# Atypical pneumonia caused by *Chlamydia psittaci* during the COVID-19 pandemic

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ABSTRACT

*Objectives:* Here, we retrospectively described the diagnosis and treatment of 32 cases diagnosed with *Chlamydia psittaci* pneumonia during the COVID-19 pandemic.
*Methods:* Clinical information was collected from all the patients. Reverse transcription–PCR and ELISAs were conducted for the detection of COVID-19 using nasal swabs and bronchoalveolar lavage fluid (BALF) samples. Metagenomic next-generation sequencing (mNGS) was performed for the identification of causative pathogens using BALF, peripheral blood and sputum samples. End-point PCR was performed to confirm the mNGS results.
*Results:* All 32 patients showed atypical pneumonia and had infection-like symptoms that were similar to COVID-19. Results of reverse transcription–PCR and ELISAs ruled out COVID-19 infection. mNGS identified *C. psittaci* as the suspected pathogen in these patients within 48 hours, which was validated by PCR, except for three blood samples. The sequence reads that covered fragments of *C. psittaci* genome were detected more often in BALF than in sputum or blood samples. All patients received doxycycline-based treatment regimens and showed favorable outcomes.
*Conclusion:* This retrospective study, with the highest number of *C. psittaci* pneumonia enrolled cases in China so far, suggests that human psittacosis may be underdiagnosed and misdiagnosed clinically, especially in the midst of the COVID-19 pandemic.

## Introduction

The COVID-19 pandemic has spread worldwide and caused a globally massive loss with regard to the economy and health (Bartleson et al., 2021). The diagnosis and screening of COVID-19 have been proceeding urgently in recent years (Yüce et al., 2021). Under this disastrous epidemic, however, atypical pneumonia caused by other uncommon pathogens, which has a series of clinical symptoms similar to COVID-19, is easily underdiagnosed and misdiagnosed.

Psittacosis is a zoonotic disease caused by *Chlamydia psittaci*. The clinical manifestations of human psittacosis can present as rapidly progressing severe pneumonia, acute respiratory distress syndrome, sepsis, and multiple organ failure. *C. psittaci* pneumonia has a wide range of clinical signs, such as fever, headache, fatigue, joint pain, and loss of appetite (Andrews et al., 1981;

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Cunha et al., 2006). Although previous reports indicated that psittacosis could be diagnosed by serology, isolation of *Chlamydia psittaci*, and molecular detection (Smith et al., 2005), psittacosis is sometimes difficult to diagnose correctly due to its nonspecific symptoms and the limitation of traditional testing methods (de Gier et al., 2018).

In recent years, sequencing technologies have been used clinically in pathologic and etiologic diagnosis of various diseases, including cancers, genetic diseases, and infectious diseases. Metagenomic next-generation sequencing (mNGS) can identify underlying pathogens regardless of whether they are bacterial, viral, fungal, or parasitic (Schlaberg et al., 2017). Several studies revealed that mNGS can facilitate the precise and rapid diagnosis of intractable infections, especially severe pneumonia caused by uncommon and rare pathogens (Langelier et al., 2018; Van Boheemen et al., 2020; Zinter et al., 2019). Therefore, mNGS is expected to be an effective diagnostic tool for *C. psittaci* pneumonia (Chen et al., 2020; Gu et al., 2020).

In China, we collected 32 suspected cases diagnosed with *C. psittaci* pneumonia by mNGS, from April 2020 to June 2021. On admission, because of the strict epidemic prevention policy on COVID-19 in China, these patients with pneumonia of unknown etiology initially put the entire hospital staff on high alert under the threat of COVID-19. Here, we retrospectively summarized the diagnosis and treatment process for these patients to remind physicians that atypical pneumonia, except for COVID-19, should also be considered during the COVID-19 pandemic.

## Materials and methods

### Enrolled subjects

A total of 32 patients with *C. psittaci* pneumonia were enrolled in this retrospective study. These patients were admitted from April 2020 to June 2021, when COVID-19 pandemic was supposed to spread in China. The hospitals where these patients were diagnosed and treated included Zhejiang Provincial People's Hospital, The First People's Hospital of Yongkang, and Pujiang People's Hospital. This study was approved by the ethics committees of these hospitals.

### Clinical data collection

Information was collected, including clinical data, demographic characteristics, basic medical conditions, clinical signs and symptoms, chest radiograph results, clinical laboratory test results, travel histories, recent contact with animals, etc.

