, patients were classified as ≤60 years or >60 years. As common respiratory pathogens such as HCoVs and influenza are more prevalent in winter and spring in temperate and subtropical regions, seasonal grouping was considered with November-March as the winter-spring endemic season and April-October as the non-endemic season according to the climatic characteristics of Shanghai region.

The chi-square test and Fisher's exact test were used for the univariate analyses of factors associated with co-infection and with HCoV infection. The univariate associations were used to select variables for further multivariable logistic regression to determine factors associated with co-infection and with HCoV infection. The Hosmer-Lemeshow method was used to test the goodness-of-fit of the regression model. IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis. *P*-values <0.05 were considered statistically significant.

Results

Prevalence of HCoVs

A total of 4541 respiratory specimens collected from January 2015 through December 2020 were examined for respiratory pathogens, with a positive rate of 40.4% (1833/4541), including 37.8% (1716/4541) positive for respiratory viruses and 3.6% (163/4541) positive for common HCoVs.

The positive rate for HCoVs differed by year ($P < 0.001$) and was highest in 2017 (7.0%) and lowest in 2018 (1.7%) (Figure 1(a)). The incidence of HCoV infection was higher during the non-endemic season (April to October) ($P < 0.001$). The peaks in detection rates of HCoVs were all in the non-endemic seasons, in contrast to the peaks in the overall detection of respiratory pathogens (Figure 1(b)). The incidence of common respiratory virus infection by month is shown in Figure 2. Of the 163 specimens in which common HCoVs detected, HCoV-OC43 had the highest detection rate (41.7%, 68/163), followed by HCoV-NL63/HKU1 (39.3%, 64/163) and HCoV-229E (19.6%, 32/163).

The median age of HCoV-positive cases was 65.0 years (interquartile range [IQR]: 47.0–75.0 years), with patients with ILI having a median age of 56.0 years (IQR: 32.0–66.0 years) and patients with SARI having a median age of 70.0 years (IQR: 59.3–84.0 years). SARI cases were more frequent in patients aged >60 years, while ILI cases were more common in patients aged ≤60 years ($P < 0.001$). The factors associated with HCoV type are shown in Table 1. There was no significant difference in the incidence of specific HCoV types according to age, specimen type, sampling interval, or antibiotic use.

Co-infection of HCoVs with other pathogens

Among all the cases, 167 cases of co-infection were identified with at least two pathogens, including 92 ILI cases and 75 SARI cases. The most common co-infection pattern was IAV and MP (25.8%) and 30.5% (51/167) of the co-infections included HCoV infection. Among the 163 specimens from which HCoVs were identified, 51 (31.3%) had a co-infection detected. The prevalence of HCoV co-infection was higher with PIV, EV/RV, IAV and AdV infections. One specimen was found to be co-infected of HCoV-OC43 and HCoV-NL63/HKU1 with no other pathogens detected.

The logistic regression analysis adjusted for disease type (ILI and SARI), revealed that endemic season (odds ratio [OR]: 2.13, 95% confidence interval [CI]: 1.53–2.95), with lower respiratory tract specimens (OR: 4.39, 95% CI: 2.80–6.88), and having used antibiotics prior to specimen collection (OR: 1.48, 95% CI: 1.06–2.09) were associated with a significantly higher risk of co-infection. Among patients with HCoV infections, co-infection with other pathogens was more likely to be detected in lower respiratory tract specimens (OR: 7.22, 95% CI: 2.41–21.69).

