BMC Microbiology

RESEARCH

Open Access

Evaluation of clinical characteristics and risk factors associated with Chlamydia psittaci infection based on metagenomic next-generation sequencing

Lei Yuan[1†], Qiang Chen[1†], Xin Yu Zhu[1†], Lan Min Lai[1], Rui Zhao[1*] and Yang Liu[1*]

Abstract

Introduction Psittacosis is a zoonosis caused by *Chlamydia psittaci*, the clinical manifestations of Psittacosis range from mild illness to fulminant severe pneumonia with multiple organ failure. This study aimed to evaluate the clinical characteristics of *Chlamydia psittaci* infection diagnosed based on metagenomic next-generation sequencing(mNGS), as well as the risk factors affecting the progress of *Chlamydia psittaci* infection, in order to improve the effect of therapeutics.

Methods We retrospectively analyzed the clinical data of patients infected with *chlamydia psittaci* in the First Affiliated Hospital of Nanchang University from January 2021 to December 2021. The patient's past medical history, clinical manifestations, laboratory examinations, chest CT results, treatment status, and prognosis data were collected. we also investigated both the pathogenic profile characteristics and the lower respiratory tract microbiota of patients with *Chlamydia psittaci* pneumonia using mNGS.

Results All cases of *Chlamydia psittaci* in our research have been confirmed by mNGS. Among 46 cases of *Chlamydia psittaci* pneumonia, Poultry exposure was reported in 35 cases. In severe cases of *Chlamydia psittaci* pneumonia, Neutrophils, Procalcitonin (PCT), Lactate Dehydrogenase (LDH), Hydroxybutyrate Dehydrogenase (HBDH), Creatine Kinase Isoenzymes-B (CK-MB) and D-Dimer levels were remarkably higher than that of non-severe cases, except for lymphocytes (all $P<0.05$). Chest CT scans showed Bilateral (77.8%), multiple lobar lungs (85.2%), pleural effusions (44.4%) involvement in those suffering from severe *Chlamydia psittaci* pneumonia, whereas its incidence was 0%, 21.1% and 10.5% in non-severe patients, respectively ($P<0.05$). Multivariate analysis revealed that higher lymphocyte concentrations (OR 0.836, 95% CI 0.714–0.962, $P=0.041$) were the only protective factor for survival. mNGS results indicated that 41.3% of patients (19/46) had suspected coinfections with a coinfection rate of 84.2% (16/19) in the

[†]Lei Yuan, Qiang Chen, and Xin Yu Zhu have equal contributions to this work.

*Correspondence:
Rui Zhao
zhaoruisc@163.com
Yang Liu
ly13767160474@sina.com

Full list of author information is available at the end of the article

severe group, much higher than that in the non severe group ($p < 0.05$). No significantly different profiles of lower respiratory tract microbiota diversity were found between non severe group and severe group.

Conclusion A history of poultry exposure in patients can serve as an important basis for diagnosing *Chlamydia psittaci* pneumonia, and patients with severe *Chlamydia psittaci* pneumonia are more likely to develop elevated inflammatory biomarkers as well as elevated cardiac markers. Higher lymphocyte concentrations are protective factors associated with severe *C. psittaci* pneumonia. The higher proportion of patients with coinfections in our study supports the use of mNGS for comprehensive early detection of respiratory infections in patients with *C. psittaci* pneumonia.

Keywords Metagenomic next-generation sequencing, *Chlamydia psittaci*, Pneumonia, Diagnosis, Therapeutic, Lower respiratory tract microbiota

Introduction

Community acquired pneumonia (CAP) is one of the most universal infectious diseases in intensive care unit(ICU), with a mortality rate of 30–50% [1], *Chlamydia Psittacosis*(*C. psittaci*), as the pathogen of CAP, accounting for 0-6.7% of the CAP infection [2]. *C. psittaci* is an obligate intracellular bacterium that grows in eukaryotic cells and can cause zoonotic disease psittacosis, which can lead to widespread infection in humans and animals, causing serious economic losses to the breeding industry, and posing a huge menace to human public health security [3, 4]. Humans mainly cause infection through the respiratory system. Bacteria that enter the human body diffuse into the reticuloendothelial system through the bloodstream, causing lung lesions and potentially affecting other organ systems,, including the liver, spleen, meninges and central nervous system [5]. The clinical manifestations of *C. psittaci* pneumonia range from mild symptoms to severe pneumonia. The main symptoms are respiratory infections, manifested as non-specific influenza-like symptoms such as fever, chills, sore throat and headache, as well as fatigue and muscle pain. Other non-specific symptoms include skin rash, vomiting, diarrhea [6, 7].

In recent years, severe *C. psittaci* pneumonia has been reported more frequently in clinical practice [8]. Early diagnosis of *C. psittaci* infection is one of the critical approachs for its prevention and control, and accurate detection results play an important role in controlling the spread of pathogens. However, Early diagnosis of *C. psittaci* pneumonia is challenging because the disease is characterized by non-specific symptoms [9], The traditional detection methods for *C. psittaci* include pathogen detection, serological detection, and molecular biology detection, among others. Culture is still the gold standard for the diagnosis of *C. psittaci* infection, but the diagnosis and treatment of *C. psittaci* infection will be delayed due to its strict nutritional requirements and long culture time. Besides, serological detection techniques such as immunofluorescence and complement binding assay have drawbacks such as long detection cycles, insufficient specificity or sensitivity [10]. With the development of detection technology, while ensuring specificity and sensitivity, researchers have further increased their requirements for detection speed. Molecular biology detection technology stands out because it is more suitable for laboratory testing and epidemiological investigations.

Metagenomic next-generation sequencing (mNGS) can quickly detect bacteria, viruses, fungi, protozoa, and other multicellular eukaryotic pathogens [11], achieving rapid screeningwithout specific amplification, which is helpful to identify pathogens in time to customize specific anti infective treatment [12], in addition, mNGS also plays a good auxiliary role in the clinical diagnosis of unexplained pneumonia, especially suitable for the diagnosis of difficult and complicated cases. In view of this, we conducted a retrospective study to investigate the clinical characteristics of severe *C. psittaci* pneumonia diagnosed by mNGS and identify the risk factors for poor prognosis of *C. psittaci* infection, in order to provide clinical suggestions and new insights into the pathogenesis of infectious pneumonia caused by *C. psittaci*.

