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Clinical diagnostic value of throat swabs in pediatric acute lower respiratory tract infections using targeted next-generation sequencing

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Abstract

Background To evaluate the clinical utility of targeted next-generation sequencing (tNGS) for pathogen detection of pediatric acute lower respiratory tract infections (ALRTIs), with a particular focus on the use of throat swab samples.

Methods In this diagnostic accuracy study involving 132 children, throat swabs and bronchoalveolar lavage fluid (BALF) samples were collected and analyzed by tNGS, and the results were compared with those obtained from conventional diagnostic methods. The impact of prior antibiotic use on the detection rate of tNGS was evaluated, the consistency between throat swabs and BALF was assessed, and the economic cost and invasiveness of the sampling methods were examined.

Results This study enrolled 132 children, of whom 79 (60%) were boys and 53 (40%) were girls. Ninety-two (70%) of the patients had fever, and 128 (97%) had a cough. The detection rates of bacteria, viruses, and atypical pathogens in BALF samples by tNGS were 89.5% ($n=68$), 98.2% ($n=108$), and 77.8% ($n=63$), respectively. Compared to traditional detection methods, tNGS showed significantly higher detection rates for bacteria and viruses ($P<0.001$), but there was no statistically significant difference in the detection of atypical pathogens ($P=0.59$). The use of antibiotics had no significant effect on bacterial detection by tNGS ($P=0.237$). Using BALF-tNGS as the "gold standard," the sensitivities of tNGS of throat swabs for detecting bacteria, viruses, and atypical pathogens were 95.83%, 88.16%, and 92.06%, respectively, with specificities of 55.95%, 83.93%, and 100%. In the analysis of economic costs and invasiveness, the cost of throat swab sampling was significantly lower than that of BALF sampling, and the associated pain score and complication rate were significantly lower ($P<0.05$).

Conclusions tNGS with throat swabs offers higher sensitivity and specificity than traditional methods for diagnosing pediatric ALRTIs. As such, it offers a less invasive, more cost-effective alternative to BALF sampling.

Keywords Throat swabs, Acute lower respiratory tract infections (ALRTIs), Targeted next-generation sequencing (tNGS), Pediatric pathogen diagnosis, Non-invasive diagnostics methods

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Background

Acute lower respiratory tract infections (ALRTIs) in children constitute a significant global health burden, and are a leading cause of pediatric hospitalizations and mortality. The high incidence and severe outcomes associated with ALRTIs underscores the urgent need for accurate and timely methods of pathogen detection [1–3]. Traditional diagnostic techniques, such as bacterial culture, antigen detection, antibody assays, and nucleic acid tests, are often limited by low sensitivity and specificity [4, 5]. In addition, these methods typically require invasive sampling procedures, which can be particularly traumatic for pediatric patients.

The limitations of conventional diagnostic methods for ALRTIs have been well-documented. Bacterial culture, although considered the gold standard, is time-consuming, and commonly fails to detect fastidious or slow-growing organisms [6, 7]. Antigen and antibody detection methods, although quicker, have lower sensitivity and specificity, leading to potential false negatives or positives [8]. Further, while nucleic acid tests are more sensitive, they are limited by their inability to detect a wide range of pathogens simultaneously [9]. Additionally, obtaining lower respiratory tract samples, such as bronchoalveolar lavage fluid (BALF), involves invasive procedures that carry risks of complications, and are not always feasible in a pediatric setting. These challenges highlight the need for a more efficient, accurate, and less invasive diagnostic approach.

Targeted next-generation sequencing (tNGS) offers several advantages over traditional diagnostic methods. This technology allows for high-throughput, as well as rapid and precise, detection of multiple pathogens in a single assay. Unlike conventional methods, tNGS can simultaneously identify a wide range of bacterial, viral, and atypical pathogens, providing a comprehensive overview of the infectious agents involved [10, 11]. Furthermore, tNGS can be performed on non-invasive samples, such as throat swabs, which are easier to collect and cause minimal discomfort to patients. These attributes make tNGS a promising tool for improving the diagnosis and management of ALRTI in children.

The present study aimed to evaluate the clinical utility of throat swab samples in tNGS at detecting pathogens in children with ALRTIs. By comparing the diagnostic performance of throat swabs to BALF samples, we aimed to determine the sensitivity, specificity, and overall concordance of the tNGS results between these sample types. Additionally, we assessed the practicality of collecting throat swabs in a clinical setting, considering sampling complexity, patient discomfort, and potential complications. Given the potential of tNGS to enhance pathogen detection and guide targeted therapy, this research could significantly improve the management of pediatric ALRTIs, by validating a less invasive sampling method, improving diagnostic accuracy, reducing the burden on patients and healthcare providers, and enhancing clinical outcomes.

Materials and methods

Study design

This diagnostic accuracy study aimed to evaluate the clinical utility of throat swab-tNGS for pathogen detection among children with ALRTIs. The study was conducted at the Department of Respiratory Medicine at Fujian Children's Hospital from December 2022 to June 2023.

Study population

This study included 132 children treated for ALRTIs at Fujian Children's Hospital. All participants met the below inclusion and exclusion criteria.

The inclusion criteria were: (1) age ≤ 12 years old; (2) hospitalization due to respiratory symptoms, such as cough, fever, and shortness of breath, ultimately diagnosed as ALRTIs based on clinical symptoms, radiographic findings, and laboratory test results; (3) requirement to undergo BALF; and (4) informed consent signed by the guardian(s).

The exclusion criteria included: (1) a history of congenital heart disease, hematological malignancy, immunodeficiency, or use of immunosuppressants; (2) having undergone bronchoalveolar lavage via bronchoscopy owing to factors other than pneumonia, such as detection of bronchial foreign bodies and airway stenosis.

