ORIGINAL RESEARCH

Atypical Pathogen Distribution in Chinese Hospitalized AECOPD Patients: A Multicenter Cross-Sectional Study

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Purpose: The proportion of atypical pathogens in patient with AECOPD within mainland China is unknown. The objectives of this study were to determine the distribution of atypical pathogens among Chinese patients with AECOPD, to evaluate the clinical characteristics of different atypical pathogen infections, and to compare different detection methods for atypical pathogens.

Patients and Methods: Specimens were collected from patients with AECOPD from March 2016 to November 2018 at eleven medical institutions in eight cities in China. Double serum, sputum, and urine samples were obtained from 145 patients. Serological and nucleic acid tests were used to assess for *Mycoplasma pneumonia* and *Chlamydia pneumoniae*; serological, urinary antigen, and nucleic acid tests were applied to detect *Legionella pneumophila*. The clinical characteristics of atypical pathogen-positive and -negative groups were also compared.

Results: The overall positivity rate for *Mycoplasma pneumoniae* was 20.69% (30/145), with the highest rate being 20.00% (29/145) when determined by passive agglutination.The overall positive rates for *Chlamydia pneumoniae* and *Legionella pneumophila* were 29.66% (43/145) and 10.34% (15/145), respectively. The most common serotype of *Legionella pneumophila* was type 6. The maximum hospitalized body temperature, ratio of eosinophils, C-reactive protein (CRP) level, and procalcitonin (PCT) level of the *Mycoplasma pneumoniae*-positive group were significantly higher than those of the *Mycoplasma pneumoniae*-negative group. Patients in the *Chlamydia pneumoniae*-positive group smoked more, had higher proportions of comorbidities and frequent aggravations in the previous two years than those in the *Chlamydia pneumoniae*-negative group. Furthermore, the forced expiratory volume in one second to forced vital capacity (FEV_1/FVC) ratio assessment of lung function was higher, and the concentration of arterial blood bicarbonate (HCO_3^-) was lower in the *Legionella pneumophila*-positive group than in the *Legionella pneumophila*-negative group.

Conclusion: Overall, atypical pathogens play an important role in AECOPD. Regarding the testing method, serological testing is a superior method to nucleic acid testing.

Keywords: COPD, exacerbations, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila*

Introduction

Chronic obstructive pulmonary disease (COPD) is a common chronic airway disease that poses a serious health risk to humans.[1] Acute exacerbations of COPD (AECOPD) result in dramatic deterioration of lung function and significantly

decreased life quality and are clearly associated with a high patient mortality rate.[2,3] Respiratory tract infections are the most common cause of AECOPD.[4] Common atypical pathogens associated with respiratory infections include *Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Legionella pneumophila*. In clinical practice, atypical pathogen infections are usually diagnosed indirectly by serological antibody testing and nucleic acid testing of secretions (sputum) without culturing these pathogens. Thus, although the etiological factors of AECOPD have been discussed in many studies, the prevalence of atypical pathogens in patients with AECOPD varies considerably among studies.[5–7] Furthermore, few studies have evaluated the prevalence of atypical pathogens among patients with AECOPD in mainland China, which poses a challenge for selecting empirical treatment for these pathogens. In this study, specimens (serum and sputum) were prospectively collected from patients AECOPD from multiple centers, and serological and nucleic acid tests were performed. The distribution of atypical pathogens in patients with AECOPD in mainland China was determined, and the detection methods for these pathogens were compared.

Patients and Methods

Data from patients with AECOPD were collected from eleven medical institutions in eight cities in China during the period from March 2016 to November 2018. The inclusion and exclusion criteria are as follows:

Inclusion Criteria

1. Patients meeting the diagnostic criteria for COPD;
2. Patients in the acute exacerbation stage of COPD requiring hospitalization; and
3. Complete collection of eligible sputum specimens and two serum specimens (14 ± 4 days interval).