Materials and methods

Patients and specimens

Retrospective analysis was conducted in patients admitted to the First Affiliated Hospital of Nanchang University in 2021 with *C. psittaci* infection. For each case, data on prodromal symptoms, disease severity, dynamics, and comprehensive computer tomography and detection indicators were extracted from electronic medical records. Other data on therapeutic drugs and outcomes were also collected. In our study, pathogen diagnosis was judged by clinicians according to imaging, clinical features and clinical examinations.

Diagnostic criteria

The diagnostic criteria for *C. psittaci* pneumonia was defined as follows: (I) Individuals fulfilling the diagnostic criteria for CAP according to Chinese adult guidelines for diagnosis and treatment of community-acquired pneumonia [13]. (II) The existence of specific *C. psittaci* gene

fragments in samples detected by mNGS. (III) The results are consistent with the diagnosis of clinicians. Diagnostic criteria for severe pneumonia are as follows [13]: Severe pneumonia can be diagnosed if one or more of the following signs occur. (1) Disturbance of consciousness; (2) Respiratory rate≥30 times/min; (3) PaO2<60mmHg; PaO2/FiO2<300, requiring mechanical ventilation; (4) Arterial systolic blood pressure<90mmHg; (5) Septic shock; (6) The X-ray chest film showed bilateral or multiple pulmonary lobe involvement, or the lesion expanded≥50% within 48 h of Hospital; (7) Oliguria: urine volume<20 ml/h, or <80 ml/4 h, or complicated with acute renal failure, requiring dialysis treatment.

Metagenomic next-generation sequencing and analysis

Sample collection

34 samples of alveolar lavage fluid, 5 sampless of sputum, and 7 samples of peripheral blood samples were collected for analysis. Blood samples were centrifuged at 1600 g for 10 min in order to separate plasma supernatant for extracting DNA. The sample was stored at -80 ° C for long-term storage.

Nucleic acid extraction

Nucleic acid extracted from all samples including the negative controls (water and extraction buffer) were acquired using the TIANamp Micro DNA Kit (TIANGEN Biotech, Beijing, China) in accordance with the manufacturer's manual. Qubit dsDNA HS Assay Kit (ThermoFisher Scientific) were used for nucleic acid concentration determination according to the manufacturer's manual.

Library construction and sequencing

Libraries were constructed by using the Nextera XT kit (Illumina) following the manufacturer manual. Illumina NextSeq-550Dx sequencer was employed for the sequencing reaction to gain the sample sequence information in the sample. The sequencing depth of each sample≥20 million reads was sequenced using a 75-cycle single-end sequencing strategy.

Bioinformatics analysis

Bcl2fastq v2.20.0.422 with default parameters was applied to demultiplex the primary sequencing output. For quality control adapter contamination and low-quality and low-complexity reads, raw reads and adapter removal were filtered by fastp (v0.19.5) [14] and Komplexity v0.3.6 [15]. Samples with <10 million reads after QC are treated as unqualified and need to be re-sampled and tested. Reads that were mapped to the human reference assembly GRCh38 were removed with Bowtie2 v2.3.4.3 [16]. Then, reads were aligned to the microorganism database consisting of approximately 12,000 genomes with SNAP v1.0 beta.18 [17] as previously described [18]. The mapped reads were classified based on the NCBI RefSeq genome database, or the NCBI GenBank genome database (http://ftp.ncbi.nlm.nih.gov/genomes/) was selected for each species. After filtering low-complexity reads [19], we counted the species or genus abundance with Perl scripts.

Reporting

The following were criteria for positive results of mNGS [20]:

1. For intracellular bacterium (*C. psittaci*, *Mycobacterium spp*,ect) and parasites, due to the difficulty of DNA extraction and low possibility for contamination, the result was considered positive if the number of detected sequences had the reads per million (RPM) ≥ 1.
2. For opportunistic pathogens, fungi and virus, the result was considered positive if the number of detected sequences were found > 5 folds in samples than in controls.

Statistical analysis

All statistical analyses were conducted using SPSS 26.0 software, with counting data described in terms of frequency and component ratio. Continuous variables of normal distribution are expressed as means ± SD(standard), or as medians (25th, 75th percentiles) for non-normal distribution. Continuous variables were compared by Mann-Whitney U-test or t-test, and categorical variables were analyzed using the Fisher's exact test. Using multivariate logistic regression analysis to identify risk factors associated with severe *C. psittaci* pneumonia. $P<0.05$ in univariate analysis was considered for the multivariate model. $P<0.05$ was considered statistically significant for the difference.

Results

General characteristics of the enrolled cohort

Among the 46 patients, 33 were male and 13 were female; The age range is 20–86 years old; The length of hospitalization time ranged from 3 to 52 days, and 35 patients had a clear history of bird contact; The incidence was mainly concentrated in autumn and winter (from September of that year to February of the next year). The most common clinical symptoms included fever (82.6%, 38/46), cough (37.0%, 17/46), expectoration (26.10%, 12/46), chest tightness (8.70%, 4/46), dyspnea (8.70%, 4/46), limb fatigue (10.87%, 5/46), etc. Past medical history included hypertension (3.04%, 14/46), diabetes (10.87%, 5/46), cirrhosis (4.34%, 2/46), cerebral infarction (6.52%, 3/46), nephrotic syndrome (2.17%, 1/46), myelodysplastic

syndrome (2.17%, 1/46), coronary heart disease (4.34%, 2/46), nodulosis (2.17%, 1/46), gastric ulcer (2.17%, 1/46). Clinical manifestations of the enrolled patients are shown in Table 1.

