

Original Article

Detection of pathogens and epidemiological characteristics of community-acquired pneumonia in children using metagenomic next-generation sequencingShiQu Deng¹, YaLi Wu¹, PeiYun Hu¹, JianLiang Lin², Jingming Yao³, Bin Wang¹¹Clinical laboratory, Fujian Children's Hospital (Fujian Branch of Shanghai Children's Medical Center), College of Clinical Medicine for Obstetrics and Gynecology and Pediatrics, Fujian Medical University, Fuzhou 350001, China²Fuzhou Jinyu Inspection Company, Fuzhou 350001, China³Clinical laboratory, Minhou County Hospital, Fuzhou, Fujian, China**Abstract**

Introduction: Community-acquired pneumonia (CAP) is a common respiratory disease in children and a significant factor in child mortality.

Methodology: We aimed to investigate metagenomic next-generation sequencing (mNGS) technology to explore pathogens and epidemiological characteristics of pediatric CAP. We retrospectively analyzed mNGS detection and microbiological culture results of bronchoalveolar lavage fluid (BALF) and sputum samples from children with CAP.

Results: *Mycoplasma pneumoniae* was the predominant pathogen. Bacteria/fungi detection rates using mNGS in sputum and BALF were higher than those using microbiological culture ($p < 0.05$). *Cytomegalovirus* was the predominant pathogen in children aged 0-1; *Mycoplasma pneumoniae* was the predominant pathogen in those over 1. *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *human bocavirus 1*, and *Streptococcus pneumoniae* were the predominant pathogens in spring, summer, autumn, and winter, respectively.

Conclusions: mNGS is superior to traditional microbiological culture for pediatric CAP potential pathogen detection. CAP pathogenic infection characteristics at different ages and seasons detected by mNGS will benefit clinical practitioners in the prevention and treatment of pediatric CAP in their local/regional areas.

Key words: Community-acquired pneumonia; CAP; metagenomic next-generation sequencing; mNGS; pathogen; children.

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Introduction

Community-acquired pneumonia (CAP) is a common respiratory disease in children and a significant factor in child mortality [1,2]. Because of the complexity of pathogenic infections, patients often do not receive the timely and accurate etiological diagnosis crucial for the proper treatment of pediatric CAP [3,4]. Most hospitals, especially grassroots hospitals, rely primarily on traditional microbial culture, immunological assays and traditional polymerase chain reaction (PCR). However, microbial culture analysis is time-consuming and has low sensitivity. In addition, the results of immunological tests that rely on immunoglobulin (Ig)M and IgG testing are often not available until one week after infection, and the types of bacterial pathogen detection using PCR is limited [5,6]. Therefore, physicians primarily perform empirical treatment based on white blood cell count, neutrophil-to-lymphocyte percentage, C-reactive protein, procalcitonin, and other laboratory tests, resulting in the misuse or overuse of antibiotics.

CAP poses a serious threat to children's health. The pathogens that cause CAP are primarily bacteria, viruses, fungi, and atypical pathogens. In the absence of effective pathogen identification markers, clinical treatment is primarily empirical [7]. Therefore, rapid and accurate determination of pathogenic assay is crucial for the diagnosis and treatment of pediatric CAP. Due to the rapid development of metagenomic next-generation sequencing (mNGS) technology, its application in respiratory tract infections is becoming increasingly extensive. mNGS is a microbial identification technique based on nucleic acid detection that has emerged recently and has since been widely used because of advantages such as unpredictability and high throughput [8,9]. Compared to traditional methods, the most important advantages of mNGS are its short turnaround time and high accuracy, especially for the diagnosis of rare, novel, and unknown pathogens, indicating its suitability for clinical use [10,11]. Existing literature has focused on the effectiveness of mNGS using bronchoalveolar lavage

fluid (BALF) for the detection of causative pathogens of pneumonia in children [12-14]. However, few studies have simultaneously explored the diagnostic value of etiology and the application of mNGS technology in the distribution of pathogens by age and season using BALF and sputum.

In this study, we described the pathogen spectrum of CAP in children using BALF and sputum samples, evaluated the effectiveness of mNGS compared to that of traditional microbial culture, and explored the differences in pathogen infection among CAP infections at different ages and seasons.