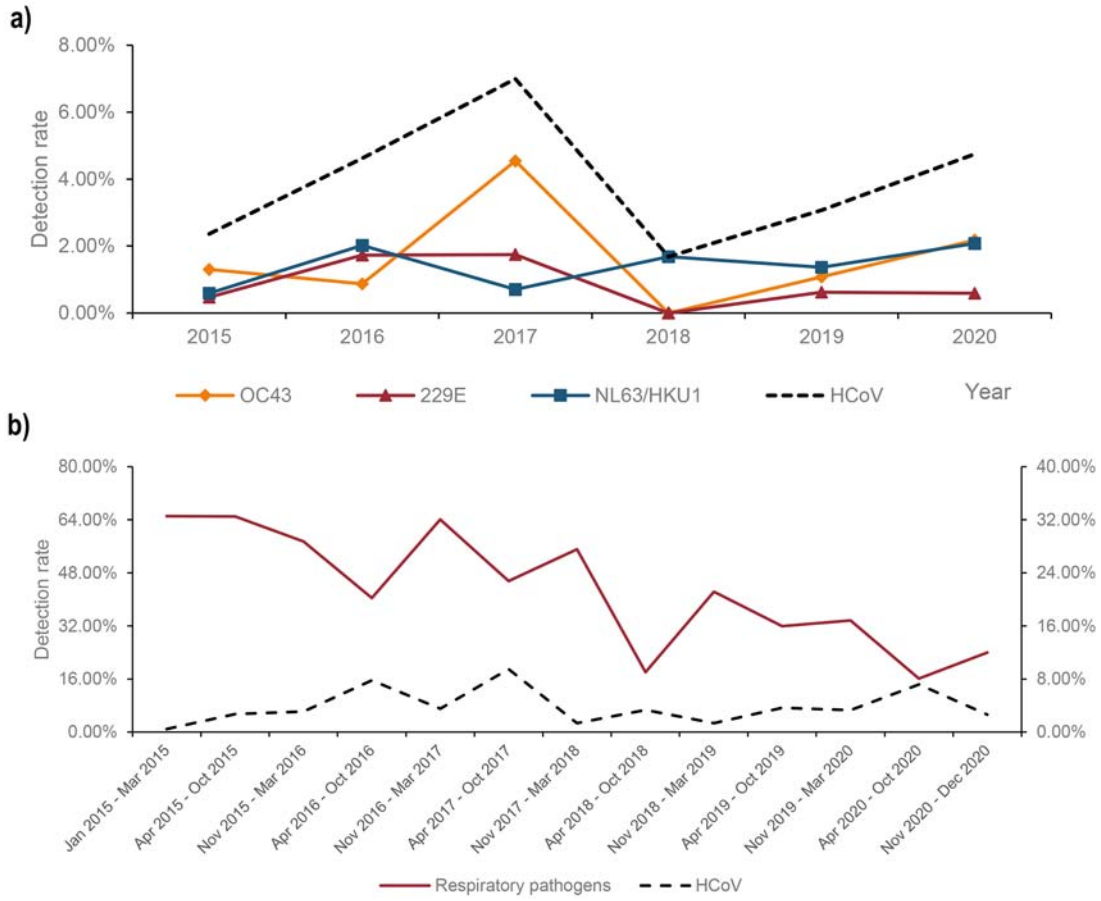

Figure 1. Detection of human coronavirus infections according to year and season, 2015–2020. (a) Positive rate of common human coronaviruses (HCoVs) by year. (b) Positive rate of different types of common HCoVs by endemic season. HCoV: total detection of common HCoVs; Respiratory pathogens: total detection of all tested respiratory pathogens.

The details of each type of HCoVs co-infecting with other respiratory pathogens are shown in Table 2. HCoV-OC43 was associated with co-infections with IBV, LP and MP while HCoV-NL63/HKU1 was associated with CP co-infection, and HCoV-229E was associated with HMPV coinfection. There were no HCoV-BP co-infections. The prevalence of PIV co-infection was higher with of HCoV-229E compared with all other types of coronaviruses ($P < 0.001$). Of the 51 HCoV co-infections, only five (9.8%) were associated with two or more other pathogens.

The patterns of co-infection are shown in Figure 3. Among the five viruses most commonly associated with HCoV co-infection, IAV-HCoV co-infection accounted for less than 25% of all IAV infections. Of the patients with co-infection with specific pathogens, the prevalence of HCoV co-infection was below 10% for IAV, IBV, MPV, and MP.

Total number of co-infections detected was 25 of 53 ILI cases (47%) and 26 of 54 SARI cases (48%). Common HCoV co-infections with MP and IBV were only seen in ILI cases, and common HCoV co-infections with MPV, LP and CP were only seen in SARI cases. The number of with IAV co-infections was higher in ILI cases ($n = 7$) than in SARI cases ($n = 1$), while co-infection with EV/RV was more common in SARI cases ($n = 7$) than in ILI cases ($n = 3$). The pattern of co-infection differed according to the HCoV type (Figure 4).

Discussion

In this study, the total positive rate of respiratory pathogens was 40.37% in 4541 samples collected during a 6-year retrospective, observational, and multicentre study in Shanghai. The prevalence of HCoVs was 3.59%, with HCoV-OC43 being the most commonly detected type. Several studies have shown the consistent findings [8,9,23]. The reason may be that HCoV-OC43 induces an immune response to suppress infection by HCoV-HKU1 or HCoV-229E [4].

