Preplanned Studies

Human Infection with *Chlamydia pneumoniae* ST16 — Lishui City, Zhejiang Province, China, 2024

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Summary

What is already known about this topic?

Chlamydia pneumoniae (*C. pneumoniae*) is an important pathogen associated with respiratory infections. In China, *C. pneumoniae* pneumonia is not a notifiable infectious disease and is frequently overlooked in clinical detection protocols for community-acquired pneumonia. Consequently, the prevalence and genotypic distribution of chlamydial infections remain inadequately characterized.

What is added by this report?

We investigated four patients with *C. pneumoniae* pneumonia in Lishui City, Zhejiang Province, China, between April and May 2024. All patients exhibited decreased levels of retinol-binding protein and prealbumin, with two patients presenting with co-infections. Analysis of the 16S rRNA and *ompA* gene sequences demonstrated 98% to 100% homology with known *C. pneumoniae* strains. To further characterize these isolates, we sequenced seven housekeeping genes, which revealed that all four patients were infected with the ST16 sequence type.

What are the implications for public health practice?

Our findings underscore the necessity for enhanced surveillance and research on chlamydial infections, as well as the implementation of next-generation sequencing methodologies to improve pathogen identification, particularly in complex cases involving co-infections.

ABSTRACT

Introduction: This study aims to analyze the clinical characteristics and pathogenic features of *Chlamydia pneumoniae* pneumonia, providing a scientific basis for the diagnosis and treatment of *C. pneumoniae* infections.

Methods: Clinical data from four patients diagnosed with *C. pneumoniae* pneumonia in Lishui City, Zhejiang Province, between April and May 2024, were collected to analyze clinical manifestations and pathogenic findings. Multi-locus sequence typing (MLST) analysis of the pathogen was conducted using seven housekeeping genes.

Results: All patients exhibited decreased levels of retinol-binding protein and prealbumin, findings not previously reported in earlier studies. Additionally, co-infections were identified in two cases. Analysis of the 16S rRNA and *ompA* gene sequences indicated a homology of 98% to 100% with known *C. pneumoniae* strains. To further characterize these strains, sequencing of the seven housekeeping genes confirmed that all cases were infected with the ST16 genotype.

Conclusions: *C. pneumoniae* infections in Lishui City are predominantly caused by the ST16 genotype, highlighting the need for enhanced research into these infections. The decrease in retinol-binding protein and prealbumin levels may serve as auxiliary diagnostic biomarkers in clinical practice. Next-generation sequencing methods demonstrate significant potential for pathogen identification, particularly in diagnosing co-infections.

Humans are the only known reservoir of *Chlamydia pneumoniae* (*C. pneumoniae*), a gram-negative, obligate intracellular bacterium. This pathogen represents a common etiological agent of community-acquired pneumonia, with diagnosis and treatment frequently delayed due to its presentation with atypical clinical manifestations. Beyond respiratory infections, emerging evidence suggests associations between *C. pneumoniae* and various pathological conditions, including asthma, atherosclerosis, and Alzheimer disease (*1*).

In China, neither *C. pneumoniae* pneumonia nor *C. psittaci* pneumonia are classified as notifiable infectious diseases, and these pathogens are not routinely included in standard clinical diagnostic

panels for respiratory infections. Consequently, the epidemiological prevalence and genotypic distribution of chlamydial infections remain inadequately characterized. To date, only a single serovar of *C. pneumoniae* has been identified. Previously, our research team investigated the prevalence of *C. psittaci* infections in Lishui, revealing the presence of a novel *C. psittaci* strain responsible for local infections (*2*). In the current study, to elucidate the prevalence and genotypic characteristics of *C. pneumoniae* in Lishui, we conducted comprehensive clinical and laboratory investigations of four cases of severe community-acquired pneumonia.

In this study, an analysis was conducted for consecutive cases of *C. pneumoniae* infection in patients admitted to Lishui People's Hospital of Zhejiang Province between April 23 and May 14, 2024. Nested polymerase chain reaction targeting Chlamydia-specific genes of 16s rRNA and outer membrane protein A (*ompA*) were amplified. To further characterize the *C. pneumoniae* isolates through multi-locus sequence typing (MLST), we employed nested polymerase chain reaction methods to sequence seven housekeeping genes (*enoA*, *fumC*, *gatA*, *gidA*, *hemN*, *hflX*, and *oppA*) (*3*). Targeted next-generation sequencing (tNGS) was utilized for pathogen diagnosis in patients 2 and 3. Additionally, we attempted to obtain the complete genome sequence of *C. pneumoniae* using the NGS-based hybrid capture method.

