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International Journal of Infectious Diseases 105 (2021) 617–620

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

SARS-CoV-2 respiratory co-infections: Incidence of viral and bacterial co-pathogens

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ARTICLE INFO

Article history:
Received 28 November 2020
Received in revised form 25 January 2021
Accepted 23 February 2021

Keywords:
COVID-19
Co-infections
Pandemic
SARS-CoV-2
Respiratory co-infections

ABSTRACT

The global coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in an unprecedented challenge to our healthcare system. Secondary and concurrent bacterial and viral co-infections are well documented for other viral respiratory pathogens; however knowledge regarding co-infections in COVID-19 remains limited. In the present study, concurrent testing of 50 419 individual samples for the presence of SARS-CoV-2 and other bacterial and viral respiratory pathogens was performed between March and August 2020. Overall, a lower rate of viral co-infection was observed in the SARS-CoV-2-positive population when compared to the population testing negative for the virus. Significant levels of *Staphylococcus aureus* and Epstein–Barr virus co-infections were detected in the SARS-CoV-2-positive population. This is one of the largest surveys looking into the co-infection patterns of SARS-CoV-2 infection in the United States. Data from this study will enhance our understanding of the current pandemic and will assist clinicians in making better patient care decisions, especially with respect to antimicrobial therapy.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus, has resulted in the largest mobilization of public health resources and policy in recent memory. At the time of writing this report, the virus has infected 92.3 million people resulting in over 1.98 million deaths globally. The United States, representing 4.25% of the world population, has been disproportionately affected by the pandemic. The total SARS-CoV-2-positive cases in the United States stands at 23.1 million with 384 000 fatalities, which represents 20% of the world tally (https://www.cdc.gov/coronavirus/2019-ncov).

The co-evolution of viral and bacterial respiratory pathogens has created an environment in which a viral infection allows concurrent or secondary bacterial co-infections, leading to increased morbidity and mortality associated with respiratory viral infections and greatly enhancing the disease burden on society (McCullers, 2014; Gupta et al., 2008). Although viral–bacterial co-infections are well researched, studies detailing their impact during the COVID-19 pandemic remain sparse. With mass vaccination efforts against SARS-CoV-2 still in their initial stages, underlying co-infections and their treatment could have a significant impact on disease morbidity and associated patient care (Cox et al., 2020; Kim et al., 2020). The study was performed with a number of goals in mind and the data prsented in the paper is one aspect of it and not the sole purpose.

Materials and methods

Nasal, oropharyngeal, and sputum swabs were received and tested in the HealthTrackRX laboratory located in Denton, Texas, USA. Nucleic acid extraction and real-time PCR on the Open Array platform (Thermo Fisher Scientific, San Francisco, CA, USA) were performed as described previously (Singh et al., 2019). The following microbial pathogens were tested for: (1) bacteria, including *Bordetella pertussis*, *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Legionella pneumophila*, and *Haemophilus influenzae*; (2) viruses, including adenovirus, coronavirus (NL63, HKU1, 229E, OC43), human metapneumovirus, rhinovirus, enterovirus, influenza virus (A, B), parainfluenza virus (1, 2, 3, 4), respiratory syncytial virus (A, B), bocavirus, Epstein–Barr virus (EBV), human herpesvirus 6, and varicella zoster virus. Real-time PCR detection of SARS-CoV-2 virus was performed using the TaqPath COVID-19 Combo Kit, which targets the N, S, and Orf1ab

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https://doi.org/10.1016/j.ijid.2021.02.087

regions of the viral genome (Thermo Fisher Scientific, San Francisco, CA, USA), under an emergency use authorization granted to HealthTrackRX by the US Food and Drug Administration (https://www.fda.gov/media/137374/download). The limit of detection for the SARS-CoV-2 detection assay was determined to be 10^1 RNA copies/μl.

