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Analysis of pathogens and risk factors of secondary pulmonary infection in patients with COVID-19

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ABSTRACT

To investigate the distribution and risk factors of pathogens in secondary pulmonary infection in patients with COVID-19.142 patients with confirmed COVID-19 from Shanghai Public Health Clinical Center were collected, and 32 patients with pulmonary infection were taken as the infection group. The distribution of pathogens in the sputum specimens was applied for retrospective analysis. Meanwhile, 110 patients diagnosed with COVID-19, but without pulmonary infection were regarded as the asymptomatic group. The risk factors of pulmonary infection were analyzed with generalized linear models and logistic regression. The pathogens in the lung infection group were mainly gram-negative bacteria (22, 68.8%), especially *Klebsiella pneumoniae*. Gram-positive bacteria and fungi accounted for 13 (40.6%), mainly *Staphylococcus aureus*, and 11 (34.4%), mainly *Candida albicans*. There were 14 cases (43.8%) infected with two or more pathogens. The comparison between the two groups found that, patients with elder age, underlying diseases, more lung lesions and low protein contents, were more likely to develop lung infections. At last, univariate analysis showed that 6 factors, including indwelling gastric catheter, the number of deep vein catheters, tracheal intubation tracheotomy, invasive mechanical ventilation, hormonal application, and the use of more than three antibacterial drugs, are risk factors for COVID-19 secondary pulmonary infection. Generalized linear models and logistic regression analysis showed antimicrobial use as an independent risk factor for COVID-19 secondary lung infection. There are many risk factors for secondary lung infection in severe COVID-19 patients, and it is recommended to use antibiotics reasonably.

1. Introduction

Since December 2019, unexplained pneumonia cases reported in Wuhan, Hubei Province have spread to most parts of the country, causing widespread concerns, at home and abroad. The World Health Organization (WHO) named the virus as "2019 New Coronavirus", referred to as *SARS-CoV-2*. The clinical symptoms are similar to *SARS-CoV* and *MERS-CoV* infections, and fever and cough are the most common symptoms. The typical CT image of the chest is the opaque of bilateral ground glass and the sub-consolidated area. However, *SARS-CoV-2* is highly contagious. Previous analysis pointed that *SARS-CoV-2* is similar to *β-coronavirus* detected in bats. It is a new *β-coronavirus*, belonging to the *Rotavirus* subgenus of the *Coronaviridae* family [1]. It is highly contagious, susceptible, and showed serious threatens for public health. After more than a month of joint efforts across the country, the number of new cases was significantly reduced, and the number of discharged patients continued to increase.

As some patients with new-type coronavirus pneumonia showed dangerous onset and rapid disease progression, as well as susceptible to critical diseases, such as acute respiratory distress syndrome (ARDS), respiratory failure, septic shock, and renal failure, which increases the mortality of the disease. Existing studies found that elderly patients, with underlying diseases are more likely to develop critical infections [2,3]. For critical COVID-19 patients, tracheal intubation and ventilator correction are usually required to correct hypoxia. Some patients also need invasive operations, like tracheotomy, gastric tube insertion, urinary catheter, and deep vein catheterization, and some critically patients need continuous renal replacement therapy (CRRT) and Extracorporeal Membrane Oxygenation (ECMO) adjuvant therapy. These invasive procedures and treatments may cause or exacerbate lung

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infections, and even cause patients to die from severe lung infections. Therefore, we conducted a detailed analysis of the pathogen types and risk factors of secondary pulmonary infection, in patients with COVID-19. This study provides a reference basis for early warning and diagnosis of pulmonary infection, and reliable basis for the choice of early clinical experience for drug use, in patients with COVID-19.

2. Materials and methods

2.1. Data collection

A total of 142 confirmed COVID-19 patients admitted to the emergency ward, from January 2020 to October 2020 in Shanghai Public Health Clinical Center, were selected and 32 patients with secondary pulmonary infection were identified by sputum culture examination as the infection group. Correlation was analyzed for clinical data, including patient's age, gender, history of diabetes, hypertension and smoking, albumin level, procalcitonin level, range of lung lesions, hormone application, use of antibacterial drugs, invasive non-invasive mechanical ventilation, urine and nasal feeding, deep vein catheterization, tracheal intubation, tracheotomy, CRRT and ECMO use, etc. The other 110 patients with negative sputum culture and no clear secondary pulmonary infection were regarded as the asymptomatic group. All patients or their families signed informed consent and were reviewed and approved by the Ethics Committee of Shanghai Public Health Clinical Center.

