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Respiratory bacterial pathogen spectrum among COVID-19 infected and non–COVID-19 virus infected pneumonia patients

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Abstract

COVID-19 positive (194) and negative (212) pneumonia patients were selected to analyze bacterial pathogens coinfection. Results showed that 50% of COVID-19 patients were coinfected or carried bacterial pathogens. Bordetella pertussis infection rate was significantly higher in positive patients. Consequently, precautions should be taken to control bacterial pathogens coinfection in COVID-19 patients. © 2020 Elsevier Inc. All rights reserved.

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During February 8 to February 15, 2020, in Suizhou city, Hubei province, 2216 pneumonia patients were diagnosed based on chest X-ray or computed tomography (small slide shadows and interstitial change, obvious extrapulmonary bands, or even ground-glass opacity and infiltration shadows, lung consolidation, and pleural effusion), blood routine (reduction of lymphocytes), and blood biochemistry (increase of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, myozyme, myoglobin, and C-reactive protein, and normal procalcitonin) tests. These patients were admitted to hospital after being diagnosed as pneumonia patients. Then, the COVID-19 pneumonia patients were determined by throat swab nucleic acid test in Suizhou Center for Disease Control and Prevention. Total nucleic acids (DNA and RNA) were extracted from throat swab samples by Liferiver Nucleic acids extraction Kit (Shanghai ZJ Bio-Tech Co., Ltd.). The COVID-19 nucleic acid test was based on real-time polymerase chain reaction (PCR) according to the Chinese Center for Disease Control and Prevention’s proposal (Chinese National Health Commission, 2020), and the used primers were shown in Table S1. Among them, there were 292 COVID-19 positive and 1924 COVID-19 negative pneumonia patients. In this study, 194 cases (47.78%) were selected from 292 positive cases randomly and 212 cases (52.22%) from 1924 negative cases randomly. Among COVID-19 positive patients, 99 (51.03%) were male and 94 (48.45%) were female, while the males and females in the COVID-19 negative patients were 119 (56.13%) and 93 (43.87%).
respectively. The age range was 1–88 years old including 12 children, and the average age was 45 years old.

The residual total nucleic acids (DNA and RNA) after COVID-19 diagnosis were sent to Hubei Center for Disease Control and Prevention for bacterial pathogens analysis from Suizhou Center for Disease Control and Prevention. Chlamydia pneumoniae, Streptococcus pneumoniae, Bordetella pertussis, Streptococcus pyogenes, Staphylococcus aureus, Corynebacterium diphtheria, L. pneumophila, M. pneumonia, Mycobacterium tuberculosis, Neisseria meningitides, Haemophilus influenzae, Streptococcus agalactiae, Pseudomonas aeruginosa, and Moraxella catarrhalis were detected by real-time PCR (Hiramu et al., 2011; Schwartz et al., 2006; World Health Organization, 2011; Yang et al., 2015). All the primers and probes used in this study were shown in Table S1. The bacterial coinfection or carriage rate showed no difference among COVID-19 positive and negative patients, while 8.25% were coinfect or carried 1 species of bacterial pathogen, while 8.25% were coinfect or carried 1 species of bacterial pathogens (Fig. 1B). Among them, 41.24% were coinfect or carried 1 species of bacterial pathogen, while 8.25% were coinfect or carried 2 or 3 species of bacterial pathogens (Fig. 1B). Although some patients had been treated for only 1–2 days (the first time for COVID-19 test), the others had taken this treatment for 5–9 days already (the second time for COVID-19 test), however, all the negative patients accepted this treatment for only 1–2 days (the first time for COVID-19 test). Hence, the longer time treatment might reduce the infection of *P. aeruginosa* carriage in this area was very high and might not be reduced by moxifloxacin and these Chinese traditional medicines. It is necessary to prevent *P. aeruginosa* infection in the treatment of COVID-19 because it is an opportunistic pathogen and its infection is common in some pulmonary diseases (Faure et al., 2018). In addition, none of *C. pneumonia*, *C. diphtheria*, *L. pneumophila*, *M. tuberculosis*, and *S. agalactiae* were detected in both COVID-19 positive and negative patients. *L. pneumophila* co-infection with COVID-19 in Wuhan and Qingdao (Xing et al., 2020) might be due to high-level contamination of *L. pneumophila* compared to Suizhou.