### mNGS procedure

Samples from the 32 enrolled patients were collected for mNGS, including 18 bronchoalveolar lavage fluid (BALF) samples, nine peripheral blood samples, and five sputum samples. Nucleic acids were extracted from each sample with the Direct-zol RNA Miniprep kit (Zymo Research, Irvine, CA) and Trizol LS (Thermo

Fisher Scientific, Carlsbad, CA) according to the manufacturer's instructions in a biosafety level three laboratory. Hangzhou D.A. Medical Laboratory and Hangzhou D.A. Precision Diagnosis Center used independently developed kits to construct a paired-end library. After being qualified in quality inspection, high-throughput sequencing was performed with HiSeq4000, and the sequencing mode was PE150. The main steps of library construction included genomic DNA interrupted by ultrasound, DNA fragment end repair, adding 'A' base to the 3' end of DNA fragment, adding sequencing connector, fragment selection, PCR amplification, library quality inspection, and machine sequencing.

At least 25 million single-end 76-bp reads were generated for each sample on the Illumina NextSeq platform. Quality control processes included removal of low-complexity reads by bb-duk (entropy = 0.7, entropy-window = 50, entropy k = 5; version: January 25, 2018), adapter trimming, low quality reads removal, short reads removal by Trimmomatic (adapter: TruSeq3-SE.fa:2:30:6, LEADING: 3, TRAILING: 3, SLIDING WINDOW: 4:10, MINLEN: 70, version: 0.36), host removal by bmtagger (using human genome GRCh38 and yh-specific sequences as reference), and ribosomal reads removal by SortMeRNA (version: 2.1b). Taxonomic assignment of the clean reads was performed with Kraken 2 (Wood et al., 2019) against the reference databases (ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/), including archaea, bacteria, fungi, humans, plasmid, protozoa, univec, and virus sequences (software 2.0.7-beta, database version: August 2, 2019) after filtering of the adapters and human-origin reads. A negative control (no template) was processed and sequenced in parallel for each sequencing run as contamination control. The mNGS data in this study were deposited in China National GeneBank DataBase (https://db.cngb.org/; ID: CNP0003194).

### Data interpretation

The mNGS data processing method was adapted from the study by Yin et al. (2021). Unique nonhuman sequence reads that were >10-fold higher than that in negative controls were considered to be derived from the samples. A list of suspected pathogenic microorganisms from samples was obtained after the removal of background microorganisms through comparison with the negative controls. For a specific microbe (except *Legionella pneumophila*, *Mycobacterium*, and *Nocardia*), the results were considered positive when over three specific non-overlapping reads were detected by mNGS. Then, the top-ranked taxa were further screened based on the clinical features of the patient. Pathogenic microorganisms that could cause a clinical phenotype concordant with the clinical symptoms of the patients were considered as the candidate infectious agents.

### Confirmation by C. psittaci-specific PCR

The cases with suspected *C. psittaci* infection were confirmed by *C. psittaci*-specific end-point PCR analysis, which Hangzhou D.A. Medical Laboratory performed. Two *C. psittaci*-specific primer sets targeting the *OmpA* gene were used according to the study by Li et al. (2016) (Table 1). AccuPrime™ *Taq* DNA Polymerase, High

**Table 1**
*C. psittaci*-specific primers used in this study.

| Primer set | Sequence (5'-3') | F/R | Target | Size(bp) |
|---|---|---|---|---|
| 1 | GCAGGATACTACGGAGATTATGTTTTC | F | *OmpA* | 426 |
| | ACGTGCACCTACGCTCCAAGA | R | VD1-2 | |
| 2 | CAATACGCTCAATCTAATCCTAAAATTGA | F | *OmpA* | 341 |
| | CCTAAAAGGGTTGGGTTCCATGT | R | VD3-4 | |

Abbreviations: F/R= forward/reverse.

Fidelity (Invitrogen, Thermo Fisher Scientific, USA) was used for amplification, according to the manufacturer's instructions. Amplification was carried out on an ABI Veriti thermocycler (Applied Biosystems) using the following cycling parameters: an initial cycle of heating at 94°C for 2 minutes (single denaturation step), 40 cycles of 94°C for 15 seconds, 60°C (primer set 1) or 57°C (primer set 2) for 30 seconds, and 68°C for 30 seconds (annealing and extension). Three DNA extract samples from the blood of three healthy individuals were set as the controls. Gel electrophoresis verified the amplicons (1.0% agarose; Invitrogen, China).