Exclusion Criteria

1. Women who were pregnant or breastfeeding;
2. Patients with bronchiectasis;
3. Patients with active tuberculosis;
4. Patients with aspiration pneumonia or obstructive pneumonia;
5. Patients with a history of hospitalization 2 weeks before the onset of the illness (as the infection at the time of admission may be a hospital-acquired infection);

6. Human immunodeficiency virus (HIV)-positive patients (not required to be tested for HIV)
7. Patients who could not and/or did not understand and/or implement the investigation protocol; and
8. Patients with pneumonia.

This study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University.All study participants signed informed consent prior to commencement of the study and gave consent to have their data published.

Demographic and Clinical Data

The following patient information was collected: 1) basic information: sex, age, body mass index, primary vital signs (including temperature, heart rate, and respiration) on admission, and care unit admission status; 2) incident information: time (days) from the incident to hospital admission, history of suspicious environmental exposures (eg, air conditioning, bathing, rain, tree watering, or oral treatment), travel history 2 weeks before the incident, and details on the prevalence of influenza in the community 2 weeks before the incident; 3) past history: smoking history, drinking history, history of concomitant diseases, usage of long-term inhaled or oral hormone therapy, number of acute exacerbations in the previous two years, lung function (prior six months), COPD assessment test findings, and results of the British modified Medical Research Council (mMRC) scale; 4) laboratory tests: blood gas analysis after hospitalization, routine blood tests, C-reactive protein (CRP), procalcitonin (PCT) and related biochemical tests [eg, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin (ALB), blood urea nitrogen, serum electrolytes, lactate dehydrogenase (LDH), and creatine kinase (CK)]; and 5) clinical data: mechanical ventilation (including noninvasive ventilation and invasive mechanical ventilation), days of hospitalization, and transfer on the third day of hospitalization.

Microbiology Research
Sputum Specimens
After admission, spontaneous sputum was collected in a routine manner with or without the use of expectorant. Sputum specimens were collected before antibiotics were administered in the hospital. The collected sputum specimens were included in the study only if they met the

standards of Gram staining (<10 epithelial cells and >25 polymorphonuclear leukocytes). A polymerase chain reaction (PCR) method was applied to detect DNA fragments of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* in these specimens.

Paired Serological Specimens

Serum was collected on admission and 2 weeks (14 ± 4 days) after the initial serum collection for two paired serological examinations.

Urine Specimens

Morning urine specimens were collected from patients at the time of admission to test for urine antigens of *Legionella pneumophila*.

Detection of Atypical Pathogens

QIAamp@DNAmini kit (Qiagen, Germany) was used to extract genomic DNA from the sputum specimens included in the study. DNA fragments of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* were detected by PCR using Roche's *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* (both *Legionella pneumophila* and non-*Legionella pneumophila*) kits. Specifically, the 89-bp fragment of the repMp1 gene was detected for *Mycoplasma pneumoniae*, the 85-bp fragment of the major outer membrane protein gene was detected for *Chlamydia pneumoniae*, and the 162-bp fragment of the 16S RNA gene was detected for *Legionella pneumophila*. The result determination criteria are shown in Table 1.

Paired serological specimens included in the study were tested to detect serological antibodies for atypical pathogens.

Serum *Mycoplasma pneumoniae* antibodies were detected using a Serodia®-Myco II *Mycoplasma pneumoniae* antibody test kit (Fujirebio, Japan) (passive agglutination method). The RIDASCREEN® *Mycoplasma pneumoniae* IgM kit (R-Biopharm AG, Germany) (enzyme

Table 1 The Result Determination Criteria of PCR

Result	Sample	Internal Reference	Negative Control
Negative	No amplification	Detectable	Negative
Positive	Amplification Cp < 37		Negative

Notes: If the internal reference is not detected, it indicates PCR failure; If the negative control is positive, it indicates PCR contamination.

immunoassay) was used for the detection of *Mycoplasma* IgM antibodies.[8]

Serum *Chlamydia pneumoniae* IgG antibodies were detected using a *Chlamydia pneumoniae* microimmuno-fluorescence kit (FOCUS, USA) (microimmunofluorescence (MIF) assay).