Laboratory characteristics

All 25 patients with *C. psittaci* pneumonia had a total leukocytes count within the normal range, of which 17 had leukocytes exceeding 9.5×10^9/L, 1 case below 3.5×10^9/L.The number of neutrophils increased in some cases, the proportion of neutrophils in 26 cases was higher than normal, and 1 case was lower than the normal range. hs-CRP was detected in 42 patients and increased in almost all cases (40/41). 32 patients underwent Erythrocyte sedimentation rate(ESR), of which 31 were higher than the normal range. The PCT data of 42 patients showed that 20 cases were higher than normal, and 22 cases had PCT values within the normal range.All patients were tested for coagulation function, including D-D dimer, prothrombin time(PT), Activated partial thromboplastin time (APTT), FIB and thrombin time(TT). The more characteristic laboratory abnormalities are D-D dimer, PT and FIB. 76.1%, 73.9% and 95.7% of the cases increased respectively. The number of patients with elevated AST (73.8%, 31/42) was higher than that of patients with ALT (50%, 21/42). Among the patients who performed myocardial zymogram, LDH and CK increased in 82.9% and 35.9% respectively. Compared to patients with the non-severe infection, those with severe *C. psittaci* pneumonia exhibited significantly lower lymphocyte and higher Neutrophils, PCT, LDH, HBDH, CK-MB and D-Dimer levels($p<0.05$). Laboratory results are shown in Table 2.

Radiological manifestations

All patients underwent computerized tomography(CT) of lung before admission. Most patients with psittacosis developed lobar pneumonia (37 cases, 80.4%), and the rest showed flake or strip-shaped high-density shadow; The lesions were unilateral in 25 cases (54.3%), including 10 cases on the left and 15 cases on the right; Bilateral 21 cases (45.6%); There were 17 cases of single leaf involvement, including 10 cases of upper leaf lesions, 7 cases of middle and lower leaf lesions and 29 cases of multiple

Table 1 Clinical manifestations of patients with *C. psittaci* pneumonia

Characteristics	*C. psittaci* pneumonia (*n* = 46)	Non severe *C. psittaci* pneumonia (*n* = 19)	Severe *C. psittaci* pneumonia (*n* = 27)	P value
Demographics				
Male/female	33/13	12/7	21/6	0.2782
Age, median (range, years)	61.7 ± 14.5	63.6 ± 14.2	60.3 ± 19.5	0.4749
Length of stay (days)	16.6 ± 10.8	12.5 ± 9.7	15.1 ± 12.3	0.0563
Season of admission, n				0.4110
Spring	8	2	6	
Summer	6	3	3	
Autumn	12	5	7	
Winter	20	9	11	
Clinical manifestations, n				
Fever	38	17	21	0.4395
Cough	17	5	12	0.2352
Expectoration	12	4	8	0.7346
Chest tightness	4	1	3	0.6322
Dyspnoea	4	0	4	0.1313
Hypodynamia	5	1	4	0.3870
Chills	1	0	1	1.0000
Headache	1	1	0	0.4419
Disturbance of consciousness	2	0	2	0.4950
Underlying disease, n				
Hypertension	14	4	10	0.3352
Diabetes	5	2	3	1.0000
Cirrhosis	2	1	1	1.0000
Cerebral infarction	3	2	1	0.5607
Nephrotic syndrome	1	0	1	1.0000
Myelodysplastic syndrome	2	0	2	0.5043
Coronary heart disease	2	1	1	1.0000
Nodulosis	1	0	1	1.0000
Gastric ulcer	1	0	1	1.0000

Table 2 Laboratory examination on admission of patients with *C. psittaci* pneumonia

Characteristics	*C. psittaci* pneumonia (*n* = 46)	Non severe *C. psittaci* pneumonia (*n* = 29)	Severe *C. psittaci* pneumonia (*n* = 27)	P value
WBC (3.5–9.5 × 10^9 /L)	7.60(5.50, 9.88)	6.92(5.36, 9.24)	9.01(0.66,10.11)	0.0725
Neutrophils(1.8–6.3 × 10^9 /L)	6.61(4.35, 9.28)	6.25(4.25, 8.06)	8.06(5.66, 9.76)	**0.035**
Lymphocyte (1.1–3.2 × 10^9 /L)	0.65 ± 0.53	0.74 ± 0.51	0.48 ± 0.46	**0.0016**
CRP (0–8 mg/L)	184.61 ± 97.91	185.63 ± 95.55	201.55 ± 101.65	0.2531
ESR (0–20)	53.5(35.75, 72.5)	53.5(42, 68.5)	52(30, 72)	0.4727
PCT (0–0.5 ng/mL)	0.47(0.25, 2.42)	0.39(0.25, 1.87)	1.75(0.44, 4,32)	**0.001**
ALT (9–50 U/L)	50(25.5, 93.1)	54(27, 94.8)	54(31, 91.4)	0.4464
AST (15–40 U/L)	78.4(37.25, 133)	75(44, 121)	97(45, 168.5)	0.0786
LDH (120–250 U/L)	364.4(266.45,535.4)	327.2(216.63, 503.5)	449.35(296.08, 741)	**0.0473**
HBDH (72–182 U/L)	226.7(203.25, 438.2)	214.95(200.5, 438.2)	331.8(217, 592)	**0.0388**
CK (50–310 U/L)	172.9(83.45, 536)	174.45(90.475,485.93)	230.9(109.5, 1152.9)	0.0595
CK-MB(2–25U/L)	18.6(13.7, 34.4)	17.2(12.35, 25.23)	24(14.15, 49)	**0.0441**
IL-6 (0–11.09 pg/mL)	223.11 ± 406.95	106.69 ± 277.29	292.96 ± 395.44	0.1554
D-Dimer(0-0.55 mg/L)	2(0.61, 5.06)	1.46(0.59, 4.19)	2.83(0.94, 5.63)	**0.0137**
PT(9.8-12.1s)	12.9(11.98, 14.08)	12.9(12.2, 14)	12.9(12.3, 13.95)	0.4889
APTT(25.3-33.8s)	30.9(28.58, 34.85)	30.5(28.5, 33.3)	31(28.65, 37.9)	0.4752
FIB(1.8-3.5 g/L)	5.74(4.72, 6.38)	5.55(4.77, 6.17)	5.93(4.58, 6.51)	0.6472
TT(14-21s)	15.5(14.23, 16.63)	15.4(14.2, 16.7)	15.3(14.2, 16.3)	0.4481

Table 3 Radiological manifestations of patients with *C. psittaci* pneumonia

Characteristics	*C. psittaci* pneumonia (*n* = 46)	Non severe *C. psittaci* pneumonia (*n* = 19)	Severe *C. psittaci* pneumonia (*n* = 27)	P value
Imaging, n				
Scope of lesions				
Unilateral, Left	10	8	2	**< 0.01**
Unilateral, Right	15	11	4	
Bilateral	21	0	21	
Single lobe	19	15	4	**< 0.01**
Multiple lobar	27	4	23	
Pleural effusions, n				**0.0218**
No pleural effusions	32	17	15	
Unilateral	4	2	2	
Bilateral	10	0	10	

leaf involvement. Patients in the severe group had more bilateral lung lesions(77.8%)than the non-severe group, pleural effusion(44.4%) and multiple lobar lesions(85.2%) were more observed in the severe group(p<0.05). The imaging features during admission are shown in Table 3.