Methodology

Study subjects

A total of 339 children with CAP admitted to Fujian Children's Hospital between December 2021 and January 2023 were considered as the research subjects. The inclusion criteria were as follows:

1) The diagnostic criteria for CAP in the children complied with the "Zhu Futang's Practice of Pediatrics" (8th edition) [15]: a) Respiratory symptoms such as fever, cough and so on; b) high respiratory rate and lung moist rales.

2) Children' BALF or sputum samples were collected for mNGS testing and traditional microbiological culture; Criteria for qualified BALF including: a) squamous cells < 10/low power objective (LP); b) white blood cell \geq 25/LP; c) columnar epithelium < 5% of all nucleated cells; d) recovery was > 40% and more than 95% of surviving cells; e) red blood cells were < 10% of all cells; Criteria for qualified sputum samples was the number of leukocytes is more than 25/LP, squamous cells are less than 10/LP, and squamous cells / leukocytes are less than 1: 2.5.

Sample acquisition

Sputum and BALF samples were collected within 48 hours of hospitalization from all patients who underwent bronchoscopy according to standard

procedures.

mNGS analysis

In summary, our approach integrated PCR and next-generation sequencing technologies to target highly conserved regions of respiratory pathogens. Specific primers were used to enrich target pathogens via PCR, incorporating sequencing adapters for sample source identification in a second PCR round. High-throughput sequencing followed by bioinformatics analysis was performed to filter and align data with reference genomes for pathogen detection. Sample quality was assessed by concurrent human DNA detection. The detailed steps included: (1) nucleic acid extraction using reagents (KS118-BYTQ-72) from Guangzhou KingCreate Biotechnology; (2) library preparation and sequencing according to kits (MR100) and sequencer (KM MiniSeqDx-CN) instructions; and (3) bioinformatics analysis using the "Pathogen Microbial Data Analysis System" with quality standards of Q30 \geq 75%, \geq 50k original reads, and normalized reads of \geq 200 (internal reference) or \geq 3000 (target pathogen). The mNGS analysis, along with the bioinformatics analyses and interpretations of sputum and BALF specimens, was conducted by Fuzhou Jinyu Inspection Company.

Microbial culture

The clinical laboratory at Fujian Children's Hospital conducted microbial cultures as follows: Briefly, bronchoalveolar lavage fluid (BALF) or sputum samples were inoculated onto blood agar and chocolate agar plates for microbial isolation and identification. These plates were then incubated at 38 °C in an atmosphere containing CO₂ (5%) for a period of 24–48 hours to optimize growth and isolation of microorganisms. Subsequently, experienced laboratory technicians carefully selected and identified suspicious colonies utilizing a bacterial mass spectrometer (Microflex LT/SH) to accurately determine species.

Statistical analysis

Data are expressed as mean \pm standard deviation. The counting data are expressed as the number of cases (percentage) (n [%]), and the data between groups were compared using the Chi-square test or Fisher's exact test. Statistical significance was considered at $p < 0.05$.

Results

The general characteristics of the patients

The patients' general characteristics are presented in Table 1. In addition, the primary noted clinical

Table 1. The general characteristics of the patients.

Characteristics	n (%)
Gender	Total 339 (100)
Male	213 (62.83)
Female	126 (37.17)
Age	years old
Range	0-14
Average	3
NGS	
BALF	268 (79.06)
Sputum	71 (20.94)
Microbial culture	
BALF	101 (29.79)
Sputum	339 (100)

symptoms included fever accompanied by cough in 40.3% of cases, cough only in 29.0%, and fever only in 12%. Radiographic findings revealed bilateral lung inflammation in 60.2%, single lung inflammation in 23.9%, increased lung texture in 4.4%, no significant abnormalities in 0.3%, and no radiographic results in 11.2% of the patients. Furthermore, 93.8% of the children had no underlying diseases, while 85.2% did not receive empirical treatment prior to sample collection. All children with CAP participating in the study had no prior hospitalization records within the three months preceding their admission. Furthermore, no children with immunosuppression or cystic fibrosis were included in the study.