Differences in the co-infection status of the SARS-CoV-2-positive and negative population were calculated as proportions by means of Chi-square analysis ($P < 0.05$). All statistical analyses were performed using R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

Between March 16, 2020 and August 1, 2020, a total of 50 419 respiratory samples (nasopharyngeal, oropharyngeal, and sputum swabs) were collected and concurrently tested for the presence of SARS-CoV-2 and other respiratory pathogens.

SARS-CoV-2-positive patients (n = 4259) represented 8.44% (95% confidence interval (CI) 0.082–0.087; P = 0.084) of the total samples analyzed in this study. The sex distribution of the SARS-CoV-2-positive population (55.5% female and 44.4% male) was similar to that of the total population (55.3% female and 44.6% male). The average age of the SARS-CoV-2-positive patients was 45.21 ± 20.43 years. The distribution of the SARS-CoV-2-positive population by age range revealed higher incidences of infections in the younger age groups (20–49 years), which comprised approximately 50% of the total positive cases (Figure 1). Early reporting demonstrated higher rates of SARS-CoV-2 infection in the older population (Stokes et al., 2020). However, the latest trends of the COVID-19 pandemic in the United States (Boehmer et al., 2020) are in agreement with our data that show higher infection rates in the younger population.

Both bacterial and viral respiratory co-infections can be secondary or concurrent. A variety of synergistic biological interactions between viruses and bacteria have been reported, leading to an increased risk of bacterial infections (where a primary viral infection is present) and vice versa (Lee et al., 2016). In the case of SARS-CoV-2, this is substantiated by the co-infection data from the present study, by the data of Massey et al. (2020), and by our previous work on co-infections present in 'influenza-like illnesses' (Singh et al., 2019).

It is now widely accepted that in all of the influenza pandemics over the last century, the leading causes of mortality were secondary or concurrent bacterial co-infections including *S. pneumoniae*, *S. aureus*, and *H. influenzae* (Gupta et al., 2008). An initial study originating from Wuhan, China reported bacterial co-infections in 50% of the patients who did not survive COVID-19 (Zhou et al., 2020). A review of 13 studies reporting SARS-CoV-2 co-infection rates disclosed co-infection and secondary infection rates ranging from 0.6% to 45.0% (Lai et al., 2020).

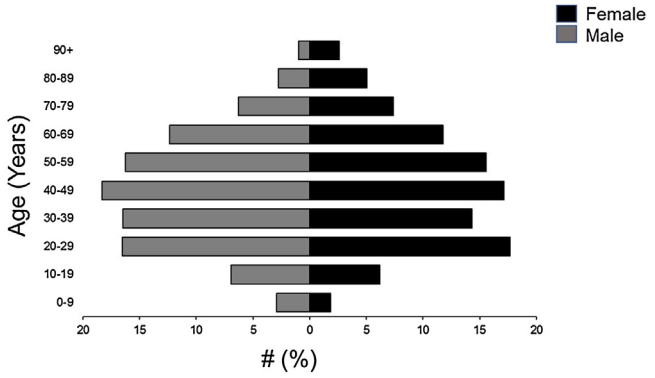

Figure 1. Distribution of SARS-CoV-2-positive patients according to age and sex.

In the present study, significant bacterial and viral co-infections were observed in both the SARS-CoV-2-positive and SARS-CoV-2-negative populations. In general, the SARS-CoV-2-positive patients had lower incidences of co-infections when compared to the SARS-CoV-2-negative patients (Figure 2). This trend is similar to that reported previously in Stanford University data (Kim et al., 2020), and differs from the data of Massey et al. (2020). However, the bacterial co-infections were comparable (33.17% SARS-CoV-2-positive, 35.45% SARS-CoV-2-negative). The viral co-infection rate was significantly lower in the SARS-CoV-2-positive patients (3.42%) in comparison to the SARS-CoV-2-negative patients (8.66%).