2.2. The diagnostic criteria for pulmonary infection are as follows

The diagnosis of pulmonary infection must meet four of the following conditions at the same time: ① Respiratory symptoms such as cough, yellow purulent sputum, rapid breathing, etc.; ②Fever, axillary temperature ≥38.0 °C; ③ White blood cells increased during routine blood tests; ④ Dry and wet rales can be heard on auscultation of lung; ⑤ Sheet shadow or chest CT showed sheet shadow; ⑥ pathogens were cultured in sputum culture; ⑦ tracheotomy or intubation, aspiration, pulmonary edema, and atelectasis. This diagnostic criteria refer to the international ERS/ESICM/ESCMID/ALAT guidelines for the management of pneumonia and the infection diagnostic criteria issued by the Ministry of Health of China in 2001 [4]. The final grouping is mainly based on the results of sputum culture.

2.3. Collection and processing of sputum samples

Instruct the patient to rinse the mouth with Boric acid solution first, and then rinse with water to remove bacteria in the oral cavity. After inhaling deeply, cough up 1–2 sputum in a Petri dish or bottle and send it to the test in time. Patients with endotracheal intubation used anti-contamination brushes to remove respiratory sputum samples for examination. AUTOMA automatic microbe drug sensitivity analysis system was applied for identification and drug sensitivity test [4]. Three consecutive sputum cultures of the same pathogen, and each time the sputum retention time interval is more than 24 h, according to three sputum culture results positive for the infection group, three sputum culture negative for the asymptomatic group. (All sputum samples were processed in the P3 laboratory).

2.4. Clinical and experimental data analysis

Clinical data of COVID-19 patients was collected, and pathogens in sputum specimens of patients were analyzed, with retrospective analysis. Sputum specimens were collected for three consecutive times, each time with more than 24 h interval. The medical history and related clinical indicators of the infected group and the asymptomatic group were compared, and combining the results of sputum culture, the risk factors of secondary lung infection were analyzed.

2.5. Statistical analysis

SPSS 20.0 software was used to analyze the data. The measurement data were expressed as mean ± standard deviation. The comparison was performed by *t*-test. The count data were analyzed by $\chi 2$ test. The risk factors were analyzed by univariate and multivariate analysis of variance, and factors with $P < 0.05$ was considered significant. Significant factors were applied for generalized linear models and logistic regression with R base package.

3. Results

3.1. Comparison of clinical data between two groups of COVID-19 patients

As shown in Table 1, the average age of patients in the infected group was 59.4 years, which was higher than the asymptomatic group of 45.6 , and the difference between the two was statistically significant ($P < 0.05$). No significant gender difference between the two groups of patients ($p > 0.05$) was observed, and 28 patients in the infected group had higher previous underlying diseases, mainly including hypertension, diabetes and coronary heart disease, compared with 12 cases in the asymptomatic group, with statistical significance ($p < 0.001$). The smoking history of the two groups was not significant. According to imaging analysis, more than 2 lung segments were infected by COVID-19 in the infected group, which was statistically significant, compared with the asymptomatic group. There were 22 hypoproteinemia patients in the infected group, higher than 11 in the asymptomatic group, and the difference was significant. Further comparison found no difference in the procalcitonin level between the infected group and the asymptomatic group.

3.2. Pathogen distributions in two groups of COVID-19 patients

According to sputum culture results, the pathogens in the lung infection group were mainly gram-negative bacteria (22, 68.8%), especially *Klebsiella pneumoniae*. Gram-positive bacteria and fungi accounted for 13 (40.6%), mainly *Staphylococcus aureus*, and 11 (34.4%), mainly *Candida albicans*. There were 14 cases (43.8%) of two or more mixed infections. The results were shown in Table 2.

Analysis of risk factors for secondary pulmonary infection with COVID-19:

Variance analysis showed that the use of the following two antibacterial drugs were not statistically significant between the infected group and the asymptomatic group. Gastric tube, Urinary catheter,

Table 1
Comparison of clinical parameters between two groups of COVID-19 patients.

Index	Asymptomatic group (n = 110)	Infection group (n = 32)	P value
Age (Year)	45.6	59.4	**0.017***
Gender			0.210
Male	62	22	
Female	48	10	
Underlying diseases	12	28	**0.000*****
Hypertension	7	10	
Diabetes	2	4	
Coronary heart disease	1	5	
Malignant tumor	1	2	
Hyperlipidemia	1	7	
Smoking history	10	5	0.290
Lung lesions> 2 lung segments	19	32	**0.000*****
Low protein (Cases) (≤35 g/L)	11	22	**0.000*****
PCT	0.178	0.201	0.325

*, P value < 0.05; **, P value < 0.01; ***, P value < 0.001.

Table 2
Distribution of sputum pathogens in lung infection group.