In total, 50% of COVID-19 positive patients were coinfect or carried common bacterial pathogens (Fig. 1B). Among them, 41.24% were coinfect or carried 1 species of bacterial pathogen, while 8.25% were coinfect or carried 2 or 3 species of bacterial pathogens (Fig. 1B). The rate of co-infection or carrying 2 species of bacterial pathogens in COVID-19 negative patients was significantly higher than that in COVID-19 positive patients (Fig. 1B). Meanwhile, 1- and 3-species bacterial pathogens co-infection or carriage rate showed no difference between COVID-19 positive and negative patients.

### Table 1

| Sample source | Age (frequency) | Gender (frequency) | Frequency of pathogen carriage |
|---------------|----------------|--------------------|--------------------------------|
| **COVID 19 positive pneumonia patients** | | | |
| 0–10 (1) | Male (0) | 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| | Female (1) | 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| 10–18 (1) | Male (0) | 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| | Female (1) | 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| 18–45 (78) | Male (40) | 0 3 5 1 0 0 0 0 0 5 0 14 0 |
| | Female (38) | 0 6 3 0 0 0 0 0 0 3 0 8 0 |
| 45–65 (94) | Male (52) | 0 2 4 1 1 0 0 0 4 6 0 16 0 |
| | Female (42) | 0 1 4 1 0 0 0 0 1 0 0 14 0 |
| 65–(20) | Male (7) | 0 1 3 0 0 0 0 0 7 0 0 1 0 |
| | Female (13) | 0 1 0 0 0 0 0 0 2 3 0 4 0 |
| Total (194) | Male (99) | 0 6 12 2 1 0 0 0 4 11 0 31 0 |
| | Female (95) | 0 8 8 1 0 0 0 0 3 6 0 26 0 |
| **COVID 19 negative pneumonia patients** | | | |
| 0–10 (11) | Male (7) | 0 2 1 0 0 0 0 1 0 1 2 0 5 1 |
| | Female (4) | 0 1 1 0 0 0 0 1 0 0 0 2 0 2 |
| 10–18 (4) | Male (2) | 0 0 0 0 0 0 0 0 0 0 1 0 1 0 |
| | Female (2) | 0 0 0 0 0 0 0 0 1 0 1 0 0 0 |
| 18–45 (96) | Male (60) | 0 6 3 1 3 0 0 2 0 7 5 0 19 0 |
| | Female (36) | 0 1 1 1 2 0 0 2 0 4 0 0 12 0 |
| 45–65 (80) | Male (40) | 0 4 1 0 0 0 0 0 7 5 0 17 0 |
| | Female (40) | 0 3 1 0 1 0 0 0 4 5 0 11 0 |
| 65–(21) | Male (10) | 0 0 0 0 0 0 0 0 0 0 3 0 |
| | Female (11) | 0 0 1 0 0 0 0 0 1 0 3 0 |
| Total (212) | Male (119) | 0 12 5 1 3 0 0 3 0 15 13 0 45 1 |
| | Female (93) | 0 5 4 1 4 0 0 4 0 8 0 30 0 |

**CP = C. pneumonia; SP = S. pneumonia; BP = B. pertussis; SPY = S. pyogenes; SA = S. aureus; CD = C. diphtheria; LP = L. pneumophila; MP = M. pneumonia; MT = M. tuberculosis; NM = N. meningitides; HI = H. influenzae; SAG = S. agalactiae; PA = P. aeruginosa; MC = M. catarrhalis.**
Although there are some limitations in this study including 1) bacterial culture and susceptibility studies were not performed on these samples to corroborate PCR findings, 2) lower respiratory tract samples for PCR or culture were not obtained to further determine whether PCR findings were reflective of actual infection, and 3) viral coinfections were not studied, it can provide some insights for COVID-19 prevention and treatment. In summary, 50% of COVID-19 positive patients were coinfectected or carried the tested common bacterial pathogens according to this study. Usually, when a single or multiple pathogens invade the upper respiratory tract and damage the respiratory system, the other pathogens can easily infect the patient at that moment and further lead to the clinical diagnosis and treatment being more complicated.
The increased risk of *B. pertussis* infection in COVID-19 positive patients and high carriage of *P. aeruginosa* in this area might worsen the pathogenic condition. Consequently, some precautions should be taken to treat the common bacterial pathogens coinfection, especially for *B. pertussis*. The reason why *B. pertussis* increased in COVID-19 positive patients needs to be clarified and further confirmed in further study.