Examination results of the predominant pathogens of children with CAP detected using mNGS

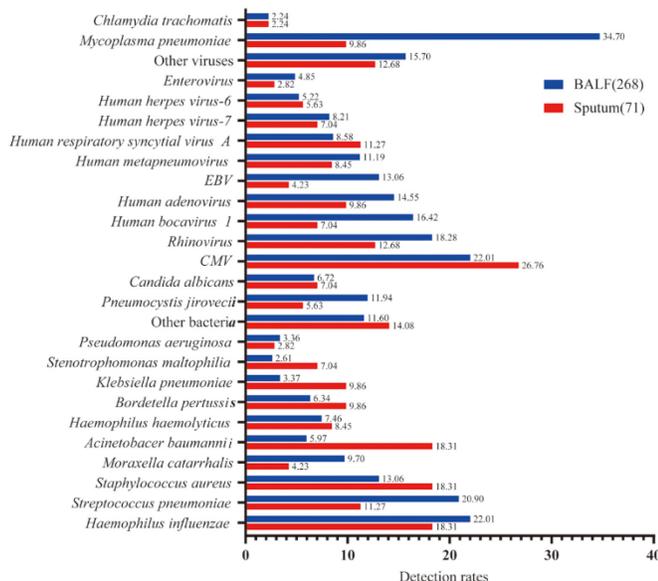
We obtained 71 sputum and 268 BALF samples from 339 pediatric patients with CAP for mNGS detection. The pathogen detection rates in the sputum and BALF were 91.55% and 96.27%, respectively, which were not significantly different. A total of 205 cases (60.47%) of bacterial infections, 56 cases (16.52%) of fungal infections, 257 cases (75.81%) of viral infections, and 121 cases (35.69%) of atypical pathogens were detected in all specimens. Among these, only one pathogen was detected in 64 cases (18.88%) and two or more pathogens were detected in 259 (76.40%) cases. As shown in Figure 1, the top three pathogens detected in sputum were *Cytomegalovirus (CMV)* (26.76%), *Haemophilus influenzae* (18.31%), and *Staphylococcus aureus* (18.31%). The top three

pathogens in BALF were *Mycoplasma pneumoniae* (34.70%), CMV (22.01%), and *Haemophilus influenzae* (22.01%). There was no statistically significant difference in the overall detection rates of bacteria, viruses, and fungi between the sputum and BALF samples; however, the detection rates of *Mycoplasma pneumoniae* and *EBV* in the BALF samples were higher than those in the sputum samples ($p < 0.05$). The detection rates of *Acinetobacter baumannii* and *Klebsiella pneumoniae* in the sputum samples were higher than those in the BALF samples ($p < 0.05$).

Pathogen examination results using microbial culture

Sputum was collected from 339 pediatric patients with CAP for traditional microbial culture, among which 101 samples were also collected from BALF for microbial culture. A total of 49 bacterial infections (14.45%) and one (0.29%) fungal infection were detected in 339 sputum samples. Among the 101 BALF specimens, 25 samples (24.75%) had bacteria and one (0.99%) had fungi. Among the two types of specimens, the detection rate of bacteria in the BALF was higher than that of the sputum ($p < 0.05$). The top three bacteria detected in the microbial cultures of 339 children with CAP were *Haemophilus influenzae* (4.72%), *Staphylococcus aureus* (4.72%), and *Pseudomonas aeruginosa* (3.24%). The detected fungi included *Candida albicans* in two samples (0.59%). Among the 101 patients, 70 (69.3%) were simultaneously negative for infection in both the BALF and the sputum, whereas only five samples (5%) of BALF and sputum had the same pathogen.

Figure 1. Pathogen detection rates of BALF and sputum using mNGS.



Comparison of the results of microbial culture and mNGS

A total of 65 cases of bacterial infection were detected in the sputum and BALF by microbial cultures of 339 children with CAP (including nine cases of bacteria detected in both sputum and BALF). Two cases of fungi were detected. Among the 65 positive cases, 51 (78.47%) were consistent with the results of bacterial detection using mNGS. The results of the two fungus-positive cases were consistent with those of the fungi detected using mNGS. Because traditional microbiological cultures can only detect bacteria and fungi, we compared the detection rates of bacteria and fungi between mNGS and microbial culture. The detection rates of bacteria in the sputum and BALF using mNGS were 64.79% and 59.33%, respectively, and those in the sputum and BALF using microbial cultures were 14.45% and 18.28%, respectively.