Serum *Legionella pneumophila* IgA, IgM, and IgG antibodies (indirect fluorescent antibody types 1–14) were detected using a *Legionella pneumophila* antibody IgA/IgG/IgM test kit (EUROIMMUN MedizinischeLabordiagnostika AG, Germany). Serum *Legionella pneumophila* IgM antibodies were detected using a *Legionella pneumophila* antibody IgM ELISA test kit (EUROIMMUN MedizinischeLabordiagnostika AG, Germany).[9] The BINAX NOW® Legionella Urinary Antigen Card kit was used to detect *Legionella pneumophila* urinary antigens.

Positive Result Determination Criteria

Mycoplasma pneumoniae: The result is considered to be positive when 1) the antibody titer in the second serum sample is four times higher or lower than that in the first serum sample, 2) IgM is positive, or 3) the nucleic acid test is positive.[10–12]

Chlamydia pneumoniae: The result is considered to be positive when 1) the IgG antibody titer in the second serum sample is four times higher or lower than that in the first serum sample or 2) the nucleic acid test is positive.[13–16]

Legionella pneumophila: The result is considered to be positive when 1) the antibody titer in the second serum sample is four times higher or lower than that in the first serum sample, 2) IgM is positive, 3) the nucleic acid test is positive, or 4) *Legionella pneumophila* urinary antigen is positive.[17,18]

Statistical Methods

Statistical analysis was performed using the statistical software SPSS 17.0. Categorical variables are expressed as percentages, and numerical variables are expressed as the mean ± standard deviation. Continuous variables and categorical variables were evaluated by an independent samples *t*-test and the Chi-square test, respectively, to identify differences among groups. A p-value < 0.05 was considered statistically significant.

Results

Basic Information of Patients with AECOPD

During the study, a total of 366 patients with AECOPD were admitted to the eleven centers, and 145 patients with

AECOPD were included in the study according to the inclusion and exclusion criteria. The general demographic data are shown in Table 2.

Overall Positive Rate for Atypical Pathogens in Patients with AECOPD

The overall positive rate for *Mycoplasma pneumoniae* was 20.69% (30/145), with the highest positive rate being 20.00% (29/145) when determined by passive agglutination. The overall positive rate for *Chlamydia pneumoniae* was 29.66% (43/145). The overall positive rate for *Legionella pneumophila* was 10.34% (15/145), and the most common serotype was type 6. The specific results for atypical pathogens detected by various methods are shown in Table 3. The serotype results for *Legionella pneumophila* are shown in Table 4.

Clinical Characteristics of Patients Who Tested Positive for Atypical Pathogens

The clinical characteristics of patients who tested positive for *Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Legionella pneumophila* are shown in Table 5.

Discussion

A total of 366 patients were initially included in this study. To improve the reliability of the results of atypical pathogens, only patients with paired serum samples (one obtained on admission and one obtained 2 weeks (14 ± 4 days) later) were included. However, more than half of this initial patient group (221 patients) did not have a second serum sample (14±4 days) and were thus excluded. There were three main reasons for this: 1) Many patients with mild disease did not stay in hospital for 10 days. 2) Chinese medical insurance policies require that the length

Table 3 The Specific Results of Atypical Pathogens Detected by Various Methods

Mycoplasma pneumoniae	
Detection method	**Positive rate (positive/ total)**
Passive agglutination	20.00%(29/145)
IgM(enzyme immunoassay)	2.75%(4/145)
PCR(sputum)	1.75%(2/114)
Total	20.69%(30/145)
Chlamydia pneumoniae	
Detection method	**Positive rate (positive/ total)**
Microimmunofluorescence assay(MIF)	29.66%(43/145)
PCR(sputum)	0%(0/114)
Total	29.66%(43/145)
Legionella pneumophila	
Detection method	**Positive rate (positive/ total)**
Indirect fluorescent	10.34%(15/145)
IgM(ELISA)	0.69%(1/145)
PCR(sputum)	0%(0/115)
Urinary Antigen	0%(0/145)
Total	10.34%(15/145)

of stay cannot be too long (once patients have improved, they may return to local hospitals for further treatment). 3) Some patients declined to have blood collected again after they improved. Thus, the selected patients may have had worse lung function (mean forced expiratory volume in one second (FEV1), 41.81%) and longer hospitalization

