Haemophilus Influenza Coinfection is Common in COVID-19 Patients

junping yan (768444919@qq.com)
Guangdong Second Provincial General Hospital
liangshan Hu
Guangdong Second Provincial General Hospital
Seyin Zou
Guangdong Second Provincial General Hospital
guochen liu
Sun Yat-sen University Cancer Center
donglin cao
Guangdong Second Provincial General Hospital

Research

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Abstract

Background: The combined infection rate and bacterial spectrum has not been reported in COVID-19 patients.

Methods: Constant temperature amplification reaction and real-time fluorescence analysis was performed for common bacterial respiratory pathogens on sputum samples (spontaneous or induced) from a prospective cohort study of infections. Forty-nine suspected COVID-19 cases from Guangdong Second Provincial General Hospital were included in the study, 49.0% were male.

Results: Probable bacterial infection was detected in 33 participants. Haemophilus influenzae was the most common bacterial pathogens detected.

Conclusions: Multiple coinfections were commonly detected. Sputum multiplex PCR could become a useful diagnostic tool for bacterial respiratory infections in COVID-19 infected inpatients.

1. Background

Lower respiratory tract infection is one of the most common clinical disease, most of which are bacterial infections[1]. According to statistics released by the World Health Organization in 2013, lower respiratory tract infection and chronic obstructive pulmonary disease rank third and fourth in the world's top ten causes of death respectively. In late 2019 and early 2020, a novel coronavirus was discovered to be the cause of a large and rapidly spreading outbreak of respiratory disease, including potentially fatal pneumonia, in Wuhan, China. Due to its similarity to SARS-CoV, the virus was given the official name SARS-CoV-2. The disease caused by the virus was officially named COVID-19 by WHO. SARS-CoV-2 belongs to the β-coronavirus cluster and is recognized as the seventh discrete coronavirus species capable of causing human disease. Current research shows that it has more than 85% homology with bat SARS-like coronavirus[2–5].

Pneumonia of other viral or bacterial origin especially Streptococcus pneumonia, Haemophilus influenzae, methicillin-resistant Staphylococcus aureus and Respiratory syncytial virus must be included in the diagnosis and antidiastole of SARS-CoV-2. Other febrile viral diseases like seasonal and avian influenza should also be included in the differential diagnosis. To avoid blind or inappropriate use of antimicrobials, rapid antigen detection and multiple PCR nucleic acid detection should be conducted.

Modern PCR diagnostic techniques are comparatively more sensitive than traditional methods for the detection of bacteria, therefore, some patients with ‘viral-only’ pathogens may also have microbiologically unrecognised bacterial infection.

This study collected suspected cases of COVID-19 in Guangdong Second Provincial General Hospital from January 25, 2020 to March 1, 2020, and performed SARS-CoV-2 nucleic acid detection and qualitative detection of 13 clinically common lower respiratory tract pathogens in sputum extracted

nucleic acid {Streptococcus pneumoniae (SPN), Staphylococcus aureus (SAU), Methicillin-resistant Staphylococcus (MRSA), Klebsiella pneumoniae (KPN), Pseudomonas aeruginosa (PAE), Acinetobacter baumannii (ABA), Stenotrophomonas maltophilia (SMA), Haemophilus influenzae (HIN), Escherichia coli (ECO), Legionella pneumophila (LPN), Mycoplasma pneumoniae (MPN), Chlamydia pneumoniae (CPN), Mycobacterium tuberculosis complex (MTB) }.

2. Methods

2.1. Experimental principle

Use fluorescent dye incorporation method to perform a constant temperature (65℃) reaction on a constant temperature amplification microfluidic chip nucleic acid analyzer and real-time fluorescence analysis, under the action of a polymerase with a strand displacement function, amplification-positive samples will generate a "S" -shaped amplification curve, which completes the target gene amplification and detection in one step. This is a loop-mediated isothermal amplification method, and based on the fact that DNA is in a dynamic equilibrium state at about 65℃. When any primer is extended to the complementary part of double-stranded DNA, the other strand will dissociate into a single strand. Four specific primers (including two internal primers and two external primers) are designed for the six regions of the target sequence, and DNA polymerase with the function of chain replacement is used to continuously replicate and amplify DNA at constant temperature. Generally, in order to improve the reaction efficiency, two loop primers are also added to the reaction system, which are respectively combined with the stem-loop structure, start the chain displacement synthesis, and cyclically replicate.