Furthermore, the detection rates of fungi in the sputum and BALF using mNGS were 12.68% and 17.54%, respectively, and the rates in the sputum and BALF using microbial culture were only 0.30% and 0.99%, respectively. Thus, the bacterial and fungal detection rates using mNGS were higher than those using microbial cultures. There were no significant differences among the bacterial detection rates of *Pseudomonas aeruginosa*, *Klebsiella aeruginosa* and *Escherichia coli* between mNGS and microbial culture. The detection rates of *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were significantly higher using mNGS than those when using microbial culture (Figure 2).

The predominant pathogens detected using mNGS in different age groups

According to the enrollment age of the Chinese children, 339 patients with CAP were divided into a 0-1 year-old group (146 cases, 43.1%), 1-3 year-old group

Figure 2. Difference in the detection rate between microbial culture and mNGS.

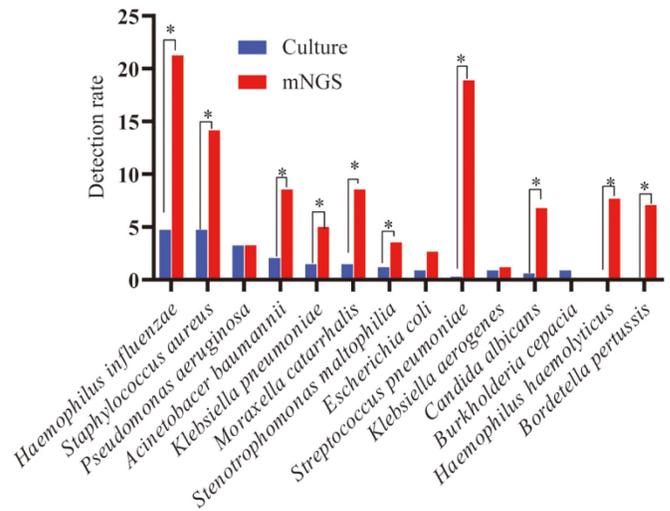
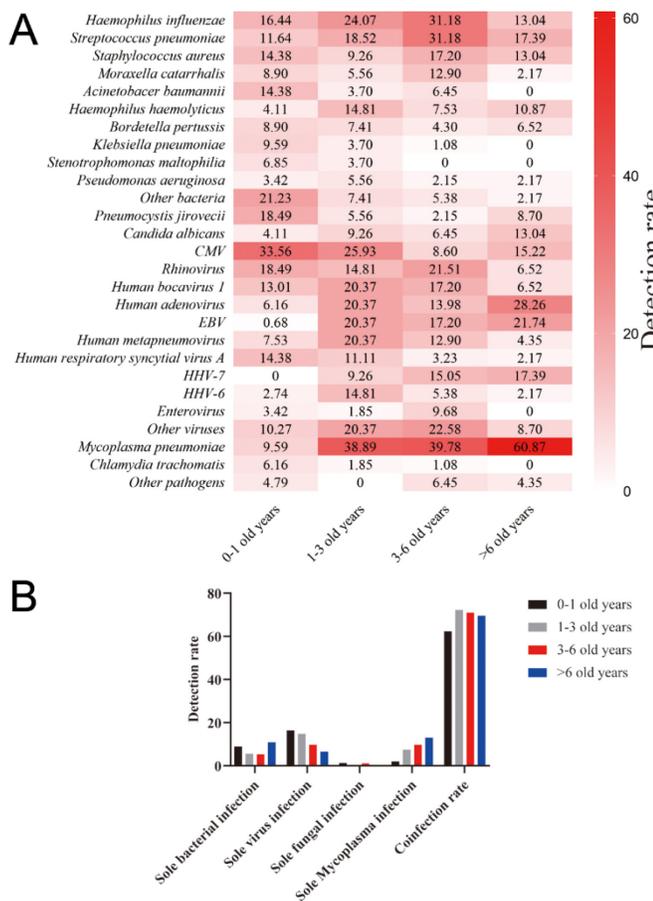


Figure 3. Pathogens detected in different age groups using mNGS.



A. The numbers in the figure show the positive detection rate of pathogen according to age; **B.** Bar graphs illustrate positive infection percentages for bacterial, viral, fungal, and mycoplasma cases, alongside coinfection rates.