The study cohort comprised three females and one male, with a median age of 22 years (range: 14–36 years). Medical records indicated that three patients presented with high fever. All four patients were hospitalized with cough, with three exhibiting productive sputum. Only one patient reported chills and myalgia. Computed tomography (CT) chest scans revealed unilateral pulmonary inflammation in all patients. Hematological analysis demonstrated markedly decreased retinol-binding protein and prealbumin levels across all four cases. Three patients exhibited elevated plateletcrit values. Additional abnormal laboratory parameters were observed in two patients, including neutrophilia, elevated high-sensitivity C-reactive protein, hyperfibrinogenemia, increased d-dimer, prolonged activated partial thromboplastin time, and elevated total bile acids, concurrent with lymphopenia and hypoalbuminemia. Serological testing for IgM antibodies against *Legionella pneumophila* and *Mycoplasma pneumoniae* yielded negative results in three patients. Throat swab specimens from two patients were analyzed for common respiratory pathogens, including influenza virus, parainfluenza virus, metapneumovirus, bocavirus, respiratory syncytial virus, coronavirus, rhinovirus, *M. pneumoniae*, and adenovirus. Patient 4 tested positive for adenovirus, while no other pathogens were detected in any patient. All patients recovered following treatment with azithromycin or doxycycline and were subsequently discharged.

tNGS of bronchoalveolar lavage fluid samples from Patients 2 and 3 identified *C. pneumoniae* in Patient 2 (10,681 reads), while Patient 3 demonstrated a polymicrobial infection with *C. pneumoniae* (87 reads), *Haemophilus influenzae* (35,231 reads), and human adenovirus 5 (157 reads) (Table 1). Sanger sequencing confirmed *C. pneumoniae* infection, with 99%–100% homology to known *C. pneumoniae* 16S rRNA sequences and 98%–100% homology to the *ompA* gene. The sequences were deposited in GenBank (accession numbers PQ510372-PQ510375 for 16S rRNA, PQ522244-PQ522247 for *ompA*). Complete *C. pneumoniae* genomic sequences were attempted to be obtained using NGS-based hybrid capture methodology. Only Patient 4 yielded a sufficient genomic draft (GenBank BioProject PRJNA1177653), with phylogenetic analysis demonstrating close clustering with the prototype strain AR39 (Figure 1). MLST analysis revealed that all four patients were infected with sequence type 16 (ST16) (*enoA*:5, *fumC*:4, *gatA*:4, *gidA*:6, *hemN*:3, *hflX*:5, and *oppA*:7).

DISCUSSION

In this study, we identified *C. pneumoniae* infection in four patients with severe pneumonia from Lishui City, Zhejiang Province, China. Analysis of the 16S rRNA and *ompA* gene sequences demonstrated 98% to 100% homology with known strains of *C. pneumoniae*. To further characterize the molecular epidemiology of these isolates, we sequenced seven housekeeping genes for MLST. Results revealed that all four patients were infected with the ST16 genotype. Based on currently available sequencing data for *C. pneumoniae*, MLST classification encompasses four distinct sequence types: ST16, ST17, ST18, and ST215. Among these, ST215 is exclusively derived from Australian koalas and *Perameles bougainville*, while the remaining three sequence types (ST16, ST17, and ST18) are associated

TABLE 1. Demographic and basic clinical features of the four patients.

Characteristics	Case #1	Case #2	Case #3	Case #4
Age (years)/sex	28/Female	36/Female	16/Male	14/Female
Signs/symptoms	37.1 ℃ Productive cough	37.5 ℃ Chills, Myalgia Productive cough	36.5 ℃ Productive cough	38.5 ℃ Fever, Cough Moist rales
IgM detection*	(−)	(−)	/	(−)
Nucleic acid detection of common respiratory pathogens†	/	(−)	/	Adenovirus (+)
tNGS	/	*C. pneumonia* (10,681 reads)	*C. pneumonia* (87 reads) *Haemophilus influenzae* (35,231 reads) Human Adenovirus 5 (157 reads)	/
Blood test	↑ (APTT, D-dimer, FIB, PCT, TBA) ↓ (PA, RBP)	↑ (D-dimer, FIB, hs-CRP, NEUT, PCT) ↓ (ALB, LYM, PA, RBP)	↑ (PCT, TBA) ↓ (ALB, PA, RBP)	↑ (ADA, APTT, AST, β-2-MG, CK, HBDH, hs-CRP, LDH, NEUT) ↓ (LYM, PA, RBP)