Table 4 Frequency of the Various *Legionella pneumophila* Serotype Identified in the 11 Hospital Admissions

Legionella pneumophila	N(%)
Legionella pneumophila-6	9 (60.00%)
Legionella pneumophila-14	5 (33.33%)
Legionella pneumophila-1	4 (26.67%)
Legionella pneumophila-7	3 (20.00%)
Legionella pneumophila-8	3 (20.00%)
Legionella pneumophila-10	3 (20.00%)
Legionella pneumophila-12	3 (20.00%)
Legionella pneumophila-4	2 (13.33%)
Legionella pneumophila-11	2 (13.33%)
Legionella pneumophila-2	1 (6.67%)
Legionella pneumophila-5	1 (6.67%)
Two serotypes positive	3 (20.00%)
≧three serotypes positive	6 (40.00%)

Table 2 General Demographic Data

Clinical Characteristics	
Number of patients	145
Age in years(Mean±SD)	74.35±10.07
Gender (male)	70.34%
BMI(kg/m^{2})(Mean±SD)	22.58±4.12
Smoker	66.21%
Spirometry(post)	
FEV$_1$% pred(Mean±SD)	41.81±19.31
FVC % pred(Mean±SD)	59.57±20.21
FEV$_1$/FVC(Mean±SD)	51.75±14.35
Comorbidities	63.45%

Table 5 The Clinical Characteristics of Patients Tested Positive for for the Three Atypical Pathogens