Each dish chip is provided with 24 reaction cells, and a set of primers can be embedded and fixed in a specific reaction cell for the amplification and detection of a nucleic acid target sequence. Independent constant temperature amplification reactions and real-time fluorescence detection are performed in each reaction cell of the chip at the same time. When the amplification curve is “S” shape, the detection index corresponding to the reaction cell is positive.

DNA was extracted from sputum sample according to the manufacturer’s instructions.

2.2. Inclusion criteria

According to the seventh edition of COVID-19 diagnosis and treatment plan in China. Inclusion criteria for suspected cases includes epidemiological history and clinical manifestations:

Epidemiological history: 1. travel or residence history in Wuhan or surrounding areas, or other communities with case reports within 14 days, 2. contact history with SARS-CoV-2 infected persons (positive nucleic acid test) within 14 days, 3. contact history with patients with fever or respiratory symptoms that from Wuhan and surrounding areas within 14 days.

Clinical manifestation:1. Fever or respiratory symptoms, 2. the imaging features of COVID-19, 3. the total number of white blood cells is normal or decreased in the early stage of disease, and the lymphocyte

count is normal or decreased.

Patients who had one or more epidemiological history with two of the three clinical manifestations, or had 3 of the clinical manifestations were considered as suspected cases.

COVID-19 diagnosis was confirmed based on positive viral nucleic acid from throat swab samples.

2.3. Statistical analysis

SPSS software (SPSS Inc., Chicago, IL, version 19) was used for data analysis. The Wald chisquare (χ^2) test was used to compare the difference of categorical parameters. All *P* values were two-sided, and *P* values of less than 0.05 were considered statistically significant.

3. Results

Forty-nine suspected COVID-19 cases were included in the study.

Among them, 23 patients were finally confirmed SARS-CoV-2 infection, including 15 males and 8 females, patients were 12 to 74 years old, and 87.0% (20 cases) were older than 30 years. One of the 23 patients was 74 years old and had a history of tuberculosis and chronic obstructive pulmonary disease. Now he is suffering from diabetes, and being treated with subcutaneous insulin injections, and the blood glucose control is stable. Another three patients have background disease. The rest of the patients were generally in good health, denied history of infectious diseases, hypertension, coronary heart disease and chronic diseases, denied history of surgery, trauma, blood transfusion and allergy.

Twenty-six patients were SARS-CoV-2 negative, including 9 males and 17 females, patients were 18 to 67 years old. Nine of the 26 patients have background disease. The rest of the patients were generally in good health.

It seems that men were more common in COVID-19 group, while no statistical difference was found in age and past medical history between the two groups. (Table 1)

Table 1
Demographic and Clinicopathologic Characteristics

Variables	Gruop		*P*
	COVID- n = 26 (%)	COVID+ n = 23 (%)	
Gender			
Male	9 (34.6%)	15 (65.2%)	
Female	17 (65.4%)	8 (34.8%)	0.032
Age (year)			
Median	39	48	
Range	18 ~ 67	12 ~ 74	0.158
Background disease			
Negative	17 (65.4%)	19 (82.6%)	
Positive	9 (34.6%)	4 (17.4%)	0.173
Pathogens co-infection			
Negative	9 (34.6%)	7 (30.4%)	
Positive	17(65.4%)	16 (69.6%)	0.755

Nucleic acid detection of the above mentioned 13 clinically common lower respiratory tract pathogens by constant temperature amplification microfluidic chip nucleic acid analyzer and real-time fluorescence analysis were applied to the 49 suspected COVID-19 cases. Sixteen patients were negative, another 33 were positive for one or more of the following pathogens: SAU, MRSA, KPN, HIN, ABA, SPN, SMA, MPN. Among them, HIN was detected in 29 cases, including 14 males and 15 females; the age ranged from 12 to 74 years. Both in the two groups and the whole enrolled patients, HIN was the most common co-infection(P□0.001). (Fig. 1)

