Methods and materials

Patients

In this retrospective study, we recruited COVID-19 patients from the First Affiliated Hospital of Guangzhou Medical University and Guangzhou Eighth People's Hospital between January 1, 2020 and March 1, 2020. COVID-19 was diagnosed by the diagnostic criteria of COVID-19 according to the guidelines of diagnosis and treatment of COVID-19.[16] The study was granted ethical approval from the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China (Ethics number: Number 36, 2020).

Data and specimen collection

The data of clinical features, imaging and laboratory findings for all patients were recorded on standardized case reports from electronic medical records. Sputum specimens were collected in a sterile specimen container. Specimens were stored between 2°C and 8°C until ready for shipment to the standardized laboratory, which was accredited by Guangdong Centers for Disease Control and Prevention (CDC).

Pathogen detection

RNA was extracted by commercial extraction kits according to the instructions, and RNA concentration was determined by a Qubit. The absorbance A260/A280 ratio was qualified between 1.9 and 2.0. Samples were tested using TaqMan real-time PCR assays (Beijing Applied Biological Technologies Co., Ltd.) for the following 15 respiratory pathogens: influenza A virus (FluA), influenza B virus (FluB), RSV, adenovirus (ADV), coronavirus NL63/229E/OC43/HKU1, human rhinovirus (HRV),

human metapneumovirus (HMPV), human bocavirus (HBoV), parainfluenza 1/2/3/4 (HPIV) and enterovirus (EV). PCR was performed with an amplification protocol consisting of one cycle at 45°C for 20 minutes, one cycle at 95°C for 5 minutes, and 45 cycles at 95°C for 15 seconds and 60°C for 30 seconds. Furthermore, the species of pathogenic bacteria and fungi in the sputum specimens were identified by culture.

Data analysis

All data were analyzed with SPSS statistical software (version 23.0; SPSS Inc., Chicago, IL), and a p-value <0.05 was used to indicate statistical significance. Categorical data are presented as numbers or numbers and percentages, and continuous measurements are presented as the mean (SD) if they are normally distributed or median (IQR) if they are not. Comparison of continuous data between patients was performed using Student's t test or Mann-Whitney test; Fisher’s exact test was used to compare categorical variables. Univariable analysis and multivariable analysis for risk factors for coinfection were used for logistic regression analyses.