In our study setting, we found that the increasing and decreasing trend of HCoV detection rates between non-endemic and endemic seasons varied inconsistently with the overall detection rates of all tested respiratory pathogens; however, there were yearly variations in the detection rates of the predominant HCoV species. The incidence of HCoV-OC43 infection was higher during the non-endemic season. These results differ from previous studies that have shown that HCoV is a winter virus that is prevalent

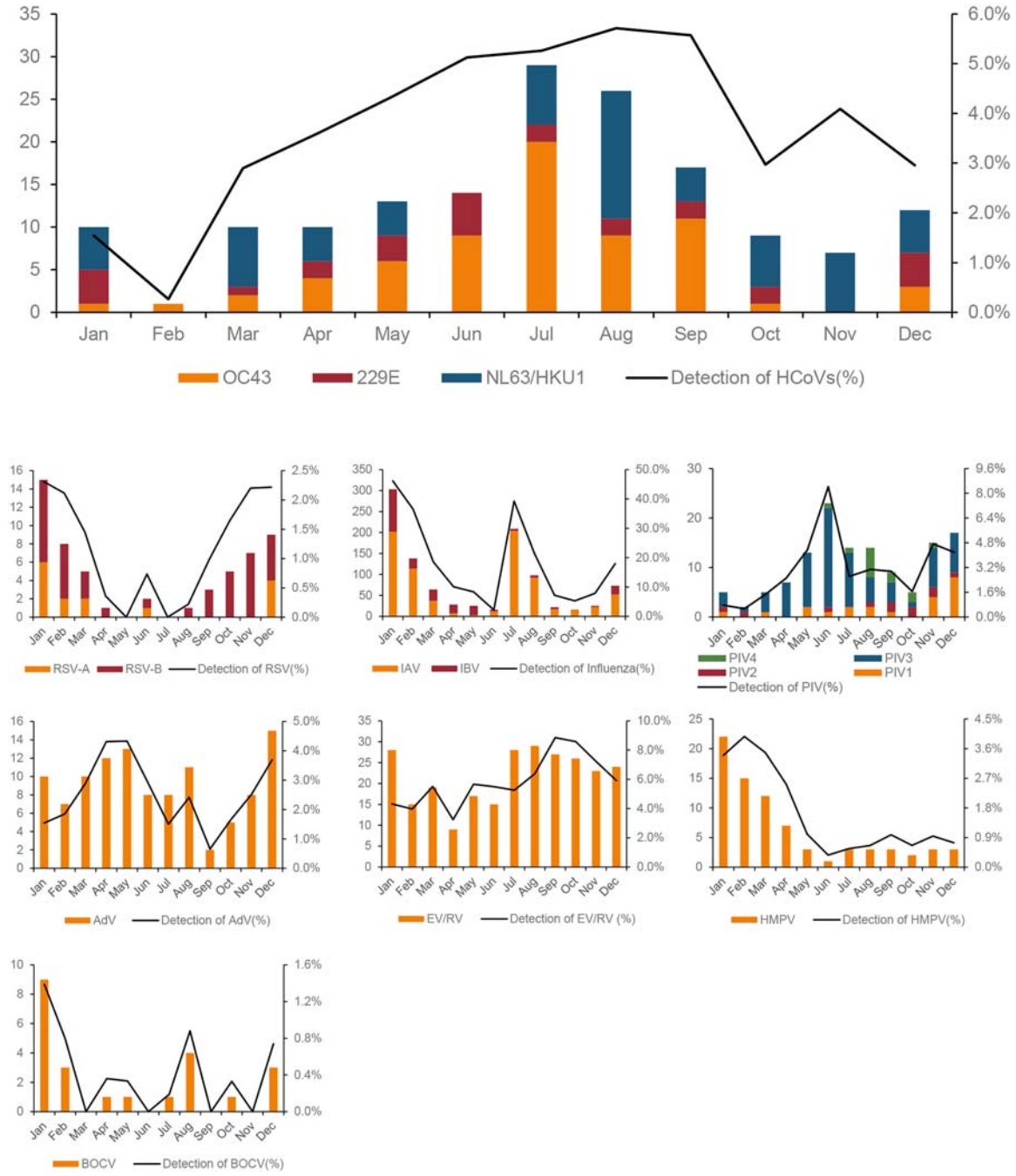

Figure 2. Detection of respiratory pathogens by month. AdV: respiratory adenovirus; BOCV: bocavirus; EV/RV: rhinovirus/enterovirus; HCoVs: human coronaviruses; HMPV: human metapneumovirus; IAV: influenza A virus; IBV: influenza B virus; PIV: parainfluenza virus; RSV: respiratory syncytial virus.