Note: "/" means the test was not performed; (−) means a negative result; ↑ and ↓ means increased and decreased test values, respectively.
Abbreviation: *C. pneumoniae=Chlamydia pneumoniae*; tNGS=Targeted next-generation sequencing; ADA=Adenosine Deaminase; ALB=Albumin; APTT=Activated partial thromboplastin time; AST=Aspartate aminotransferase; β-2-MG=Beta-2-microglobulin; CK=Creatine Kinase; FIB=Fibrinogen; HBDH=Alpha hydroxybutyrate dehydrogenase; hs-CRP=Hypersensitive C-reactive protein; LDH=Lactate dehydrogenase; LYM=Lymphocyte; NEUT=Neutrophil; PA=Prealbumin; PCT=Plateletcrit; RBP=Retinol-binding protein; TBA=Total bile acid.
* Simultaneous testing for IgM antibodies against *Legionella pneumophila* and *Mycoplasma pneumoniae*.
† Common respiratory pathogens include influenza virus, parainfluenza virus, metapneumovirus, bocavirus, respiratory syncytial virus, coronavirus, rhinovirus, *Mycoplasma pneumoniae*, and adenovirus.

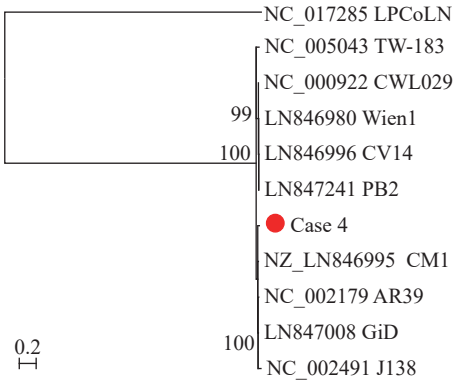

FIGURE 1. Phylogenetic analysis of *C. pneumoniae*. The phylogenetic data were obtained through the alignment of concatenated core genes with the recombination region removed.

with human infections (*4*–*5*).

All four patients with *C. pneumoniae* pneumonia initially presented with cough and fever of unknown origin, with productive cough observed in three cases. CT scans revealed unilateral pneumonia in all patients. Laboratory analyses demonstrated consistent abnormalities across all cases, notably decreased levels of retinol-binding protein and prealbumin. This finding represents a novel clinical observation not previously documented in the literature on *C. pneumoniae* infection (*6*–*8*). Both retinol-binding protein and prealbumin are hepatically synthesized proteins involved in vitamin A transport, and alterations in their concentrations serve as indirect indicators of systemic inflammatory responses (*9*–*10*). Therefore, monitoring these biomarkers in patients with *C. pneumoniae* pneumonia may provide valuable clinical insights, enabling physicians to better assess disease severity and potentially offering actionable biomarkers for clinical management.

C. pneumoniae frequently occurs as a co-infecting pathogen in pneumonia cases. In our cohort, two patients exhibited co-infections: one with a dual pathogen profile (adenovirus) and another with a triple pathogen profile (adenovirus and *H. influenzae*). Such co-infections typically present greater therapeutic challenges, underscoring the critical importance of accurate pathogen identification for targeted intervention. In this context, tNGS represents an increasingly valuable diagnostic modality for severe clinical infections, particularly for identifying complex co-infections. However, standard hospital pathogen detection protocols rarely include *C. pneumoniae*, highlighting how tNGS implementation could substantially enhance detection rates for this pathogen.

In conclusion, the epidemiology and predominant sequence types of *C. pneumoniae* in China remain inadequately characterized. While our findings

contribute meaningful data to the existing literature, the limited sample size necessitates further comprehensive investigations into *C. pneumoniae* and other chlamydial infections.

Conflicts of interest: No conflicts of interest.

Ethical statement: The study was approved by the Ethics Committee of the National Institute of Communicable Disease Prevention and Control, China CDC (Approval no. ICDC-202115).

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