Co-infection of *C. Psittaci* pneumonia patients with other pathogens based on mNGS

In this study, clinical samples identified as *C. psittaci* infection using mNGS included 34 samples of BALF, 5 samples of sputum, and 7 samples of peripheral blood. Among 46 patients,41.3% (19/46) patients had mixed infection, including 7 patients with bacterial infection, 5 patients with viral infection and 3 patients with fungal infection. 2 cases of co infection with bacteria and fungi, and 2 cases of co infection with fungi and viruses. The comprehensive detection rates of bacteria, fungi and viruses were 19.56%(9/46), 15.21%(7/46) and 15.21%(7/46) respectively. mNGS yields a higher positive detection rate for pathogens compared with the culture method. mNGS results indicated that 41.3% of patients (19/46) had suspected coinfections with a coinfection rate of 84.2% (16/19) in the severe group. much higher than that in the non severe group(p<0.05). The most common bacterial co-infection is mainly related to Acinetobacter baumannii. The most common co-infection of virus and fungus were Candida albicans and EB virus(Figure. 1).

Characterization of Lower respiratory tract microbiota in *C. Psittaci* pneumonia

A series of previous studies have shown that imbalance of microbiota in the lung may be closely associated with the development of pulmonary(infectious)

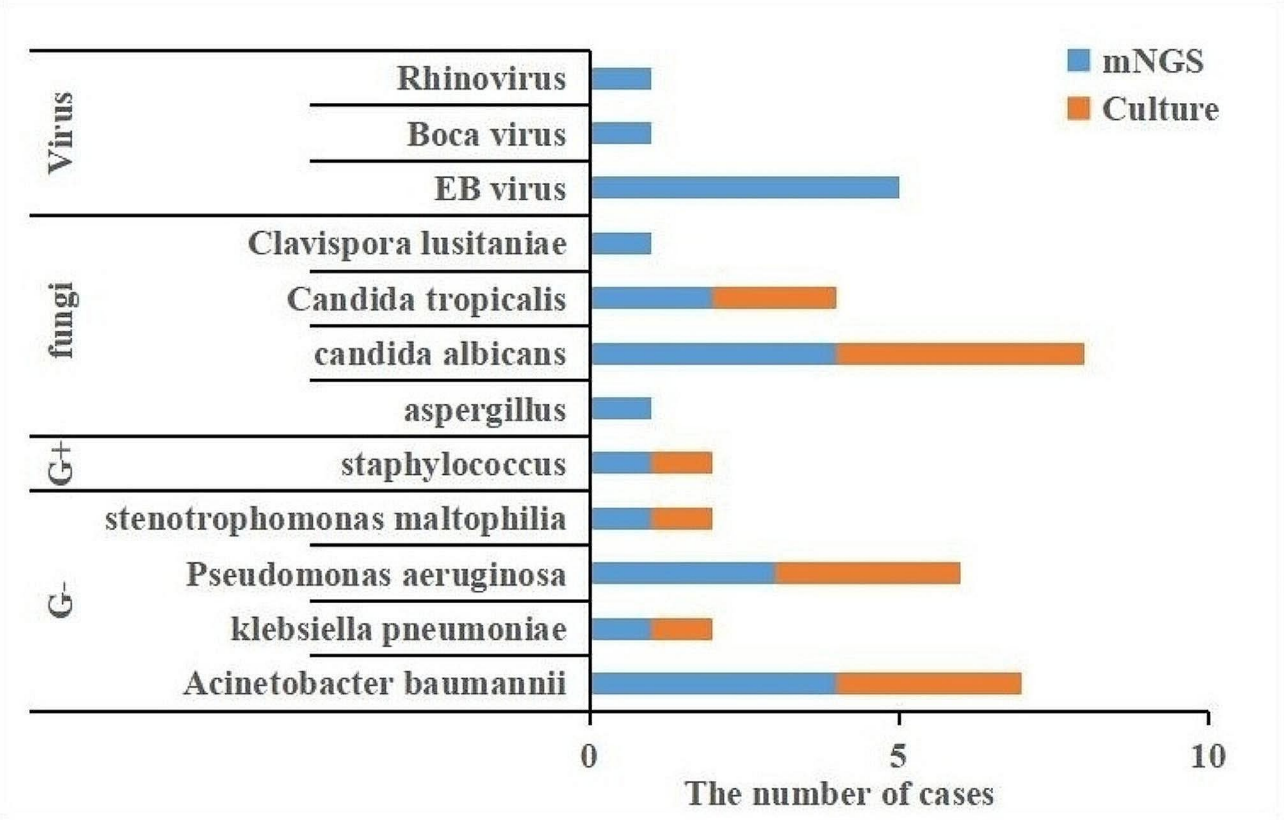

Fig. 1 Distribution of pathogens identifed by mNGS(G+: Gram positive bacteria; G-: Gram negative bacteria)

diseases. Therefore, we also investigated the effects of *C. psittaci* infection on the microbiota of the lower respiratory tract by analyzing the characteristics of the lung microbial communities detected by mNGS. BALF samples are widely used for pathogen detection in infectious diseases of the lower respiratory tract because they carry less oral microbial contamination and can yield accurate and representative lung microbial information. Among the 34 BALF samples, the mNGS fastq data of 7 patients was lost due to improper data storage and therefore not within our statistical range, only the remaining 27 patients were further analyzed. Overall, The relative abundance of the top 10 species are shown in Fig. 2A and B. The major species in the non severe *C. psittaci* group were *primate erythroparvovirus 1*, *Candida albicans* and *Cutibacterium acnes*, major species in the severe *C. psittaci* group was *Clavispora lusitaniae*, *Lacticaseibacillus paracasei* and *Candida glabrata*. In addition, further analysis indicated significant differences in the relative Abundance of *Haemophilus parainfluenzae* (Fig. 2C) and *Streptococcus pneumoniae*(Fig. 2D) in the severe and non severe groups. Subsequently, classical Alpha-diversity Shannon and Simpson index analysis and Principal coordinate analysis (PCoA) showed that there was no significant difference in the diversity of lower respiratory tract microbiota between *C. psittac*i pneumonia and severe *C. psittaci* pneumonia(Fig. 2E-G).