**Authors’ contributions**

Jing Lv and Faxian Zhan designed this study. Danwen Nie and Fang Guo collected the samples. Fei He, Hongmei Yang, Bin Fang, and Bing Hu performed the nucleic acids extraction and RT-PCR tests. Xian Xia, Honglin Jiang, Yongzhong Jiang, and Xixiang Huo analyzed the data and prepared the manuscript.

**Conflict of interest**

All authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2020.115199.

**References**

Bisgard KM, Pascual FR, Ehresmann KR, et al. Infant pertussis: who was the source? Pediatr Infect Dis J 2006;25(11):1085–8. https://doi.org/10.1097/01.inf.0000145263.37198.2b.

Bosch AA, Biesbroek G, Trzcinski K, et al. Viral and bacterial interactions in the upper respiratory tract. PLoS Pathog 2013;9(1), e1003057. https://doi.org/10.1371/journal.\_ppat.1003057.

Carlinottini NH. Immunomodulation in the pathogenesis of Bordetella pertussis infection and disease. Curr Opin Pharmacol 2007; 7(3):272–278. DOI: https://doi.org/10.1016/j.coph.2006.12.004.

Chinese National Health Commission. Prevention and control plan for novel coronavirus pneumonia3nd ed.; 2020.

Cox MJ, Loman N, Bogaert D, et al. Co-infections: potentially lethal and unexplored in COVID-19. Lancet Microbe 2020;1(1), e11. https://doi.org/10.1016/S2666-5247(20)30009-4.

Fautié E, Kwong K, Nguyen D. *Pseudomonas aeruginosa* in chronic lung infections: how to adapt within the host? Front Immunol 2018;9:2416. https://doi.org/10.3389/fimmu.2018.02416. [eCollection 2018].

Grayston MJ. *Klebsiella* the use of antibiotics. 6th ed. Melbourne, Australia: Hodder Arnold; 2010.

Hirana T, Yamaguchi T, Miyazawa H, et al. Prediction of the pathogens that are the cause of pneumonia by the battlefield hypothesis. PLoS One 2011;6(9). https://doi.org/10.1371/journal.pone.0024474.

Jose M, Desai K. Fatal superimposed bacterial sepsis in a healthy coronavirus (COVID-19) patient. Cureus 2020;12(5), e8350. https://doi.org/10.7759/cureus.8350.

Medical expert group of Tongji Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology. A quick guide to the diagnosis and treatment of pneumonia infected by new coronavirus (3rd Edition). Her Med. 2020; 1–9. [Chinese].

Schwartz T, Volkmann H, Kirchen S, et al. Real-time PCR detection of *Pseudomonas aeruginosa* in clinical and municipal wastewater and genotyping of the ciprofloxacin-resistant isolates. FEMS Microbiol Eco 2006;57(1):158–67. https://doi.org/10.1111/j.1574-6941.2006.00100.x.

World Health Organization (WHO). Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. 2nd Ed. WHO Meningitis Manual 2011.

World Health Organization (WHO). Coronavirus disease 2019 (COVID-19) Situation Report.

Yang H, Zhan J, Fang B, et al. Research on acute non-viral respiratory tract infection pathogens spectrum of four hundred influenza-like cases. Chin J Prev Med 2015;49(6):567–70.

Zhang L, Yu F, Sang W, et al. Research progress of Lianhua Qingwen in the treatment of influenza. Pharm Clin Chin Materia Medica 2019;10(1):54–8.

Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727–33. https://doi.org/10.1056/NEJMoa2001017.