Pathogens	Infection group (n = 32)
Gram-positive bacteria	**13 (40.6%)**
Staphylococcus aureus	6 (18.8%)
Staphylococcus haemolyticus	3 (9.4%)
Enterococcus	4 (12.5%)
Gram-negative bacteria	**22 (68.8%)**
Pseudomonas aeruginosa	6 (18.8%)
Klebsiella pneumoniae	11 (34.4%)
Acinetobacter baumannii	3 (9.4%)
Stenotrophomonas maltophilia	1 (3.1%)
Haemophilus influenzae	1 (3.1%)
Fungi	**11 (34.4%)**
Candida albicans	6 (18.8%)
Candida tropicalis	2 (6.3%)
Other	3 (9.4%)
≥ 2 bacteria	**14 (43.8%)**

Number of deep vein catheters, Tracheal intubation, Tracheotomy, non-invasive mechanical ventilation, Invasive mechanical ventilation, CRRT, ECMO, hormone application and the use of more than 3 antibacterial drugs were statistically significant between the two groups ($p < 0.01$), which may be a risk factor for COVID-19 secondary lung infection (Table 3). The above risk factors were included in the regression model for multivariate analysis. The results showed that antimicrobial use was an independent risk factor for COVID-19 secondary lung infection (Table 4).

4. Discussion

Coronavirus is a single-stranded positive-stranded RNA virus with an envelope, widely found in human, mammal, and bird hosts, and can cause respiratory, intestinal, liver, and nervous system diseases. SARS-CoV-2 is the seventh, among human coronaviruses and belongs to Beta coronavirus. Studies have pointed that the natural host may be bats [5–7]. The molecular mechanism of the interaction between S-protein and human ACE2 is used to infect human respiratory epithelial cells, so it has a strong ability to infect humans [8]. At present, the disease is mainly clustered in families, and medical staff was infected. SARS-CoV-2 is spread in the crowd by droplets and contact, and a few were transmitted through aerosols and digestive tract.

Table 3
Comparison of invasive operation and treatment between two groups of COVID-19 patients.

Invasive operation and treatment	Asymptomatic group (n = 110)	Infection group (n = 32)	P value
Gastric tube	5	18	**0.000*****
Urinary catheter	6	18	**0.000*****
Deep vein catheterization site			1.000
Inside the neck	12	16	
Femoral vein	6	8	
Number of deep vein catheters			**0.001****
1	14	3	
2	6	12	
3	2	10	
Tracheal intubation	2	13	**0.000*****
Tracheotomy	2	12	**0.000*****
Non-invasive ventilation (NPPV + HF)	12	12	**0.000*****
Invasive mechanical ventilation	2	13	**0.000****
CRRT	1	4	**0.002****
ECMO	1	5	**0.000*****
≤2 Antibacterial drugs	32	14	0.119
>2 Antibacterial drugs	4	18	**0.000*****
Hormone	8	20	**0.000*****

Abbreviations : ECMO = extracorporeal membrane oxygenation. CRRT= Continuous Renal Replacement Therapy
*, P value < 0.05; **, P value < 0.01; ***, P value < 0.001.

Table 4
Generalized linear models and logistic regression analysis of risk factors between two groups of COVID-19 patients.

Risk factors	Estimate	95% CI	z value	Pr (>|z|)
(Intercept)	−1.8676726	−4.8018–1.019	−1.28624	0.1983593
Gender male	0.79228003	−0.691–2.391	1.0300379	0.3029922
Age	−0.0002242	−0.062–0.053	−0.007759	0.9938092
Medical history	−0.2660297	−2.200–1.468	−0.291752	0.7704764
Smoke	−1.0382038	−5.207–2.999	−0.520299	0.6028552
Hormone	0.73686141	−1.068–2.440	0.8485237	0.3961464
Deep_vein	0.26471024	−2.246–2.806	0.2140803	0.8304845
Antibacterial	**2.51902632**	**0.674~4.831**	**2.4648072**	**0.0137087**

Current research and clinical observations found that SARS-CoV-2 mainly affects the respiratory tract and lungs. Mild COVID-19 cases showed symptoms of upper respiratory tract infections, and the severe ones can quickly progress to ARDS and respiratory failure. Severe complications include acute renal insufficiency and cardiac insufficiency, and organ dysfunction is also the leading cause of death. Rescue treatment of critically patients requires respiratory circulation support, various invasive procedures, catheter placement, etc., and the greatest potential risk of these invasive treatments is secondary to other microbial infections, which are usually respiratory system and bloodstream infections. Machine-associated pneumonia is a common complication [4,9]. In our study, we found that invasive mechanical ventilation, indwelling gastric tube, urinary catheter, and deep vein catheterization are all risk factors for secondary microbial infections. Microbial infections can lead to further deterioration of the condition, which is also the main reason for the failure of treatment.

In this study, we divided patients into infected and asymptomatic groups, and found no statistical significance in gender and smoking status. In terms of age and underlying diseases, elderly patients and patients with previous underlying diseases, such as hypertension and diabetes, are more likely to develop secondary pulmonary infections. At the same time, lung imaging changes in more than two lung segments have caused relatively severe viral invasion and are more prone to secondary lung infections. As we know, human nutrition can be reflected by serum albumin, and we found that the low protein ratio in the infection group was higher than that in the asymptomatic group, suggesting patients with poor nutrition status are more likely to have secondary pulmonary infections.