### Diagnostic criteria

According to previous studies (Chen et al., 2020; Wu et al., 2021) and our actual situation, the diagnostic criteria for *C. psittaci* pneumonia included: (i) the diagnostic criteria of community-acquired pneumonia (Mandell et al., 2007), (ii) detection of the specific DNA fragment of *C. psittaci* through mNGS, (iii) positive results in *C. psittaci*-specific PCR, and (iv) negative results for all the routine etiologic pathogen tests and no other causative organism identified by mNGS or serologic tests.

## Results

### Patient characteristics

The overall clinical features of the patients are summarized in Table 2. The 32 patients included 20 males and 12 females, with a median age of 63 years (range 45-84 years). A total of 20 patients had underlying diseases. A total of 17 patients had a fever, with a body temperature of more than 38.5°C, accompanied by cough and the expectoration of yellow-white sputum. Three patients had myalgia, two patients had a headache, and two patients had hy-

potension on admission (<90/60 mm Hg). All patients showed atypical pneumonia, including inflammatory infiltration, pleural effusion, multiple inflammatory exudative lesions with interstitial edema, lung abscesses, and white lung. These clinical symptoms could also be observed in patients with COVID-19. Notably, at their first visit to the hospital, more than three-quarters of the patients (25/32) did not admit a history of exposure to chickens or ducks in their homes, which increased the possibility of misdiagnosis.

### Technical investigations

On admission, a blood examination was performed on the patients. As a result, almost all patients were characterized by elevated neutrophil percentage and C-reactive protein levels, with a median value of 86.3% and 170.85 mg/l, respectively (Table 2). These features were also reported in other studies about *C. psittaci* pneumonia (Cheong et al., 2019; Hogerwerf et al., 2017; Su et al., 2021). In addition, of the 32 patients, more than 20 had an elevated liver enzyme level, including alanine aminotransferase and aspartate aminotransferase (Table 2). It is noteworthy that these results were also similar to those for patients with COVID-19 (Guan et al., 2020; Hu et al., 2021). Most of the patients had a normal amount of white blood cells. Pulmonary imaging showed inflammatory infiltration of the lungs, with interstitial changes (unilateral or bilateral) with pleural effusion (data not shown). The results of the blood culture were negative.

### Etiological examinations

The patients were initially suspected of having a COVID-19 infection. Therefore, nasal swabs and BALF samples were collected from each patient, and SARS-CoV-2 nucleic acid was investigated by real-time reverse transcription–PCR. ELISAs were also used to

**Table 2**
Patient characteristics.

| Characteristics | Patients, n (%) | Median value, (range) |
|---|---|---|
| *Demographics* | | |
| Male/female | 20/32 | |
| Age | | 63 (45-84) |
| History of exposure to poultry upon first admission | 7/32 (21.9) | |
| History of exposure to poultry after diagnosis | 32/32 (100) | |
| Underlying diseases | 20/32 (62.5) | |
| *Clinical manifestations* | | |
| Fever (>38.5°C) | 17/32 (53.1) | 38.5°C (36-40.1°C) |
| -Headache | 2/32 (6.3) | |
| Myalgia | 3/32 (9.4) | |
| Cough and expectoration | 26/32 (81.3) | |
| Hypotension (<90/60 mm Hg) | 2/32 (6.3) | |
| Invasive ventilator support | 13/32 (40.6) | |
| *Laboratory examinations* | | |
| Blood oxygen <60 mm Hg | 2/32 (6.3) | 88.4 (59.9-159 mmHg) |
| Elevated WBC (normal 4-10 $\times$ 10$^9$/l) | 4/32 (12.5) | 6.87 (2.99-29.5 $\times$ 10$^9$ /l) |
| Elevated percentage of neutrophils (normal 45-75%) | 30/32 (93.8) | 86.3 (73.2-97.3%) |
| Elevated CRP (normal 0-8 mg/l) | 32/32 (100) | 170.85 (44.3-245.4 mg/l) |
| Elevated PCT (normal 0-0.5 ng/l) | 18/32 (56.3) | 0.715 (0.09-22.459 ng/l) |
| Elevated BUN (normal 3.2-7.1 mmol/l) | 10/32 (31.3) | 5.175 (2.71-17.58 mmol/l) |
| Elevated CRE (normal 40-133 mmol/l) | 4/32 (12.5) | 67.5 (15-115 mmol/l) |
| Elevated ALT (normal 7-40 μ/l) | 21/32 (65.6) | 58.15 (13-309.4 μ/l) |
| Elevated AST (normal 13-35 μ/l) | 25/32 (78.1) | 70.4 (17-739.9 μ/l) |
| Elevated TB (normal 3.4-24 μmol/l) | 4/32 (12.5) | 12.5 (7-48.3 μmol/l) |
| Elevated DB (normal 0-6.8 μmol/l) | 14/32 (43.8) | 4.9 (2-27.6 μmol/l) |
| *Treatment* | | |
| Doxycycline | 19/32 (59.4) | |
| Moxifloxacin + Doxycycline | 13/32 (40.6) | |
| *Outcome* | | |
| Survival | 32/32 (100) | |