(54 cases, 15.9%) 3-6 year-old group (93 cases, 27.4%), and an over 6 year-old group (46 cases, 13.6%). CAP is highly prevalent in children aged 0-1. *CMV* and *rhinovirus* were the predominant pathogens of patients with CAP aged 0-1 years old. *Mycoplasma pneumoniae*, *CMV*, and *Haemophilus influenzae* were the predominant infectious pathogens in patients with CAP aged 1-3 years old. *Mycoplasma pneumoniae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* were the predominant infectious pathogens in patients with CAP aged 3-6 years old. Finally, *Mycoplasma pneumoniae*, *human adenovirus*, and *EBV* were the predominant infectious pathogens of patients with CAP over 6 years old. The incidence rates of *Mycoplasma pneumoniae* and human herpesvirus 7 increased with age, whereas the incidence rates of *Klebsiella pneumoniae* and *human respiratory syncytial virus A* decreased with age (Figure 3A). As illustrated in Figure 3B, various age groups were predominantly infected with a combination of multiple pathogens. Notably, the incidence of simple viral infection exhibited a declining trend as age increased, whereas the occurrence of simple MP infection demonstrated an ascending trend with age.

mNGS detection of the predominant pathogens in different seasons

A total of 339 pediatric patients with CAP were categorized based on the duration of hospitalization: 66 cases were hospitalized (19.5%) in the spring (March–May), 152 (44.8%) in the summer (June–August), 57 (16.8%) in autumn (September–November), and 64 (18.9%) in winter (December–February). Summer had

the highest incidence of pediatric CAP. The predominant pathogens detected in the spring were *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *rhinovirus*. *Mycoplasma pneumoniae*, *CMV*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* were the predominant pathogens in the summer, and *human bocavirus 1* and *CMV* were the predominant pathogens in autumn. Finally, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *CMV* were the predominant pathogens detected in the winter (Figure 4).

Discussion

Our results showed that various pathogens caused CAP in children, most of which were mixed infections. In this study, *CMV* was the most frequently detected virus; conversely, *respiratory syncytial virus* was the most frequently detected virus reported in other studies [16,17]. *CMV*, is a recognized pathogen that causes diseases such as congenital infections, posing a significant danger to the health of those with weakened immune systems, such as allograft recipients, and immunocompromised patients [18]. Our study revealed that CAP was highly prevalent among children aged 0-1, with *CMV* identified as the primary causative agent. However, most healthy individuals do not develop severe diseases, and the detection of *CMV* is not equivalent to a diagnosis of CAP [17]. Correlation with disease diagnosis is one of the challenges of using NGS for clinical diagnostics.

Our results showed that *Mycoplasma pneumoniae*

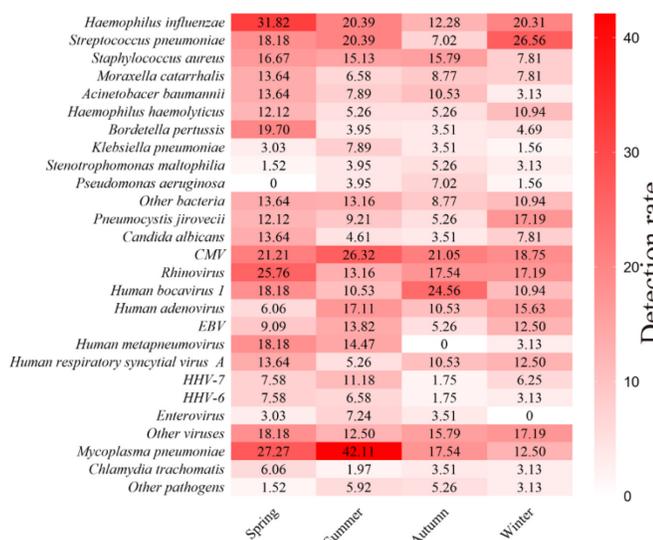
had the highest CAP detection rate. Moreover, detection rates increased with age in children, consistent with the findings in another study [19]. *Mycoplasma pneumoniae* has been confirmed as an important pathogen for causing pediatric CAP [20]. Recent reports have showed that *Mycoplasma pneumoniae* infection can develop into severe *Mycoplasma pneumoniae* infection (SMPP) and refractory *Mycoplasma pneumoniae* infection (RMPP) [21,22]. Early identification of *Mycoplasma pneumoniae* infection could effectively prevent the disease from developing into SMPP or RMPP. *Mycoplasma pneumoniae* is traditionally assessed by reverse transcription (RT) -PCR or *Mycoplasma pneumoniae*-specific immunoglobulin (Ig)M and IgG antibodies. Detection of IgM antibodies in combination with RT-PCR findings allows for precise and reliable diagnosis of *Mycoplasma pneumoniae* infections in children [23]. PCR is a method with offers higher sensitivity, specificity, and speed compared with IgM test. In clinical practice, mNGS is usually used as an effective supplement to PCR methods.