A detailed analysis of the co-infecting pathogens (Table 1) showed *S. aureus*, *H. influenzae*, *S. pneumoniae*, and *K. pneumoniae* to be the most prevalent bacterial co-infections in both SARS-CoV-2-positive and negative patients. The rates of bacterial co-infections were lower in SARS-CoV-2-positive patients as compared to SARS-CoV-2-negative patients, with the exception of *S. aureus*, which was found to be co-infecting SARS-CoV-2-positive patients at a significantly higher rate ($P > 0.05$) as compared to the SARS-CoV-2-negative patients (χ^2 (1, N = 561) = 8.73; P = 0.0039). *S. aureus* is a common respiratory pathogen responsible for causing pneumonia and has been well documented as a co-infecting pathogen during influenza infections, often with fatal outcomes, especially in cases of methicillin-resistant *S. aureus* (MRSA) co-infections (Mulcahy and McLoughlin, 2016).

A similar trend was observed with viral co-infections, wherein the SARS-CoV-2-positive patients had lower incidences of co-infections for all of the viral targets when compared to the SARS-CoV-2-negative patients, congruent with the study conducted by Kim et al. (2020). All of the viral co-infections were reported at less than 1% incidence rate apart from EBV. A possible explanation for the lower viral co-infection rate could be the time-frame in which the study was conducted. Data collection was initiated towards the end of the annual 'flu season' in the United States, well into the summer months when respiratory viral infections are generally not observed in the population. Similar observations have been reported with respect to higher EBV co-infections in COVID-19 patients in Wuhan, China (Chen et al., 2020). EBV infection is pervasive in the human population, and the virus can persist in the body in a latent form following primary infection. The higher rate of EBV co-infection in the SARS-CoV-2-positive samples as compared to other respiratory viruses, could be reflective of the high EBV instances in the general population or a result of lytic reactivation of the virus, as has been observed under conditions of immunosuppression. These conditions may be mimicked in COVID-19 patients due to an over-activation of T-cells leading to immune injury (Xu et al., 2020). Additionally, SARS-CoV-2 has been implicated in the reactivation of several herpesviruses, including varicella zoster virus (human herpesvirus 3; HHV3), roseolovirus (HHV6, HHV7), and Epstein–Barr virus (HHV4) (Massey et al., 2020; Ciccarese et al., 2020).

The distribution of all co-infections (bacterial and viral) in the SARS-CoV-2-positive population suggests a positive correlation with age (Figure 3). Even though the overall median age of the SARS-CoV-2-positive patients was 45 years, the co-infections were significantly higher in the older age group (60+ years) when compared to any other age group. Detailed analyses of the COVID-19 pandemic assert that the most severe outcomes of the disease are observed in older patients (Wu et al., 2020), and the results of the present study may provide a possible explanation for this observation.

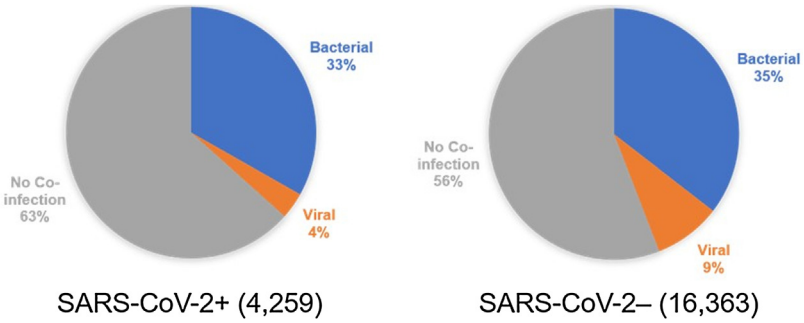

Figure 2. Bacterial and viral co-infections in the SARS-CoV-2-positive (+) and negative (−) populations. Instances of viral co-infections were found to be significantly lower in the SARS-CoV-2-positive population than in the SARS-CoV-2-negative population ($P < 0.05$).

Table 1
Observed infection rates (%) of different respiratory microbial pathogens.