in temperate regions between December and April [24]. However, the viability of respiratory viruses is higher at a relative humidity (RH) <40% or >60%. The average RH in Shanghai is >60% throughout the year and higher (>70%) in the non-endemic season, which may explain the higher detection rate of HCoV in the non-endemic season. In future studies, it would be worthwhile to consider variations in viral isolation according to geospatial climate differences. Another consideration is that there could be competitive infection and subsequent suppression between pathogens, which led to a lower detection rate of HCoV in the endemic seasons in the present study and a higher one in non-endemic seasons due to less pathogen competition [25].

A high proportion of HCoV infections were associated with co-infections, with the majority of co-infections having one another respiratory pathogen. This is consistent with a previous study showing a high co-infection rate and similar co-infection patterns between pathogens [8]. Specific patterns could be observed in the interrelationship of co-infections. The main co-infecting pathogens of HCoVs in this study were PIV, EV/RV, IAV, and AdV, whereas RSV, EV/RV, and IAV were predominant in previous studies [8,26]. In particular, in the present study, PIV

Table 1. Characteristics of human coronaviruses detected among patients with acute respiratory infections, 2015–2020.

	OC43 ($N = 36$)		NL63/HKU1 ($N = 28$)		229E ($N = 16$)	
	ILI *n* (%)	SARI *n* (%)	ILI *n* (%)	SARI *n* (%)	ILI *n* (%)	SARI *n* (%)
Sex						
Male	20 (12.27)	18 (11.04)	6 (3.68)	22 (13.50)	4 (2.45)	12 (7.36)
Female	16 (9.82)	14 (8.59)	16 (9.82)	20 (12.27)	9 (5.52)	7 (4.29)
Age (years)						
≤60	24 (14.72)	9 (5.52)	12 (7.36)	11 (6.75)	8 (4.91)	5 (3.07)
>60	12 (7.36)	23 (14.11)	10 (6.13)	31 (19.02)	5 (3.07)	14 (8.59)
Seasonality[a]						
Endemic season	5 (3.07)	3 (1.84)	4 (2.45)	20 (12.27)	5 (3.07)	9 (5.52)
Non-endemic season	31 (19.02)	29 (17.79)	18 (11.04)	22 (13.50)	8 (4.91)	10 (6.13)
Specimen type[b]						
Nasopharyngeal swab	34 (20.86)	24 (14.72)	22 (13.50)	35 (21.47)	13 (7.98)	14 (8.59)
Lower respiratory specimen	0 (0.00)	8 (4.91)	0 (0.00)	7 (4.29)	0 (0.00)	5 (3.07)
Antibiotic use						
Used	4 (2.45)	18 (11.04)	7 (4.29)	14 (8.59)	3 (1.84)	9 (5.52)
Not used	32 (19.63)	14 (8.59)	15 (9.20)	28 (17.18)	10 (6.13)	10 (6.13)
Sampling interval (days)						
≤3	35 (21.47)	14 (8.59)	20 (12.27)	27 (16.56)	12 (7.36)	14 (8.59)
4–7	0	10 (6.13)	1 (0.61)	9 (5.52)	1 (0.61)	1 (0.61)
≥8	1 (0.61)	8 (4.91)	1 (0.61)	6 (3.68)	0 (0.00)	4 (2.45)

Note: ILI: influenza-like illness; SARI: severe acute respiratory infection.
[a]The endemic season represents winter and spring (from November to March) in which coronaviruses were reported as a high incidence.
[b]Two positive specimens for coronavirus were not counted in this table due to lack of specimen type information; both of them were OC43 type.

was the most common co-infecting pathogen, with the most common co-infecting subtype being hPIV-3. As the overall prevalence of PIV in this study was not higher than in other regions (range from 1.8% to 3.8% by year), this may be due to certain seasonal prevalence factors and the relatively low suppression between these two viruses. Although RSV and HCoV co-infections were uncommon in this study, as in previous studies, the combination of HCoV accounted for 38.5% of RSV co-infections, and RSV was the second most common co-infection with HCoVs after PIV. All cases of RSV infection with HCoV co-infection were subtype B. The pattern of co-infection differed according to HCoV type, with PIV having a predilection to co-infect with HCoV-229E.