Characteristics	Mycoplasma pneumoniae-Positive (n=30)	Mycoplasma pneumoniae-Negative (n=115)	P value	Chlamydia pneumoniae-Positive (n=43)	Chlamydia pneumoniae-Negetive (n=102)	P value	Legionella pneumophila-Positive (n=15)	Legionella pneumophila-Negetive (n=130)	P value
Age (years)	76.10±9.32	73.89±10.25	0.280	74.09±8.90	74.46±10.57	0.844	74.60±9.07	74.32±10.21	0.919
Sex, Males:Female	23:7	79:36	0.395	29:14	73:29	0.619	10:15	92:130	0.742
BMI (kg/m^2)	22.92±4.52	22.48±4.02	0.236	21.78±3.97	22.92±4.16	0.135	22.81±3.41	22.55±4.20	0.823
Height(cm)	161.48±31.82	164.42±17.56	0.221	166.02±7.13	166.20±8.42	0.903	167.07±6.58	166.04±8.20	0.641
Vital signs									
Maximum body temperature in hospital(°C)	37.38±0.90	37.01±0.57	0.001**	37.09±0.75	37.09±0.63	0.988	37.19±0.65	37.08±0.67	0.552
Pulse(Mean±SD)	90.80±14.59	88.91±13.57	0.374	89.26±14.60	89.32±13.46	0.755	92.87±15.62	88.89±13.53	0.291
Rate(Mean±SD)	20.93±2.12	21.15±2.706	0.319	21.21±2.69	21.06±2.56	0.752	21.00±2.04	21.12±2.65	0.875
Proportion of patients admitted to intensive care unit	13.33%	15.65%	0.753	18.60%	13.73%	0.454	13.33%	15.38%	0.834
Days from onset to admission	8.67±7.25	14.64±22.15	0.066	13.14±13.81	13.49±22.31	0.924	9.93±7.34	13.79±21.06	0.484
Smokers	66.67%	66.96%	0.976	65.11%	66.67%	0.857	66.67%	66.15%	0.968
Tobacco quantity(pack per year)	52.02±32.78	46.51±31.16	0.689	62.33±40.11	44.10±25.60	0.036*	44.11±22.52	50.10±32.41	0.591
Comorbidities	66.67%	62.61%	0.620	80.95%	57.43%	0.007**	46.67%	66.41%	0.131
Number of acute exacerbations in the past year	1.14±1.19	1.55±1.19	0.735	1.28±1.24	1.54±1.17	0.276	1.31±1.11	1.48±1.21	0.618
Number of acute exacerbations in the past year≥2	40.00%	46.09%	0.247	34.21%	55.91%	0.024*	38.46%	50.85%	0.397
Spirometry(post)									
FEV$_1$% pred	36.43±19.67	43.84±18.96	0.649	38.81±15.86	43.39±19.45	0.376	53.56±27.13	40.60±18.20	0.091
FVC % pred	51.90±18.86	62.12±20.15	0.880	60.34±17.92	59.35±20.96	0.863	65.05±27.36	59.07±19.63	0.491
FEV$_1$/FVC	49.15±16.79	52.84±13.24	0.244	51.72±11.22	51.76±15.21	0.993	62.95±3.30	50.80±14.54	0.000**
Proportion of long-term inhaled or oral glucocorticoid therapy	30.00%	30.43%	0.963	32.35%	35.29%	0.645	26.67%	30.77%	0.743
CAT	25.53±6.51	25.98±6.68	0.601	25.21±7.03	26.20±6.49	0.422	28.13±4.07	25.65±6.85	0.051
mMRC	3.10±0.10	2.91±1.11	0.262	2.98±1.02	2.94±1.11	0.861	2.80±0.86	2.97±1.11	0.570
Blood gas									
PH	7.40±0.05	7.40±0.07	0.435	7.41±0.06	7.40±0.07	0.399	7.41±0.05	7.40±0.07	0.630
PaCO$_2$ in mmHg	47.72±15.75	49.88±15.50	0.933	48.56±15.86	49.84±15.44	0.664	43.49±14.87	50.15±15.51	0.130
HCO$_3^-$ in mmol/L	29.92±6.09	31.64±16.87	0.440	29.79±7.02	30.21±6.74	0.752	26.33±5.63	30.47±6.82	0.044*
PaO$_2$/FiO$_2$	293.28±117.16	288.28±98.19	0.537	229.20±124.89	268.42±118.95	0.127	179.27±120.90	261.17±120.41	0.068
Lactate(mol/L)	1.15±0.52	1.35±0.87	0.385	1.23±0.66	4.16±20.08	0.428	1.10±0.33	3.30±16.84	0.714

(Continued)

Table 5 (Continued).