Organism	Total	SARS-CoV-2	
		Positive	Negative
Haemophilus influenzae	11.83	9.27*	12.07
Staphylococcus aureus	11.77	13.17*	11.64
Bordetella pertussis	0.06	0.02*	0.06
Chlamydophila pneumoniae	0.08	0.02*	0.08
Klebsiella pneumoniae	2.14	1.94*	2.16
Mycoplasma pneumoniae	0.25	0.07*	0.27
Streptococcus pneumoniae	9.09	8.66*	9.13
Adenovirus	0.40	0.30*	0.41
Coronavirus[a]	0.36	0.07*	0.38
herpesevirus 5	0.09	0.07*	0.09
EBV	5.39	2.13*	5.69
RSV	0.10	0.11	0.10
Rhinovirus	1.3	0.49*	1.45
HSV	0.08	0.11*	0.08
HMPV	0.27	0.04*	0.29
PIV	0.07	0.04*	0.08
Influenza virus	0.04	0.02*	0.04

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HMPV, human metapneumovirus; HSV, herpes simplex virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a] Coronavirus represents members of the *Coronaviridae* excluding SARS-CoV-2.
* Represents statistically significant difference ($P < 0.05$).

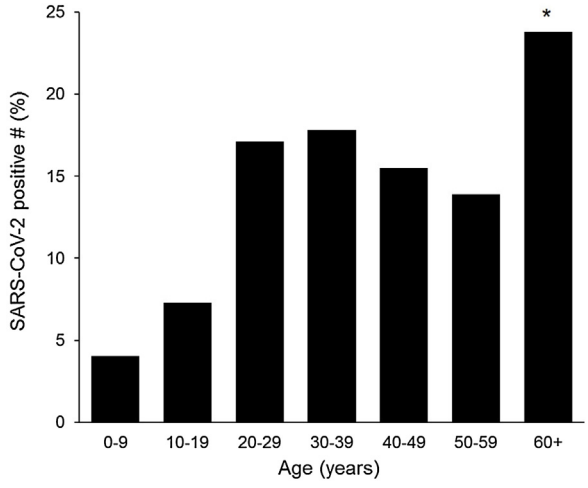

Figure 3. Distribution of bacterial and viral co-infections in SARS-CoV-2-positive patients according to age. Patients aged ≥60 years displayed significantly higher co-infection rates when compared to any other age group. The asterisk (*) represents a statistically significant difference ($P < 0.05$).

The present study, to our knowledge, is the largest such study related to the co-infections of SARS-CoV-2. The observations from this study provide significant insight into the potential risks and clinical outcomes for COVID-19 patients, especially those in the susceptible older age group. While SARS-CoV-2 has a higher mortality rate than most other respiratory viruses even without a bacterial co-infection, 33% of SARS-CoV-2-positive patients in this study had a concurrent bacterial infection and could have benefitted from an antibiotic. Novel existing therapies such as remdesivir and dexamethasone were used for the treatment of hospitalized SARS-CoV-2 patients, but as for all other respiratory viruses, bacterial co-infections cannot be ignored. It is widely known that a variety of bacteria can both colonize and, under appropriate conditions, cause infections of the entire respiratory tract. Examples include *S. pneumoniae*, *S. aureus*, and *H. influenzae*, amongst others. At the current time, immune system gene expression analysis is not in widespread use. Such an analysis, once a consensus validation of the clinical utility has been completed, could be instrumental in determining whether a given detected microbial population represents an infection or a colonization. Meanwhile, clinicians must rely upon a given patient's signs and symptoms in determining whether an infection exists. This is a potential microbiological-test interpretative limitation for both classic culture-based and current molecular diagnostic tests.

The main limitation of this study was that the patient records of hospitalization, recovery, and treatment were not available, therefore it was not possible to make further assessments of the impact of co-infections during SARS-CoV-2 infection. As a future endeavor, data will be collected during the upcoming respiratory influenza season to measure the real impact of viral co-infections, and antibiotic resistance associated with bacterial co-infections will also be studied.

Conflict of interest

The authors declare no conflict of interests.

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