Abbreviations: ALT = serum alanine aminotransferase, AST = serum aspartate aminotransferase, BUN = blood urea nitrogen, CRE = serum creatinine, CRP = C-reactive protein DB = direct bilirubin, PCT = procalcitonin, TB = total bilirubin, WBC = white blood cell.

**Table 3**
mNGS and confirmative PCR results.

| Sample Type | Sample number | Median number of reads with *C. psittaci* sequences | Mean number of reads with *C. psittaci* sequences | Positive PCR, n (%) |
|---|---|---|---|---|
| BALF | 18 | 308 | 1015 | 18 (100%) |
| Blood | 9 | 35 | 33 | 6 (66.7) |
| Sputum | 5 | 12 | 233 | 5 (100%) |

Abbreviations: PCR = polymerase chain reaction.

test for plasma IgM or IgG antibodies against SARS-CoV-2 at admission and 2 weeks after admission. All the results were negative.

Next, indirect serum immunofluorescence tests were also performed to identify common respiratory pathogens (including the H1N1 flu virus, parainfluenza virus, *Legionella pneumophila, Chlamydia pneumoniae, Mycoplasma pneumoniae*). However, no potential pathogen was detected.

*mNGS and PCR results*

mNGS was performed because our traditional tests did not identify the exact cause of pneumonia. Clinical specimens included blood, sputum, and BALF. BALF was analyzed in 18 patients, peripheral blood was analyzed in nine patients, and sputum was analyzed in five patients. mNGS took 24 48 hours from the receipt of the sample to the reporting of the results. As a result, *C. psittaci* DNA fragments were detected in all patients by mNGS. In addition, the number of sequence reads that covered fragments of the *C. psittaci* genome detected by mNGS in BALF (median: 308 and mean: 1015) was much higher than that detected in the sputum (12 and 233) and blood (35 and 33) tests (Table 3), which suggested that BALF was more suitable for the detection of *C. psittaci*. No other potential pathogens were detected in these patients.

A confirmative PCR test with two specific primer sets for *C. psittaci* (Table 1) was performed to validate the positive mNGS results. All the PCR results were positive for the 18 BALF samples and five sputum samples. However, only six of nine blood samples were positive (66.7%), indicating a lower sensitivity of PCR than mNGS for blood samples (Table 3).

*Treatment and outcome*

On admission, treatment plan options were tough due to unknown etiologies, despite the negative results of testing for SARS-CoV-2 nucleic acid and plasma IgM or IgG antibodies against SARS-CoV-2. Empirical anti-infective therapies (ceftriaxone, piperacillin, amoxicillin, aloxicillin sodium, cefoperazone, meropenem, suproxen, and cefotiam) against common pathogens were adopted out of caution. However, the clinical symptoms of the patients did not improve. Most of the patients were treated with upgraded antibiotics.

When *C. psittaci* was identified as the causative pathogen, the treatments were adjusted to doxycycline or doxycycline-based treatment regimens: 19 patients took orally doxycycline (100 mg every 12 hours), accompanied by high-flow nasal cannula therapy, whereas 14 patients received concomitant therapy of moxifloxacin (400 mg intravenous glucose tolerance test per day) and doxycycline (100 mg orally every 12 hours), as well as were treated with ventilator adjuvant therapy. Overall, the course of treatment with doxycycline (median: 8 days; range: 4-14 days) was shorter than that of concomitant therapy with moxifloxacin and doxycycline (12 days; 10-30 days). Fortunately, no dead case was observed in our study. All the patients showed normal temperature and nonobvious respiratory symptoms as well as the well-balanced neutrophil percentage and C-reactive protein levels when they were discharged from the hospital.