Treatment and outcomes

Treatment and outcomes of patients with *C. psittaci* pneumonia are shown in Table 4. 67.3% patients have a history of empirical antibiotic treatment, Antibiotics that were not active against C. psittaci were administered empirically on admission to the majority of patients with C. psittaci pneumonia. Among these empirical antibiotic treatment patients, 80.43% of patients changed the type of antibacterial drugs according to the results of mNGS. Among the 46 patients, 16 were treated with doxycycline alone, 9 with moxifloxacin alone, 8 with doxycycline and moxifloxacin in combination, 2 with doxycycline and azithromycin in combination, 4 with doxycycline and carbapenems in combination, and 3 with moxifloxacin and carbapenems in combination, another 2 cases were treated with carbapenems. Among 46 patients, 12 patients were treated with invasive mechanical ventilation, 25 patients were treated with nasal catheter, and 3 patients were treated with non-invasive ventilation. Sepsis occurred in 3 patients with severe infection. Finally, 39 patients were cured and discharged, 2 patients gave up treatment, and 3 patients died.The causes of the 3

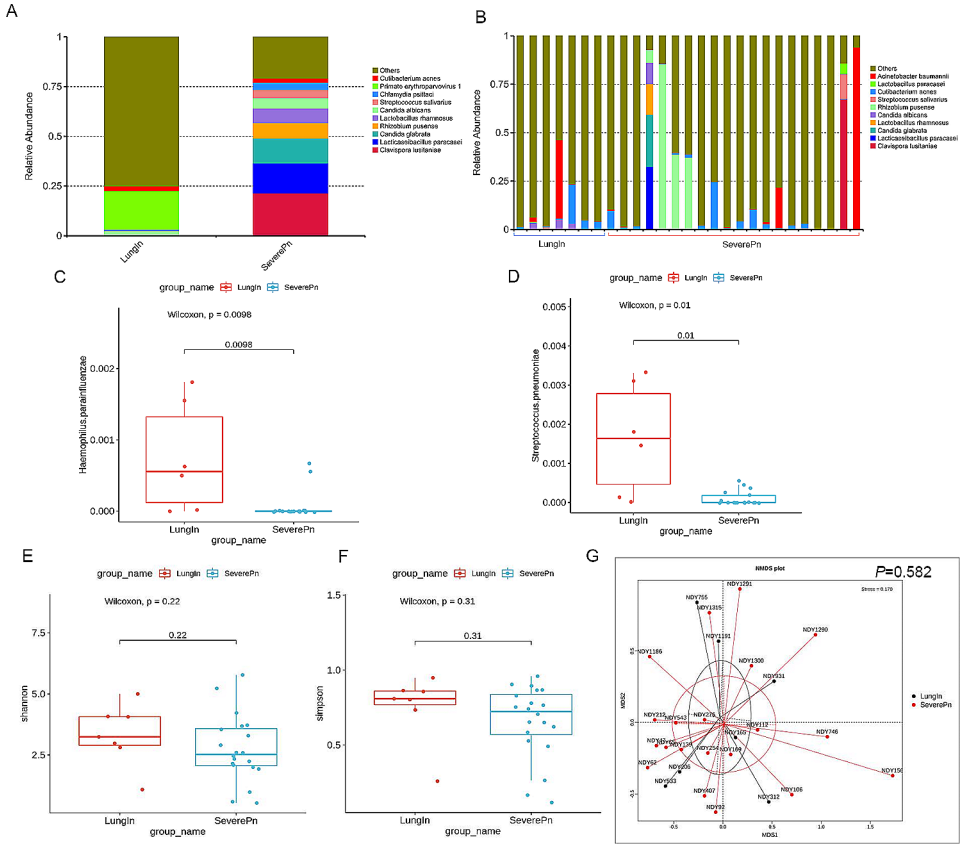

Fig. 2 (**A**) Relative abundance of the 10 most abundant species in *C. psittaci* pneumonia (LungIn)and severe *C. psittaci* pneumonia(SeverePn). (**B**) Composition of microbiome in the different samples. (**C**-**D**) The abundance of *Haemophilus parainfluenzae* and *Streptococcus pneumoniae* in different groups. The boxes represent 25th -75th percentiles, the horizontal lines indicate the median, and the whiskers were drawn from the box to the extremes (values that were lower/greater than first/third quartile minus/plus 1.5 times the interquartile range were regarded as outliers). (**E**-**F**) Alpha diversity differences of lung microbiota communities at species level between groups (Shannon index and Simpson index). (**G**) Principal coordinate analysis plot(PCoA) of samples at the species level. Each dot represents one sample from each group, and the color of the points represents the group in which the sample is located

Table 4 Treatment and outcomes of patients with *C. psittaci* pneumonia

Characteristics	*C. psittaci* pneumonia (*n* = 46)	Non severe *C. psittaci* pneumonia (*n* = 19)	Severe *C. psittaci* pneumonia (*n* = 27)	P value
History of empirical treatment	31	11	20	0.2491
Antimicrobial therapy				0.1395
Tetracycline	16	9	7	
Quinolones	9	4	5	
Combination therapy	21	6	15	
Respiratory support, n				**0.002**
Invasive ventilation	12	0	12	
Non-invasive ventilation	3	0	3	
Oxygen therapy	21	10	7	
High-flow nasal cannula	4	2	6	
No respiratory support	6	6	0	
Outcomes				0.1329
Sepsis, n	3	0	3	
Death, n(%)	3	0	3	

fatalities were Septic shock, Severe pneumonia and Cardiorespiratory failure.

Risk factors associated with severe *C. Psittaci* pneumonia

Multivariate analysis revealed that higher lymphocyte concentrations (OR 0.836, 95% CI 0.714–0.962, $p=0.041$) were the only protective factor for survival(Table 5).