We found that IAV-MP, CoV-PIV, EV/RV-MPV and IAV-BOCV were the main patterns of co-infections. Several studies have shown that typical co-infection patterns cannot be simply attributed to seasonality [27], in which there are more likely synergistic, competitive, and inhibitory effects between pathogens, resulting in specific infection patterns [25,28–30]. Although there was no significant difference in the prevalence of co-infection according to the disease type (ILI or SARI), we found that some infection combinations only occurred with ILI or SARI, suggesting that there is a complex relationship between infection and pathogenesis. The potential for disease aggravation may differ according to the co-infection pattern in HCoV infection [29].

Several researchers have hypothesized that SARS-CoV-2 will become a seasonal respiratory pathogen [14]. Therefore, it is of great practical significance to use the epidemiology and co-infection characteristics of common HCoVs as a reference. Some studies have reported the co-infection of SARS-CoV-2 with other respiratory pathogens in patients with

Table 2. Co-infecting pathogens detected with human coronaviruses.

	OC43	NL63/HKU1	229E	Total	Total number of other pathogens[a]	Co-infection with HCoVs (%)[b]
Viruses						
PIV	1	4	11	16	12	57 (16/28)
EV/RV	7	1	2	10	26	29 (10/35)
IAV	4	4	0	8	80	9 (8/85)
AdV	4	3	1	8	18	32 (8/25)
RSV	0	2	3	5	9	38 (5/13)
BOCV	0	0	2	2	15	12 (2/17)
IBV	1	0	0	1	13	7 (1/14)
HMPV	0	0	1	1	17	6 (1/18)
Other pathogens						
LP	1	0	0	1	1	50 (1/2)
BP	0	0	0	0	3	0 (0/3)
MP	2	0	0	2	47	4 (2/47)
CP	0	1	0	1	7	14 (1/7)

Note: AdV: respiratory adenovirus; BOCV: bocavirus; BP: *Bordetella pertussis*; CP: *Chlamydia pneumoniae*; EV/RV: rhinovirus/enterovirus; HCoVs: human coronaviruses; HMPV: human metapneumovirus; IAV: influenza A virus; IBV: influenza B virus; LP: *Legionella pneumophila*; MP: *Mycoplasma pneumoniae*; PIV: parainfluenza virus; RSV: respiratory syncytial virus.
[a]Total of other pathogens: Co-infection pathogens other than HCoVs.
[b]Co-infection with HCoVs%: Patients co-infected with any HCoVs/Total cases of co-infection with this pathogen × 100%.

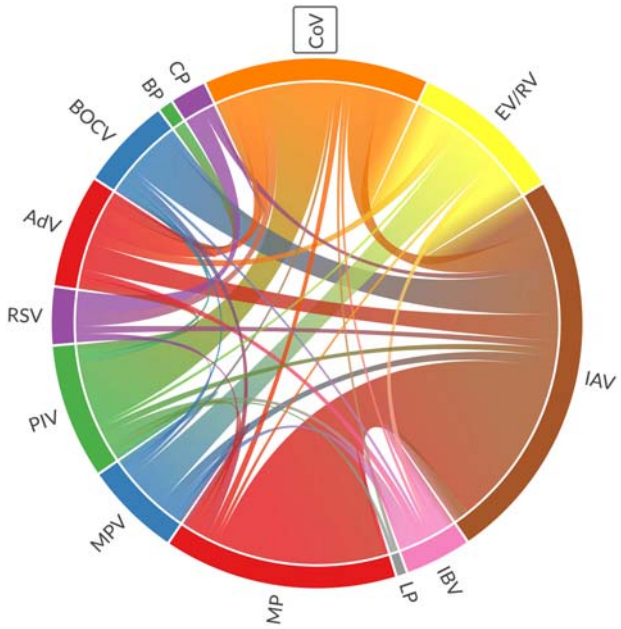

Figure 3. Interrelationship between human coronavirus infection and other pathogens. AdV: respiratory adenovirus; BOCV: bocavirus; BP: *Bordetella pertussis*; CP: *Chlamydia pneumoniae*; EV/RV: rhinovirus/enterovirus; HCoVs: human coronaviruses; IAV: influenza A virus; IBV: influenza B virus; LP: *Legionella pneumophila*; MP: *Mycoplasma pneumoniae*; MPV: human metapneumovirus; PIV: parainfluenza virus; RSV: respiratory syncytial virus. Due to emphasizing the interrelationship about HCoVs, we have rotated the image and text label so that the CoV is facing upwards now.