Characteristics	Mycoplasma pneumoniae-Positive (n=30)	Mycoplasma pneumoniae-Negetive (n=115)	P value	Chlamydia pneumoniae-Positive (n=43)	Chlamydia pneumoniae-Negetive (n=102)	P value	Legionella pneumophila-Positive (n=15)	Legionella pneumophila-Negetive (n=130)	P value
Routine blood test									
White blood cell (*10^9/L)	8.12±3.62	13.78±41.99	0.302	8.59±3.80	9.96±10.70	0.427	8.07±3.24	13.12±39.50	0.622
Neutrophil(*10^9/L)	6.31±3.07	7.15±5.56	0.306	7.72±7.74	6.67±3.56	0.269	5.82±2.70	7.12±5.35	0.358
Neutrophilt(%)	74.05±14.29	73.28±14.03	0.789	72.00±14.12	74.04±14.04	0.43	72.21±14.95	73.58±13.99	0.721
Eosinophilst(%)	3.43±7.91	1.40±1.85	0.001**	1.86±1.88	1.81±4.64	0.955	5.27±10.88	1.42±1.86	0.192
Red blood cell(*10^{12}/L)	4.40±0.72	4.89±0.85	0.813	4.42±0.76	4.42±0.74	0.982	4.03±1.41	4.43±0.73	0.289
Hemoglobin(g/L)	133.04±20.66	132.61±24.64	0.648	132.70±19.04	132.70±25.72	1.00	125.53±28.11	133.55±23.31	0.220
Hematocrit(%)	40.58±8.08	40.67±8.07	0.864	40.30±8.29	40.74±8.06	0.779	37.57±9.27	40.97±7.91	0.138
Hematocrit>50%	6.67%	9.57%	0.835	13.15%	8.42%	0.406	0%	10.92%	0.193
C-reactive protein(mg/dl)	35.74±64.95	25.48±31.42	0.048*	23.06±26.04	30.19±46.51	0.432	33.79±30.94	27.52±40.98	0.694
PCT(ng/mL)	3.21±11.00	0.21±0.95	0.000**	1.43±7.65	0.69±4.07	0.507	4.65±14.54	0.56±3.51	0.398
ALT(U/L)	17.91±12.23	29.85±63.77	0.217	20.32±17.21	30.37±67.06	0.352	17.02±10.33	28.63±60.32	0.475
AST(U/L)	23.25±11.38	28.79±28.30	0.126	27.53±32.43	27.73±22.62	0.967	24.38±13.61	28.01±26.81	0.632
ALB(g/L)	35.18±6.17	38.57±24.73	0.623	41.58±40.76	36.40±5.73	0.453	36.75±7.01	35.96±5.91	0.680
LDH(U/L)	172.72±52.54	226.12±116.33	0.051	223.19±116.33	19.57±105.07	0.291	169.09±44.21	210.79±113.43	0.231
BUN(mmol/L)	7.01±2.79	7.32±4.15	0.480	8.19±5.64	6.78±2.51	0.135	6.94±2.26	7.30±4.07	0.777
Na(mmol/L)	140.30±4.97	139.59±5.27	0.669	139.44±6.88	139.86±4.35	0.666	140.46±4.50	139.66±5.28	0.583
K(mmol/L)	4.14±0.55	4.04±0.54	0.597	3.98±0.59	4.10±0.52	0.267	4.02±0.42	4.07±0.55	0.755
Cl(mmol/L)	100.44±3.75	99.06±7.34	0.045*	99.07±9.76	99.46±5.08	0.810	100.24±4.73	99.24±6.97	0.604
Fasting blood glucose (mmol/L)	6.03±1.82	7.84±8.59	0.173	7.31±3.56	7.56±8.94	0.873	6.15±1.66	7.61±7.91	0.565
CK(U/L)	59.33±34.12	140.20±527.88	0.239	70.65±46.69	73.54±80.34	0.853	64.67±46.47	73.38±72.15	0.725
D-D(ug/L)	1.70±3.45	1.39±2.76	0.353	1.34±1.98	1.50±3.22	0.776	2.72±4.67	1.32±2.62	0.112
Mechanical ventilation(%)	16.67%	26.09%	0.283	28.21%	24.74%	0.676	20.00%	26.45%	0.590
Duration of hospitalization (days)	16.07±10.48	13.89±8.14	0.115	13.53±6.55	14.72±9.48	0.455	16.80±10.70	14.09±8.44	0.254
Improvement rate on the third day#	93.33%	88.70%	0.619	92.86%	91.00%	0.717	100%	90.55%	0.213

Notes: *p<0.05; **p<0.01; #Improvement refers to When the patients meet the following conditions on day 3: symptoms and signs of AECOPD were improved and no new symptoms appeared compared with baseline, lung infiltration shadow absorption or no obvious deterioration, and laboratory indicators related to infection were improved (including peripheral blood leukocyte count, peripheral blood neutrophil percenage, CRP and PCT, etc).

times (mean hospitalization length, ~14 days). However, this was determined by the design of this study.

In this study, all patients had chest radiographs or lung CT scans performed. If the patient's chest radiographs or lung CT scan showed new patches, patchy infiltrates, or interstitial changes in the lungs, the patient was excluded. Thus, some patients with COPD combined with pneumonia were excluded, which ensured that all patients in the group had AECODP. This is also one reason why few patients were selected.