Previous research has shown inconsistent seasonal patterns of pediatric CAP in China, with peak incidence varying between spring/winter and autumn/winter [24-27], indicating that different pathogens are present in children with CAP in different regions and seasons. This study showed that spring and summer were the high-incidence seasons of childhood CAP in Fujian Province, and *Mycoplasma pneumoniae* and *CMV* were the predominant pathogens detected in the summer. This study, employing mNGS, is the first to investigate CAP pathogen characteristics in Fujian children across seasons and age groups, offering crucial diagnostic and treatment insights for local physicians.

Traditional microbiological culture detects only bacteria and fungi, not viruses or atypical pathogens such as *Mycoplasma pneumoniae*. Our study results showed that the detection rates of bacteria and fungi in the sputum and BALF using mNGS technology was higher than those using microbiological culture. It is evident that one of the advantages of mNGS over traditional culture is that mNGS demonstrates exceptional sensitivity, enabling the detection of minute quantities of pathogen that may elude traditional culture.

Some children with CAP in this study had been treated with empirical antibiotics, thus inhibiting the detection of some bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. However, antibiotics have small impact on the mNGS detection ability [28,29]. Traditional cultivation methods mainly

Figure 4. Pathogens detected in different seasons using mNGS.



The numbers in the figure show the positive detection rate of pathogen according to seasons.

focus on a single pathogen, in mixed bacterial populations, detection can be difficult. When colonizing bacteria are more abundant than pathogenic ones, cultivation often favors the colonizers, causing pathogenic overlooked pathogen detection and potentially misleading clinical diagnosis and treatment. mNGS can simultaneously detect multiple microorganisms (including mixed bacteria and different types of microorganisms) in a single sample. Traditional microbial cultivation requires 1-2 days for identifying pathogenic bacteria, whereas mNGS can achieve bacterial identification in as little as 1 day. Therefore, compared with traditional microbiological culture, mNGS covers a wide range of pathogens, greatly shortening the pathogen detection time.

Although mNGS has many advantages, it also has some limitations. Due to potentially low specificity here since the technique can detect pathogens that are part of the normal biome or transient colonizers, such as *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia*. These colonizers are opportunistic pathogens, they are not expected to be causative agents of CAP but can be detected through mNGS. Furthermore, pathogens with low sequence counts can pose challenges for determining their clinical relevance, as distinguishing whether they are pathogenic species or merely colonizing or contaminating microorganisms can be difficult. Finally, understanding the intricate interactions and relationships among pathogens, as well as their potential implications for disease pathogenesis, is of paramount importance. Clinicians should determine pathogens based on mNGS results, clinical symptoms, and other laboratory tests, considering mNGS just as an important supplement to traditional methods, that cannot completely replace traditional methods. In addition, the cost of mNGS testing for one sample differs by region and lab; it is higher than that of traditional methods such as traditional microbiological culture and PCR in general, making it less widely used.

Conclusions

In summary, pediatric CAP involves diverse and complex pathogens. Recently, mNGS has been widely adopted in clinical practice due to its broad coverage, and high accuracy, significantly enhancing the detection rate of CAP pathogens, as it complements traditional diagnostic methods. Based on local epidemiological characteristics, clinicians can empirically treat major pathogens, reducing antibiotic

misuse and improving pediatric CAP diagnosis and treatment in the region.

Ethics statement

The study was approved by the ethics review board of the Fujian Children's Hospital (2023ETKLRK10035).

Authors contributions

ShiQu Deng: Investigation, Writing - Original Draft; YaLi Wu: Data Curation, Validation; PeiYun Hu: Methodology, Validation, Writing - Review and Editing; JianLiang Lin: Data analysis, Investigation; Jingming Yao, Bin Wang: Conceptualization, Project administration, Supervision

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Conflict of interests

No conflict of interests is declared.

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