COVID-19, including MP, RSV, IAV, PIV, EV/RV and HCoVs [31–33]. Pattern of co-infections reported with SARS-CoV-2 is similar to the pattern of co-infections with HCoVs found in this study, suggesting that the types of other respiratory pathogens that co-infect with coronaviruses is similar. In addition, there have been reports of co-infection with common HCoVs and SARS-CoV-2 [34,35]. Although present study found that HCoV-HCoV co-infection was rare, attention should be paid to the potential for SARS-CoV-2 and HCoV co-infection.

From a public health perspective, the characteristics of co-infection with different viruses are important for scientific surveillance, accurate diagnosis and individualized treatment. Over the past few decades, several studies have reported on viral co-infections with specific patterns and their impact on disease outcome. For example, influenza virus co-infections with PIV or RV may aggravate the disease [36], while co-infection of influenza virus with RSV is associated with less severe disease [37]. Studies have found that viral co-infection is less common in severe SARI cases than in mild ILI cases [38]. This suggests that in the era of exploring precision medicine, more attention should be paid to the influence of viral co-infection interrelationships on diseases and the potential to use co-infections as interventions. In addition, we are also interested in

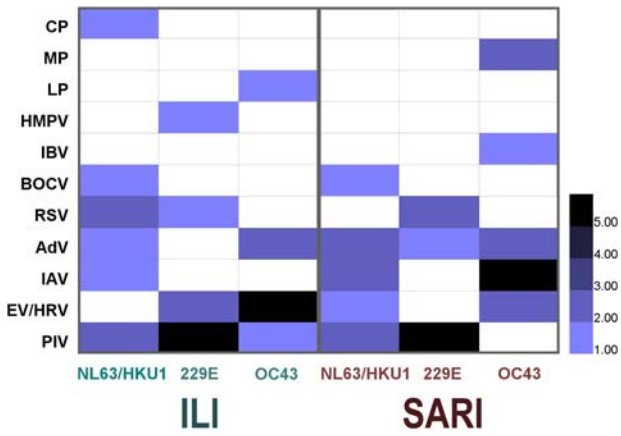

Figure 4. Frequency heatmap of human coronavirus co-infection by case type. AdV: respiratory adenovirus; BOCV: bocavirus; BP: *Bordetella pertussis*; CP: *Chlamydia pneumoniae*; EV/RV: rhinovirus/enterovirus; HCoV: human coronavirus; IAV: influenza A virus; IBV: influenza B virus; HMPV: human metapneumovirus; LP: *Legionella pneumophila*; MP: *Mycoplasma pneumoniae*; PIV: parainfluenza virus; RSV: respiratory syncytial virus.

whether different co-infection patterns could promote the evolution of viruses. The effect of co-infection on viral pathogenicity and transmission needs to be further explored as it is of public health importance.

Limitation

This study has some limitations. First, the samples in this study were obtained from sentinel hospitals, which might have led to selection bias. However, this study was based on the acute respiratory pathogen surveillance data collected over six consecutive years, including the year 2020, with sample collection at multiple hospitals to better reflect the respiratory pathogen spectrum in Shanghai and possible changes during the COVID-19 pandemic. Second, we only tested for a limited variety of pathogens; however, they are principal respiratory pathogens in Shanghai. Third, due to the limitation of detection reagents, we could not distinguish between HCoV-NL63 and HCoV-HKU1 infection, which may have affected our results. In future studies, we will try to distinguish between these two types of HCoV to obtain more accurate results and gain further insights.

Conclusion

Multiple studies suggest that the COVID-19 pandemic may enter the seasonal circulation, which raises a new public health concern. Our study found that co-infection with more than one common HCoV was rare. Furthermore, common HCoVs tend to be co-infected with PIV, EV/RV, IAV and AdV, indicating the possibility of co-infection of these pathogens with SARS-CoV-2. A better understanding of HCoV co-infections would facilitate more precise treatment and preparedness for future seasonal epidemics of coronavirus infections, including COVID-19.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The datasets analysed during the current study are not publicly available due to the reason that these data came from a long-term surveillance but are available from the corresponding author on reasonable request.

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