**Discussion**

Although psittacosis is common in birds, the infection of *C. psittaci* in humans is rare (Mair-Jenkins et al., 2018). *C. psittaci* is responsible for one to eight percent of cases involving community-acquired pneumonia (Hogerwerf et al., 2017; Wu et al., 2020). To our knowledge, however, few cases of *C. psittaci* pneumonia have been reported under the COVID-19 pandemic until now. Of the two case reports that we could find, one reported the first family outbreak of psittacosis in China under COVID-19 (Li et al., 2021), and the other described four cases of *C. psittaci* pneumonia among the medical staff in a COVID-19 screening ward (Lei et al., 2021). Combined with these rare case reports, our retrospective study, with the highest number of suspected cases with *C. psittaci* pneumonia by far, strongly suggests that physicians should be vigilant about atypical pneumonia caused by uncommon pathogens also during the COVID-19 pandemic.

Psittacosis is most commonly transmitted by the inhalation of contaminated substances, such as dry feces or nasal secretions. In addition to pigeons, poultry (including chickens, geese and ducks, etc.) is a notable source of infection by psittacosis (Cheong et al., 2019; Hogerwerf et al., 2017; Su et al., 2021). However, in the present study, 25 of our cases did not report a history of exposure to either poultry or pigeons on the first admission. We speculate that the reasons for this finding may be because the patients were unaware of their exposure to poultry. For example, one of the patients once saw geese and ducks in a vegetable market but did not report this exposure history until we received the mNGS results and asked the patient again about possible exposure. The diagnosis of *C. psittaci* pneumonia is often complicated due to unconscious exposure to poultry.

Until now, etiologic detection of *C. psittaci* is still tough clinically. The reasons can be summarized as follows: (i) the rarity of human psittacosis makes physicians not vigilant enough about this infection, especially in Asia, where the rate of pathogen carriers among parrots is lower than that in Europe/America (Cong et al., 2014), (ii) human psittacosis has a wide range of clinical symptoms and these nonspecific symptoms can easily confound physicians. Atypical pneumonia caused by mycoplasma and other chlamydial species shared similar clinical symptoms with human psittacosis in many reports (Chen et al., 2020; Dumke et al., 2015; Gu et al., 2020; Medjo et al., 2014; Zhang et al., 2020). Besides, in this study, patients with *C. psittaci* pneumonia showed elevated neutrophil percentage, C-reactive protein level, and liver enzyme level, which is consistent with clinical features of patients with COVID-19 (Guan et al., 2020; Hu et al., 2021). (iii) Traditional tests for *C. psittaci* have some limitations. In fact, there is no ideal diagnostic tool for *C. psittaci* at present. The current existing testing methods for *C. psittaci* can be divided into serologic tests, culture, and molecular detections (Li et al., 2021). Serologic tests were used mostly, but problems still exist, such as the unavailability of convalescent serum samples required for confirmation of cases or the failure of the second sample to show seroconversion, which makes interpretation and conclusions difficult (Tuuminen et al., 2000). Culture is the gold standard for the confirmation of cases but is not routine in most diagnostic laboratories and hospitals. Molecu-

lar detections for *C. psittaci,* such as PCR testing, are also unavailable in most tertiary hospitals in China and are only performed when physicians suspect the infection with *C. psittaci* (Li et al., 2021).

The prevalence of COVID-19 may increase the possibility of misdiagnosing *C. psittaci* pneumonia. The reasons are as follows: (i) as presented previously, the clinical symptoms of SARS-CoV-2 and *C. psittaci* infection are sometimes indistinguishable, (ii) family outbreaks of *C. psittaci* pneumonia under COVID-19 may be correlated to familial aggregation due to the epidemic (Li et al., 2021), (iii) the lack of medical resources due to the frequent COVID-19 screening could further reduce the detection of psittacosis, especially in countries with strict COVID-19 prevention and control policies, such as China, and (iv) in China, where COVID-19 prevention and control is a priority, pneumonia of unknown etiology is easily regarded as COVID-19.

In most of the previous studies reporting the diagnosis of human psittacosis by mNGS, the confirmative procedures for diagnosis of *C. psittaci* were usually absent. In the current study, we used an end-point PCR testing to confirm the mNGS results. The general tactic of PCR for diagnosing *C. psittaci* infection is the use of a genus-specific test as a first step, followed by a more specific (i.e., species-specific) test to exclude other chlamydial species, especially *C. pneumoniae* and *C. trachomatis* (Nieuwenhuizen et al., 2018). We did not follow this tactic because no specific DNA fragments of *Chlamydia* spp. were identified by mNGS except *C. psittaci*; thereby, other chlamydial species were excluded. Serology and culture were not used due to their low sensitivities and specificities (Mitchell et al., 2009). The *C. psittaci*-specific PCR results, combined with the favorable outcomes of patients receiving doxycycline-based treatment regimens, confirmed the accuracy of mNGS.