4. Discussion

Although the initial cases were traced to zoonotic transmission, human-to-human transmission was soon documented, both in healthcare settings and in familial clusters[6]. In fact, following the initial leap across the species barrier, human-to-human transmission quickly became responsible for widespread and rapid dissemination of the virus across populations with no preexisting immunity; the disease spread from a single focal point across the entire country of China in just 30 days[5]. Respiratory droplets and close contact transmission are the main routes of transmission. The crowd is generally susceptible. Following an incubation ranging from 1–14 days, COVID-19 manifests as respiratory illness ranging from mild to

severe, with symptoms that include fever, cough, dyspnea, fatigue and muscle pain. A small number of patients also developed expectoration, headaches, hemoptysis, and diarrhea. Judging from the current cases, most patients have a good prognosis, and a few patients are critically ill. Viral infection commonly present with fever and cough, which frequently lead to lower respiratory tract disease with poor clinical outcomes associated with older age and underlying health conditions. Confirmation of infection requires nucleic acid testing of respiratory tract samples (eg, throat swabs), but clinical diagnosis may be made based on symptoms, exposures and chest imaging. Supportive care for patients is typically the standard protocol because no specific effective antiviral therapies have been identified.

SARS-CoV-2 nucleic acids can be detected in nasopharyngeal swabs, sputum and other lower respiratory tract secretions, blood and feces. In order to increase the positive rate of nucleic acid detection, it is recommended to take sputum and lower respiratory tract secretions in tracheal intubation patients, and send them for examination as soon as possible. The samples collected in this study were sputum. The results of this study showed that many patients with SARS-CoV-2 infection were infected with other lower respiratory tract pathogens, such as SAU, MRSA, KPN, ABA and SPN, especially HIN.

HIN is gram-negative bacillus or cocci, which is a common pathogen in the genus Haemophilus. It lives in the upper respiratory tract of normal people and belongs to conditional pathogens. When the body's immunity decreases it can cause lung infection. HIN cultivate requires special nutrients for growth, resulting in a low detection rate of the bacterium. In the qualitative analysis of sputum extracted nucleic acid, HIN has a high detection rate[7]. HIN respiratory colonization is the main cause of respiratory infections and tissue damage. The acute onset of COPD patients always show a sharp increase in isolated HIN colonies. In some areas, there is a strong relationship between lower respiratory tract infections and HIN colonization. In recent years, with the widespread use of antibacterial drugs, HIN infections are becoming more common. HIN can induce the release and expression of inflammatory mediators, leading to the infiltration of inflammatory cells, which leads to lung infections.

In order to provide reference for clinical prevention of infection and rational drug use, we retrospectively analyzed the clinical data of hospitalized patients with COVID-19 in our hospital from January 25, 2020 to March 1, 2020, and analyzed the clinical etiology. Although the medical level has made great progress in recent years, the mortality rate of lower respiratory tract infection has not decreased significantly. The failure to obtain a clear etiology diagnosis in time and the inability to use antibiotics are important reasons. Patients often have mixed infections of multiple bacteria[8–11]. The traditional etiological diagnosis method takes a long time and the positive rate is not high, and a more rapid and simple diagnosis method is urgently needed in the clinical. Gene diagnosis technology provides a new way for the detection of bacteria and the results can be obtained within 1 day, and more importantly, the results are not affected by the use of antibiotics. Therefore, this method has obvious advantages over traditional culture methods.

5. Conclusions

Forty-nine suspected COVID-19 cases from Guangdong Second Provincial General Hospital were included in the study. Probable bacterial infection was detected in 33 participants. Haemophilus influenzae was the most common bacterial pathogens detected. Multiple coinfections were commonly detected. Sputum multiplex PCR could become a useful diagnostic tool for bacterial respiratory infections in COVID-19 infected inpatients.