Discussion

In this study, we performed a retrospective analysis of the clinical characteristics of patients with *C. psittaci* pneumonia.The clinical symptoms of these patients were atypical, the major clinical symptoms of *C. psittaci* pneumonia are fever, cough, expectoration. Our results discovered that there was no significant difference in the clinical symptoms between patients with severe *C. psittaci* pneumonia and those non-severe *C. psittaci* pneumonia, which is consistent with a multi-center study of 116 patients with *C. psittaci* pneumonia in Central-South China [21]. But Su et al. reported that the incidence of dyspnea in patients with severe *C. psittaci* pneumonia is higher than that in non severe *C. psittaci* pneumonia patients, and the difference is statistically significant [22]. The majority cases of *C. psittaci* pneumonia have a history of contact with poultry or pigeons, which is also a pivotal clue for the diagnosis of psittacosis. In our study, the main sources of infection were fowl and pigeons, but 14% patients denied any contact history, and the cause of infection remains obscure. Previous studies have investigated that psittacosis can spread among people [23], but fewer than 5% of *C. psittaci* pneumonia cases were community-acquired [23, 24].Our laboratory results indicated that the lymphocyte counts in severe infected patients were lower than that in non severe infected patients, which may reflect a decreased immune function in severe infected patients. In addition, multivariate analysis suggested that higher lymphocyte concentrations were the only survival protective factor for *C. psittaci* pneumonia. Due to the limited number of cases included in this study, whether lymphocyte counts could be used as a predictor of severe disease or death in severe *C. psittaci* pneumonia needs to be further studied [22]. Neutrophils counts, PCT, LDH, HBDH, CK-MB, D-Dimer were higher than that in patients with non severe *C. psittaci* pneumonia, which is consistent with the previous research results of Fang et al [25]. Our results also found that there were 6 patients with severe *C. psittaci* pneumonia complicated with rhabdomyolysis, which represents that it is not uncommon in people with severe *C. psittaci* pneumonia [26].The PCT value in our severe group was higher, indicating mixed infection from multiple pathogens [27]. The mNGS results confirmed the mixed infection findings. In our research, 41.3% (19/46) patients had mixed infection, the coinfections were mostly related to *C. psittaci*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *candida albicans* and *EBV*. Sepsis occurred in 3 patients with severe infection in this study, which may be caused by *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, respectively. Coinfections in patients with *C. psittaci* pneumonia may lead to more adverse outcomes and need further investigation [28]. two patients accompanied by bacterial infection (both pseudomonas aeruginosa) died. Screening for other respiratory pathogens during the clinical course of severe *C. psittaci* pneumonia is critical for appropriate diagnosis and treatment. Empirical antibiotic treatment should be prescribed for patients with severe *C. psittaci* pneumonia, with rapid de-escalation based on mNGS/culture results [29].

Empirical antimicrobial treatment before being diagnosed with *Chlamydia psittaci* infection is usually ineffective [30]. In our study, 67% of patients underwent empirical antimicrobial treatment, while 80.43% of patients changed their antibiotics after a clear diagnosis was made. The most effective antibacterial drugs for *C. psittaci* pneumonia include quinolones, macrolides and tetracyclines, which are first-line antibiotics for the treatment of psittacosis [31]. In our research, 31 patients were treated with doxycycline and 21 patients were treated with moxifloxacin. In a retrospective study on 116 patients with *C. psittaci* pneumonia, researchers have found that the use of quinolones was related to the reduction of days of hospitalization and days of fever after the use of antibiotics [31]. Additionally, some studies also revealed that the MIC of doxycycline was lower than that of ciprofloxacin in vitro experiments [32]. Due to the difficulty in obtaining doxycycline in China, quinolones are often given as the first choice of drugs. Although some clinical cases reflect the efficacy of quinolones in *C. psittaci* infection, there is still lack of clinical study on the efficacy of quinolones in *C. psittaci* infection at present.

In our study, three patients unfortunately passed away. All three patients underwent tracheal intubation and extracorporeal membrane lung surgery. One of the patients had myelodysplastic syndrome for 10 years, and only one pathogen, *C. psittacii*, was detected through mNGS in this patient, while the other two patients were accompanied by bacterial infections (both Pseudomonas aeruginosa), and clinical testing results showed that all three patients were hypoalbuminemia.Yang M's research indicated that higher globulin concentration was a protective factor for survival. Respiratory therapy (including high flow nasal intubation, non-invasive ventilation and

Table 5 Multivariate logistic regression analysis of factors associated with severe *C. psittaci* pneumonia

Variables	Odds ratio	95% confidence interval	P value
Lymphocyte	0.836	0.714–0.962	0.041

invasive ventilation) other than oxygen gas was a risk predictor of severe pneumonia [21], Additionally, patients with septic shock and those who require mechanical ventilation are susceptible to secondary infections, which can lead to death [27]. Our results are just in line with this. However, due to the small number of cases, we lack data to verify this statement.

Changes in the diversity or abundance of pulmonary microbiota are related to a variety of chronic respiratory diseases, such as asthma, cystic fibrosis, Bronchiectasis, and chronic obstructive pulmonary disease [28, 33, 34]. The respiratory microbiota may offer resistance to the colonization of respiratory pathogens which also engages in the maturation and maintenance of respiratory physiology and immune homeostasis. The changes in respiratory microbiota may lead to further disease progression and immune imbalance [35]. Therefore, in this study, we investigated the characteristics of the lower respiratory tract microbiota of patients with *C. psittaci* infection by mNGS. The results manifested that there was no statistical difference in the lower respiratory tract microbiota diversity between *C. psittaci* pneumonia and severe *C. psittaci* pneumonia. The microbial species with altered relative abundance in the lower respiratory tract were different in these two groups. Xie et al. found that although reduced pulmonary microecological diversity occurred in both *Chlamydi*a-infected and non-*Chlamydia*-infected patients, the microbial species with altered relative abundance in the lower respiratory tract were significantly different in two patient groups, suggesting that *Chlamydia* infection shapes the characteristics of the pulmonary microbiota in a unique disease state [36]. All these results may provide a possible research direction for unveiling the pathogenic mechanisms of pulmonary infections caused by *Chlamydia*.