However, we still found that three mNGS-positive blood samples were negative in the PCR testing, which suggested a higher detection rate of mNGS than PCR in blood for diagnosing psittacosis. The possible reasons could be concluded as follows: (i) the DNA load of the pathogen in blood was lower than that in BALF and sputum, (ii) the primer sets for PCR in this study were insufficient to detect all *C. psittaci* genotypes, and (iii) the end-point PCR, which could only be qualitative but not quantitative, had a lower sensitivity relative to mNGS. Also, a very recent psittacosis-related study (Duan et al., 2022) reported that the sensitivity of mNGS was higher than quantitative PCR, especially in blood samples, which supported our conclusion to some degree. However, Duan et al. (2022) also speculated the selected primers of quantitative PCR were not optimal. More studies comparing the detection of *C. psittaci* between mNGS and PCR/ quantitative PCR are further needed.

In recent years, many reports have proved that mNGS is an excellent diagnostic tool for meningitis, encephalitis, and lower respiratory tract infections (Langelier et al., 2018; Wilson et al., 2019). mNGS can detect a wide range of pathogens regardless of the suspected causative pathogens, which means it does not require an *a priori* hypothesis of target pathogens. Moreover, using mNGS, it only takes less than 48 hours from the receipt of the sample to the reporting of the results. Compared with the routine culture of respiratory specimens taking 5-7 days, with many negative results, mNGS is unquestionably more efficient, especially when the causative pathogens of patients are unknown and patients need to be diagnosed and treated as early as possible. Besides, mNGS can detect pathogens with an extremely low DNA load in the sample. (Duan et al., 2022). In the clinical practice of diagnosing lower respiratory tract infections, BALF and sputum samples, in which the DNA load of the pathogen is relatively high, are sometimes unavailable. This limits the use of conventional detection methods. Therefore, mNGS is particularly suitable for the detection of rare pathogens when the BALF and sputum samples are unavailable. However, the high cost of mNGS prevents it from completely replacing traditional detection methods, and the broad spectrum of pathogens by mNGS sometimes makes physicians unable to identify the causative pathogens (Simner et al., 2018).

*C. psittaci* belongs to the Chlamydiaceae family (Sachse et al., 2015). Tetracyclines, macrolides, or quinolones that interfere with DNA and protein synthesis can be selected as antimicrobial agents, whereas doxycycline represents a first-line treatment (Kohlhoff and Hammerschlag, 2015). In this study, 32 patients were adjusted to doxycycline or doxycycline-based therapy after a confirmed diagnosis. The clinical symptoms of these patients were effectively controlled and showed improvement. The prognoses of all 32 patients were good.

As mentioned previously, mNGS can detect diverse pathogens. This retrospective study only described the diagnosis of *C. psittaci* pneumonia by mNGS. In fact, in the past few years, we have used mNGS to diagnose over 80 pneumonia cases of unknown etiology and found some more causative pathogens other than *C. psittaci*, such as *Nocardia, Streptococcus parasanguis, Tropheryma whipplei, Legionella, Mycobacterium tuberculosis*, etc. We plan to use mNGS to investigate the epidemiology of community-acquired pneumonia in the future.

Although our study enrolled more patients relative to other case reports, larger-scale studies or meta-analyses are still needed to characterize the clinical features of patients with *C. psittaci* pneumonia. Given the current lack of reports for human psittacosis in China, we hope this report will raise awareness of this rare infectious disease, despite the ongoing COVID-19 pandemic. In addition, mNGS should be applied in the early diagnosis of psittacosis and other infectious diseases.

## Data Availability Statement

Data are available upon request.

## Author Contributions

Conceptualization, Q Yin and H Pan; methodology, Y Li and H Pan; formal analysis, T Hui; data curation, T Hui; writing—original draft preparation, Z Yu, H Wang, H Wu, and D Zhang; writing—review and editing, W Zheng and S Wang; supervision, Z Zhou, C Xu, W Wu, Y Tong, and H Pan. Qiaoqiao Yin, Yuecui Li, Hongyi Pan, Tianchen Hui and Zhaonan Yu contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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