Laboratory diagnostic methods for atypical pathogens include culture, serum antibody detection, and nucleic acid detection. Etiological identification of conventional bacterial pathogens causing AECOPD relies on sputum culture. However, for atypical pathogens, due to difficulty in isolation and their slow growth, which make it difficult to analyze the results in a short period of time, sputum cultures are rarely used in clinical practice.[19,20] In this study, we used a variety of detection methods such as serology, PCR, and urine antigen testing for three atypical pathogens. We listed the sensitivity and specificity of the detection methods in Table 6 after consulting many studies.[8–10,15,16,18,20] Serology-based specific antibody testing is the traditional approach for identifying atypical pathogen infections. In order to make the serological antibody results better and more accurately reflect the infection situation of the patients, the patients we collected all had double sera in this study. When there was a 4-fold change in the antibody of double sera, the test result of atypical pathogens was considered positive. The change of this antibody could better reflect the patient's recent infection of atypical pathogens.[8,14,18] Another the positive patients were IgM positive, and IgM antibodies are recognized as early antibodies when the patient were infected by atypical pathogens, so it can also reflect the recent infection of the patients.[8,19–21] We listed the sensitivity and specificity of the detection methods in Table 6 by consulting many literatures.

The specificity of nucleic acid tests of secretions (PCR method) is high, but its sensitivity varies considerably among studies.[10,20,22] We believe that this variation is related to the collection method and site of the specimens subjected to PCR testing. The diagnostic performance of Roche *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Legionella pneumophila* multiplex *Lightmix RT-PCR* kit has been evaluated by Wagner et al[23] and showed identical performance characteristics (specificity and sensitivity) to in-house singleplex RT-PCRs for pathogen

Table 6 The Sensitivity, Specificity, False Positivity and False Negativity of Each Pathogen Detection Methods

| Atypical Pathogens | Mycoplasma pneumonia[8,10,20] | | | Chlamydia pneumonia[15,16] | | Legionella pneumophila[9,18] | | | |
	Passive Agglutination Method[8]*	IgM[20] #	PCR[10,20]	Microimmunofluorescence Assay, MIF[15,16]*	PCR[15,16]*	Indirect Fluorescent Antibodies[18]	IgM[9]	PCR[18]	Urine Antigen[18]
Sensitivity	100%[8]*	7.4%[20] #	0%[10]** / 40.7%[20] #	16.5%[15]* / 82%[16]*	15.00%[15]* / 71%[16]*	40–80%	92.3%	30–100%	70–90%
Specificity	95.6%[8]*	94.9%[20] #	95.2%[10]** / 88.8%[20] #	94.55%[15]* / 40%[16]*	81.82%[15]* / 97%[16]*	95–100%	100%	95–100%	95–100%
PPV	–	28.6%[20] #	0[10]** / 50%[20] #	68.42%[15]* / 34%[16]*	37.5%[15]* / 90%[16]*	–	–	–	–
NPV	–	78.8%[20] #	85.1%[10]** / 84.5%[20] #	60.82%[15]* / 85%[16]*	56.96%[15]* / 90%[16]*	–	–	–	–

Notes: *With clinically diagnosed cases as reference standard; #With a fourfold or greater increase of IgG antibody titers of paired sera as reference standard; **With IgM positivity as reference standard.
Abbreviations: PPV, positive predictive values; NPV, negative predictive values; PLR, positive likelihood ratio.

detection. Wagner[24] and Orozco-Hoyos[25] used the kit to detect *Mycoplasma pneumoniae, Chlamydia pneumoniae*. Relevant studies have discussed the sensitivity of PCR for detecting atypical sputum pathogens in patients with AECOPD and obtained a very low positive rate,[20–22,26] which is consistent with the results of the our study. A study by Diederen et al[27] showed that all 126 sputum samples of patients with AECOPD were negative for *Mycoplasma pneumonia* and *Chlamydia pneumonia* DNA, while one sample was positive for *Legionella pneumophila* and non-*pneumophila* DNA. The sensitivity of PCR was lower than that of serology, as shown in Table 6.