Due to the lack of specific clinical manifestations and conventional laboratory tests, the incidence rate of *C. psittaci* pneumonia may be underestimated, Recently, mNGS has emerged as a novel and promising method for the detection of infectious agents. Based on non-preference and high sensitivity, mNGS provides more sensitive pathogen detection results than conventional culture, especially for rare pathogen detection involved in clinically challenging cases. In addition, mNGS has a broad pathogen spectrum, which has the potential to assist clinicians in the diagnosis and treatment of possible mixed infections.

However, There are still several limitations that should be mentioned in this study. First, we only included 46 confirmed patients with *C. psittaci*, and those with strong clinical suspicion but no evidence of the pathogen were excluded, limited research objectives may result in selection bias. Second, as this is a retrospective study, all patients were diagnosed using mNGS and no other laboratory testing methods such as polymerase chain reaction, serological testing, and pathogen culture were used to confirm the diagnosis. Third, The number of cases is not large enough and there is a lack of test results from other conventional testing methods, so it is not possible to verify the sensitivity and specificity of mNGS for detecting *C. psittaci*. we also analyzed lower respiratory tract microbiota characteristics of patients with *C. psittaci* infection by mNGS. Our research still cannot suggest that *C. psittaci* infection disrupts the dynamic balance of the pulmonary microbiome and further impact disease severity. The effects of *C. psittaci* infection on the clinical symptoms and the course of the disease in patients warrant further studies.

Conclusion

A history of poultry or pigeons exposure could be suggestive of *C. psittaci* pneumonia. *C. psittaci* pneumonia has low clinical incidence and poor clinical specificity, which is easy to cause misdiagnosis and then develop into severe disease, and the overall prognosis is well. Lower respiratory tract infection, especially severe and complex infection, Higher lymphocyte concentrations are protective factors associated with severe *C. psittaci* pneumonia. mNGS has an overall superior detection rate and broader pathogen spectrum than traditional methods and may be particularly useful for concomitant infections. Our findings help to deepen our understanding of the pathogenesis of *C. psittaci* pneumonia, especially Severe *C. psittaci* infections.

Acknowledgements

The authors wish to thank the patient for participating in this study and all the staff members at our institution.

Author contributions

YL, CQ and ZXY contributed equally to this paper and were joint frst authors. All corresponding and frst authors contributed to study concept and design. LLM extracted epidemiological and clinical data. ZXY performed the statistical analyses. CQ and YL co-drafted the initial version of manuscript. All authors provided critical revision of the manuscript and approved the fnal draft for publication. LY and ZR were responsible for the integrity and accuracy of the data and were the guarantor. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All authors read and approved the final manuscript.

Funding

This research was supported by the Science and Technology Project of Jiangxi Provincial Department of Education (grant number:GJJ200220) and the Science and Technology Plan of Jiangxi Provincial Health Commission (grant number: SKJP220212485) and the Jiangxi Province's "Double Thousand Plan"Technology Innovation High end Talent Project (grant number: jsxq2019201102). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability

The datasets generated and/or analysed during the current study are available in the National Center Biotechnology Information BioProject database under accession number PRJNA1018096.

Declarations

Ethics approval and consent to participate
According to the review and comments made by the Ethics Committee of the First Affiliated Hospital of Nanchang University, our research is in line with the exemption type of informed consent and ethics approval that "Using identifiable human body materials or data for research, it is no longer possible to locate the subject, and the research project does not involve personal privacy disclosure or commercial interests". As this is a retrospective Cohort study based on previous clinical diagnosis and treatment results, the Ethics Committee of the First Affiliated Hospital of Nanchang University granted the study exemption status. In addition, we declare that this study is in line with the ethical guidelines of the Declaration of Helsinki, and the patient related data is strictly confidential.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Clinical laboratory, The First Affiliated Hospital of Nanchang University of Nanchang University, Nanchang University, No.17, YongWaiZhengStreet, Nanchang 330006, China

Received: 14 September 2023 / Accepted: 26 February 2024
Published online: 13 March 2024