Abbreviations

Streptococcus pneumoniae (SPN)

Staphylococcus aureus (SAU)

Methicillin-resistant Staphylococcus (MRSA)

Klebsiella pneumoniae (KPN)

Pseudomonas aeruginosa (PAE)

Acinetobacter baumannii (ABA)

Stenotrophomonas maltophilia (SMA)

Haemophilus influenzae (HIN)

Escherichia coli (ECO)

Legionella pneumophila (LPN)

Mycoplasma pneumoniae (MPN)

Chlamydia pneumoniae (CPN)

Mycobacterium tuberculosis complex (MTB)

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Guangdong Second Provincial General Hospital.

Consent to publication

Not applicable.

Availability of data and material

The datasets used and analyzed in this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Junping Yan drafted the manuscript. Junping Yan and Liangshan Hu designed the study and conducted the survey. Seyin Zou and Guochen Liu performed the analysis and interpreted the data. Donglin Cao was the principal investigators, provided all facilities necessary to complete this work and was involved in editing the manuscript. All authors read and approved the final manuscript.

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Not applicable

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Figures

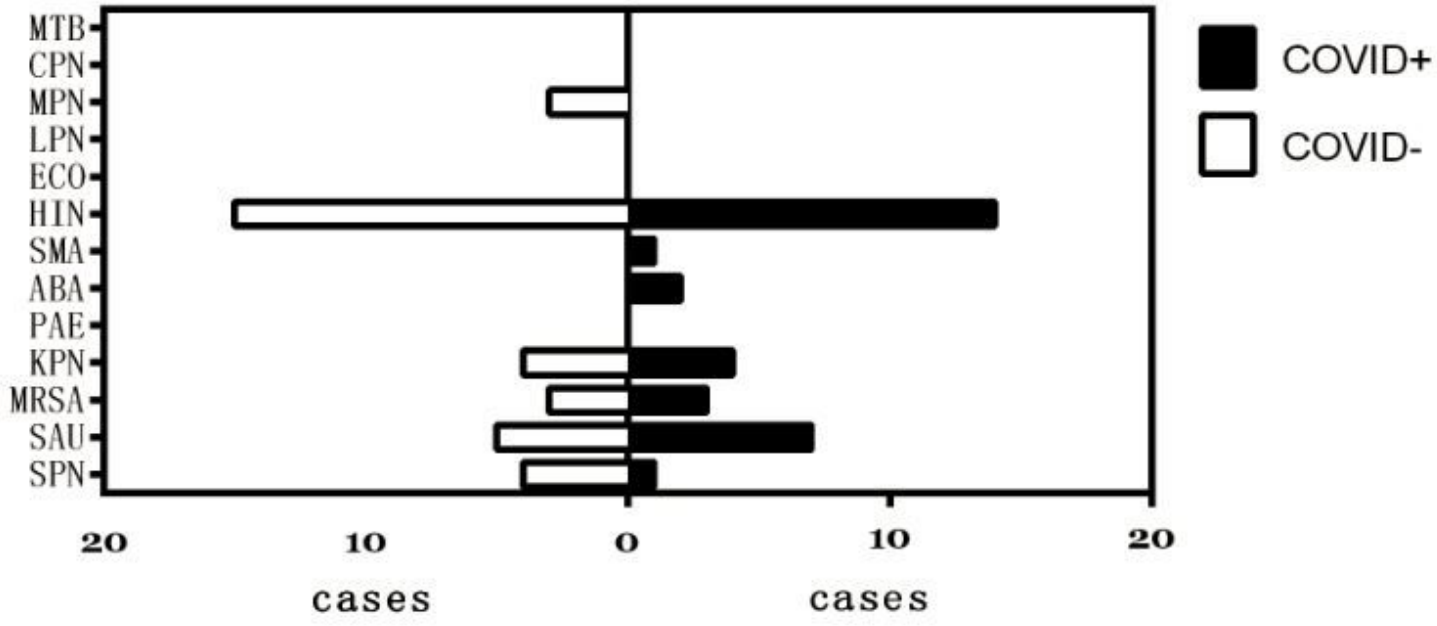

Figure 1

Nucleic acid detection of the 13 clinically common lower respiratory tract pathogens