The status of sputum culture in the diagnosis and treatment of infectious diseases is very important. According to the results of sputum culture, the pathogenic microorganisms can be clarified, and sensitive anti-infective drugs can be selected to achieve the purpose of curing the disease. Correct identification of pathogenic microorganisms is very important. We know that most patients with nosocomial infections are mainly Gram-negative bacteria, and our research found that the pathogens in the infection group are mainly Gram-negative bacteria, followed by Gram-positive bacteria and fungi. Gram-negative bacteria are mainly *Klebsiella pneumoniae*. Gram-positive bacteria are mainly *Staphylococcus aureus*. Fungus is mainly *Candida albicans*. *Candida albicans* is a common colonization or condition-causing bacteria in the respiratory tract. The infection caused by often indicates a serious condition and requires antifungal treatment. In addition, studies have shown that the effect of airway fungal colonization on lung bacterial infections. *Candida albicans* inhibits the production of reactive oxygen species in rat alveolar macrophages and is associated with an increased incidence of *P. aeruginosa* pneumonia [10–13]. Candida colonization of the respiratory tract may predispose to bacterial ventilator-associated pneumonia (VAP) [14]. This is where we need to pay special attention.

If we are diagnosed with a fungal infection, we will give antifungal treatment. We will consider the type and sensitivity of the pathogen, the

severity of the patient's condition, liver and kidney function, including fluconazole, voriconazole and caspofungin, often choose one drug, critical or resistant patients will also consider combining antifungal therapy. The treatment time is generally 2 weeks after the last sputum culture is negative and the drug is stopped. Antifungal treatment is mostly after antibacterial treatment, or combined antibacterial treatment. Fortunately, patients with fungal infections got better after antifungal treatment, and the infection was controlled, which further confirmed our judgment of the disease.

Studies have shown that for each additional 1d of mechanical ventilation and tracheotomy intubation, the chance of lung infection increases by 1%–3%. The respiratory mucosa of patients with tracheotomy is directly exposed to the air, allowing microbe to multiply. Therefore, patients with mechanical ventilation and tracheotomy cannula should be paid more attention in the later stage, which is the main risk factor of pulmonary infection [9,15,16]. In addition, long-term combined use of more than three antibacterial drugs is prone to cause dual infections and multi-drug-resistant microbe. The use of glucocorticoids is also an important factor for secondary pulmonary infections, especially prone to fungal infections or mixed infections [17,18]. This study also showed that the combined use of hormones and multiple antibacterial drugs was a risk factor for secondary pulmonary infections, of which 14 cases were mixed infections of two or more (43.8%). It is important that multiple regression analysis shows that antimicrobial use is an independent risk factor for COVID-19 secondary lung infections. Therefore, it is recommended to clinically control the use of antibacterial drugs, especially non-critical patients should avoid or reduce the use of antibacterial drugs.

In summary, we should note that COVID-19 critically patients have more risk factors for secondary lung infections. Common microbial infections are mainly Gram-negative bacilli. Clinically, we should pay close attention to the distribution of pathogenic microorganisms and drug resistance, and strengthen COVID-19 management of critically patients, including tracheotomy, ventilator control, rational use of hormones and antibacterial drugs, basic diseases, nutritional status, and other aspects of targeted interventions to reduce the incidence of secondary infections and mortality.

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Availability of data and materials

The original source data and material will be available upon reasonable request.

Author statement

All authors have read the manuscript, and agree to publish the manuscript.

All authors declare no conflict of interests.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the shanghai public health clinical center.

Declarations

This manuscript has not been previously published and is not currently being considered for publication elsewhere.

Contributors

LP, ZYZ, MW and XLZ collected the epidemiological and clinical data and processed statistical data. HCT and YBZ drafted the manuscript. FL revised the final manuscript. LP and QGW is responsible for summarizing all data related to the virus. HCT and FL are responsible for summarizing all epidemiological and clinical data.

CRediT authorship contribution statement

Haicheng Tang: Writing – original draft, Writing – review & editing, Investigation, Formal analysis, Resources, Data curation, Funding acquisition. **Zhangyan Zhao:** Investigation, Formal analysis, Resources, Data curation. **Xiaolin Zhang:** Methodology, Software. **Lei Pan:** Methodology, Software. **Qingguo Wu:** Visualization, Project administration. **Mei Wang:** Visualization, Project administration. **Yunbin Zhang:** Writing – original draft, Writing – review & editing, Conceptualization, Validation, Supervision. **Feng Li:** Conceptualization, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no competing interests to declare.

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