References

1. Torres A, Chalmers JD, Dela Cruz CS, Dominedò C, Kollef M, Martin-Loeches I, et al. Challenges in severe community-acquired pneumonia: a point-of-view review. Intensive Care Med. 2019;45(2):159–71.
2. Marrie TJ, Peeling RW, Reid T, De Carolis E. Canadian. Chlamydia species as a cause of community-acquired pneumonia in Canada. Eur Respir J. 2003;21(5):779–84.
3. Hogerwerf L, Gier DE, Baan B, Hoek BVANDER. Chlamydia psittaci (psittacosis) as a cause of community-acquired pneumonia: a systematic review and meta-analysis. Epidemiol Infect. 2017;145(15):3096–105.
4. Li N, Li S, Tan W, Wang H, Xu H, Wang D. Metagenomic next-generation sequencing in the family outbreak of psittacosis: the first reported family outbreak of psittacosis in China under COVID-19. Emerg Microbes Infect. 2021;10(1):1418–28.
5. Qu J, Zhang J, Chen Y, Huang Y, Xie Y, Zhou M, et al. Aetiology of severe community acquired pneumonia in adults identified by combined detection methods: a multi-centre prospective study in China. Emerg Microbes Infect. 2022;11(1):556–66.
6. Shen L, Tian XJ, Liang RZ, Cheng Y, Kong XL, He F, et al. Clinical and imaging features of Chlamydia psittaci pneumonia: an analysis of 48 cases in China. Zhonghua Jie He He Hu Xi Za Zhi. 2021;44:886–91.
7. Yang F, Li J, Qi B, Zou L, Shi Z, Lei Y, et al. Clinical symptoms and outcomes of severe Pneumonia caused by Chlamydia psittaci in Southwest China. Front Cell Infect Microbiol. 2022;6(11):727594.
8. Xu W, Wang Q, Li L, Zhu B, Cai Q, Yi X, et al. Case Report: metagenomic next-generation sequencing applied in diagnosing psittacosis caused by Chlamydia psittaci infection. Front Cell Infect Microbiol. 2023;9(13):1249225.
9. Liang Y, Dong T, Li M, Zhang P, Wei X, Chen H, et al. Clinical diagnosis and etiology of patients with Chlamydia psittaci pneumonia based on metagenomic next-generation sequencing. Front Cell Infect Microbiol. 2022;10(12):1006117.
10. Tang X, Wang N, Liu G, Tan H, Li A, Gao M, et al. Psittacosis caused severe community-acquired pneumonia accompanied by acute hypoxic respiratory failure: a multicenter retrospective cohort study from China. BMC Infect Dis. 2023;23(1):532.
11. Nieuwenhuizen AA, Dijkstra F, Notermans DW, van der Hoek W. Laboratory methods for case finding in human psittacosis outbreaks: a systematic review. BMC Infect Dis. 2018;18:1–16.
12. Brown JR, Bharucha T, Breuer J. Encephalitis diagnosis using metagenomics: application of next generation sequencing for undiagnosed cases. J Infect. 2018;76(3):225–40.
13. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44:27–72.
14. Wu X, Li Y, Zhang M, Li M, Zhang R, Lu X, et al. Etiology of severe community-acquired pneumonia in adults based on Metagenomic Next-Generation sequencing: a prospective Multicenter Study. Infect Dis Ther. 2020;9(4):1003–15.
15. Weiner-Lastinger LM, Abner S, Edwards JR, Kallen AJ, Karlsson M, Magill S, et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network, 2015–2017. Infect Control Hosp Epidemiol. 2020;41(1):1–18.
16. Laupland KB, Church DL. Population-based epidemiology and microbiology of community-onset bloodstream infections. Clin Microbiol Rev. 2014;27(4):647–64.
17. Corona A, Bertolini G, Lipman J, Wilson AP, Singer M. Antibiotic use and impact on outcome from bacteraemic critical illness: the BActeraemia Study in Intensive Care (BASIC). J Antimicrob Chemother. 2010;65(6):1276–85.
18. Timsit JF, Ruppé E, Barbier F, Tabah A, Bassetti M. Bloodstream infections in critically ill patients: an expert statement. Intensive Care Med. 2020;46(2):266–84.
19. Zaragoza R, Ramírez P, López-Pueyo MJ. Infección nosocomial en las unidades de cuidados intensivos [Nosocomial infections in intensive care units]. Enferm Infecc Microbiol Clin. 2014;32(5):320–7.
20. Fang X, Mei Q, Fan X, Zhu C, Yang T, Zhang L, et al. Diagnostic value of Metagenomic Next-Generation sequencing for the detection of pathogens in Bronchoalveolar Lavage Fluid in Ventilator-Associated Pneumonia patients. Front Microbiol. 2020;11:599756.
21. Speciale A, Musumeci R, Blandino G, Milazzo I, Caccamo F, Nicoletti G. Minimal inhibitory concentrations and time-kill determination of moxifloxacin against aerobic and anaerobic isolates. Int J Antimicrob Agents. 2002;19(2):111–8.
22. Su S, Su X, Zhou L, Lin P, Chen J, Chen C, et al. Severe Chlamydia psittaci: clinical characteristics and risk factors. Ann Palliat Med. 2021;10(7):8051–60.
23. Zhang Z, Zhou H, Cao H, Ji J, Zhang R, Li W, et al. Human-to-human transmission of Chlamydia psittaci in China, 2020: an epidemiological and aetiological investigation. Lancet Microbe. 2022;3(7):e512–20.
24. de Gier B, Hogerwerf L, Dijkstra F, van der Hoek W. Disease burden of psittacosis in the Netherlands. Epidemiol infection. 2018;146(3):303–5.
25. Fang C, Xu L, Lu J, Tan H, Lin J, Zhao Z. Clinical characteristics of Chlamydia psittaci Pneumonia confirmed by Metagenomic Next-Generation Sequencing. Clin Lab. 2022;1;68(11).
26. Zhang A, Xia X, Yuan X, Liu Y, Niu H, Zhang Y, et al. Severe Chlamydia psittaci Pneumonia complicated by Rhabdomyolysis: a Case Series. Infect Drug Resist. 2022;15:873–81.
27. Chen X, Cao K, Wei Y, Qian Y, Liang J, Dong D, et al. Metagenomic next-generation sequencing in the diagnosis of severe pneumonias caused by Chlamydia psittaci. Infection. 2020;48(4):535–42.
28. Sobieraj DM, Weeda ER, Nguyen E, Coleman CI, White CM, Lazarus SC, et al. Association of inhaled corticosteroids and long-acting β-Agonists as controller and quick relief therapy with exacerbations and symptom control in persistent Asthma. JAMA. 2018;319(14):1485–96.
29. Meijer R, van Biezen P, Prins G, Boiten HJ. Multi-organ failure with necrotic skin lesions due to infection with Chlamydia psittaci. Int J Infect Dis. 2021;106:262–4.
30. Segata N, Haake SK, Mannon P, Lemon KP, Waldron L, Gevers D, et al. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol. 2012;13(6):R42.
31. Ni Y, Zhong H, Gu Y, Liu L, Zhang Q, Wang L, et al. Clinical features, treatment, and Outcome of Psittacosis Pneumonia: a Multicenter Study. Open Forum Infect Dis. 2023;10(2):ofac518.
32. Beeckman DS, Vanrompay DC. Zoonotic Chlamydophila psittaci infections from a clinical perspective. Clin Microbiol Infect. 2009;15(1):11–7.
33. He Y, Li J, Yu W, Zheng Y, Yang D, Xu Y, et al. Characteristics of lower respiratory tract microbiota in the patients with post-hematopoietic stem cell transplantation pneumonia. Front Cell Infect Microbiol. 2022;13:12:943317.
34. Al Bataineh MT, Hamoudi RA, Dash NR, Ramakrishnan RK, Almasalmeh MA, Sharif HA, et al. Altered respiratory microbiota composition and functionality associated with asthma early in life. BMC Infect Dis. 2020;20(1):697.

35. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science. 2012;336(6080):489–93.
36. Xie G, Hu Q, Cao X, Wu W, Dai P, Guo W, et al. Clinical identification and microbiota analysis of Chlamydia psittaci- and Chlamydia abortus- pneumonia by metagenomic next-generation sequencing. Front Cell Infect Microbiol. 2023;13:1157540.

Publisher's Note