Overall, based on the results of this study, the sensitivity of serological testing is superior to that of sputum nucleic acid testing for atypical pathogens. Diederen's findings are consistent with our research.

Previous studies have indicated that AECOPD induced by infectious causes account for approximately 40% of the overall number of AECOPD cases.[28] In this study, we observed an overall positivity rate of 20.69% (30/145), 29.66% (43/145), and 10.34% (15/145) for *Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Legionella pneumophila*, respectively. However, the proportions of atypical pathogens identified in patients with AECOPD are different in different studies due to the use of varied detection methods in different countries and regions.

The detection rate of *Mycoplasma pneumoniae* was 0.2–6.0%[4,29–35] in a previous study. Varma-BasilM[35] conducted a study involving outpatients with AECOPD. The serological test results showed that the positive rate for *Mycoplasma pneumoniae* was 16% (compared with 18.62% by serum testing in this study). The detection rate for *Chlamydia pneumoniae* was 0.3–44.7%.[5,6,31,32,34,36–39] A study by Karnak et al[37] identified *Chlamydia pneumoniae* in 34% of patients with AECOPD. There are few studies on Legionella pneumophila. A study by Lieberman et al[40] detected *Legionella* in 16.7% of patients. The findings of the abovementioned studies are similar to the results of this study.

Due to the chronic bacterial colonization in the lower respiratory tract in patients with COPD patients,[41] the proportions of concomitant routine infections and atypical pathogens may be higher than expected.

Notably, in previous epidemiological studies, type 1 *Legionella pneumophila* infection was the most common among patients with symptomatic *Legionella pneumophila* pneumonia.[18,40] Among the 15 cases of *Legionella* infection in this study, only three cases were identified as *Legionella* type 1, whereas nine were *Legionella* type 6. *Legionella* type 1 is generally considered to be more pathogenic than other serotypes of *Legionella*, but none of the 15 patients showed typical symptoms of *Legionella* infection, such as high fever, cough with orange sputum, muscle aches, abdominal pain, or diarrhea. This finding suggests that for patients with AECOPD, the use of a urinary antigen kit that detects *Legionella* type 1 alone may not be sufficient to clarify whether a concomitant pulmonary infection caused by *Legionella pneumophila* is present.

Due to their sensitivity and specificity limitations, assays that do not rely on pathogen culture pose a clinical challenge for determining treatment strategies for atypical pathogens. We believe that empirical treatment of atypical pathogens may be beneficial if certain clinical characteristics are suggestive of infection. In the present study, the maximum body temperature, eosinophil percentage, CRP level, and PCT level were significantly higher in patients with AECOPD who tested positive for *Mycoplasma pneumoniae* than in those who tested negative. This suggests that patients with AECOPD concomitantly infected with *Mycoplasma pneumoniae* have more severe airway hyperresponsiveness and systemic inflammation; the specific mechanisms underlying this phenomenon need to be clarified in further studies. Although it is generally accepted that among atypical pathogens, *Legionella pneumophila* is more likely to cause severe sepsis, surprisingly, this phenomenon was not observed in patients with AECOPD who tested positive for *Legionella pneumophila*. Patients with AEOCPD who tested positive for *Legionella pneumophila* infection had a higher forced expiratory volume in one second to forced vital capacity (FEV1/FVC) ratio assessment of lung function and a lower arterial blood bicarbonate (HCO_3^-) concentration, suggesting that patients with COPD with mild pulmonary obstruction who do not need to be homebound and therefore have better mobility may be more susceptible to exposure to *Legionella pneumophila*. There were no statistically significant clinical characteristics observed in patients with AECOPD with *Chlamydia pneumoniae* infection.

Conclusion

In summary, we believe that the proportion of concomitant infection by atypical pathogens may be very high in patients with infection-induced AECOPD. Serological testing remains the most reliable test for atypical pathogens.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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