Recruitment was conducted throughout the entire study period; all consecutive eligible patients were approached for enrollment, ensuring that the results would be representative and generalizable to the broader population of pediatric patients with ALRTIs.

This study was approved by the Ethics Committee of the host hospital.

Sample collection

Throat swabs and BALF samples were collected from 132 children. Throat swabs were collected within one hour of bronchoscopy. Sterile swabs were rotated 2–3 times on the posterior pharyngeal wall of the children; the swabs were then placed in sterile tubes containing 1.5 mL of saline and sent for testing within two hours to ensure sample validity. Bronchoalveolar lavage fluid was collected following intravenous and local anesthesia. An electronic bronchoscope was positioned at the opening of the target bronchial segment or subsegment. Sterile saline at room temperature was rapidly instilled into the small bronchi and alveoli 2–3 times, with each instillation volume ranging from 5 to 15 mL. Post-instillation, the first aliquot of lavage fluid, potentially contaminated,

was discarded, and a 5 mL sample was collected and sent for testing within two hours at room temperature.

Pathogen detection via tNGS

Samples were immediately sent to the testing department for tNGS detection, which could identify 153 pathogens, including bacteria, viruses, fungi, and atypical pathogens. Atypical pathogens, in this study, refer to organisms commonly associated with atypical pneumonia and are not typically detected by standard bacterial culture. These include Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila, and other similar pathogens. The pathogens included in the analysis in this study were selected based on their known role in causing ALRTIs, as well as their clinical relevance in pediatric patients [12]. For a complete list of all detectable pathogens, please refer to the Supplementary Materials. The primary process included nucleic acid extraction, library construction, high-throughput sequencing, as well as data quality control and analysis [13]. The production and testing of the panels are conducted internally by Guangzhou Kingmed Diagnostics Company.

Conventional detection methods

To evaluate the efficacy of tNGS, multiple traditional detection methods were compared, including sputum culture, respiratory viral antigen detection (such as for influenza A and B, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3), Chlamydia and Mycoplasma antibody tests, and urinary pneumococcal antigen detection. All participants underwent the same traditional diagnostic methods, ensuring consistency in testing. Each patient further underwent the full range of these diagnostic tests to provide a comprehensive comparison between tNGS and traditional methods. This uniform approach to testing allowed for a more direct comparison of the effectiveness of tNGS versus conventional diagnostic methods.

Assessment of low invasiveness

Sampling complexity was rated on a scale from 1 (simple) to 5 (complex), based on the hospital's standard operating procedures (SOPs) for performing sampling. The complexity was further determined by factors such as the difficulty in accessing the sampling site, the need for additional assistance or equipment, and the potential discomfort or risk to the patient during the procedure. A higher score was assigned to samples that required more specialized techniques, or that posed a higher risk of complications. Pain score was assessed using a visual analog scale (VAS), where patients rated their pain during sampling on a scale of 0–10. Complication records documented the incidence of complications during and after sampling, including bleeding, glottic edema, and hypoxemia.

Data analysis

Data analysis was performed using SPSS v.23.0. The primary observation indicators were all quantitative data, which were tested for normality and described as the mean ± standard deviation ($\bar{x} \pm s$). Non-normally distributed data are expressed as the median and interquartile range [M (P25, P75)]. Comparisons between the two groups were performed using a t-test or a corrected t-test, as appropriate, with Bonferroni correction applied for multiple comparisons to adjust for Type I error. Categorical data were further analyzed using the chi-square or Fisher's exact tests. The sensitivity and specificity were used to assess the diagnostic accuracy of detecting pathogens in throat swabs, using the results of BALF pathogen detection as the 'gold standard.' The sensitivity, specificity, positive predictive value, and negative predictive value of detecting pathogens in throat swabs were further calculated. A P-value < 0.05 indicated a significant difference.

Sample size calculation

The sample size required to detect a minimum absolute difference of 0.3 (30%) in pathogen detection rates between the tNGS and conventional methods, with 80% power and a significance level of 0.05, was determined based on a power calculation. This effect size was chosen based on prior studies comparing molecular diagnostic methods with conventional techniques in respiratory infections. The power analysis was conducted using G*Power software to ensure that the study was adequately powered to detect clinically meaningful differences between the groups. The sample size of 132 children was considered sufficient to achieve statistically reliable results to compare the diagnostic accuracy of tNGS with conventional diagnostic methods.

Results

Patient characteristics

A total of 132 children were included in the study, including 79 (60%) males and 53 (40%) females. The median age of the children was 38 months, with an interquartile range of 15 to 65 months. Among the children, 92 (70%) presented with fever, while 40 (30%) did not. Coughing was reported in 128 (97%) children, while 4 (3%) did not have this symptom. Wheezing was observed in 40 (30%) children, while 92 (70%) did not experience wheezing. In terms of disease onset, 88 (67%) children had symptoms lasting more than 3 days, while 44 (33%) had symptoms lasting 3 days or less. Laboratory results showed that 52 (39%) children had CRP levels greater than 20 mg/L, while 80 (61%) had CRP levels of 20 mg/L or less. WBC counts above 10×10^9/L were observed in 56

Table 1 General characteristics of the study participants

Characteristic	Total cohort (*N* = 132)
Sex	
Male	79 (60%)
Female	53 (40%)
Median age (month)	38 (15, 65)
Fever	
Y	92 (70%)
N	40 (30%)
Cough	
Y	128 (97%)
N	4 (3%)
Wheezing	
Y	40 (30%)
N	92 (70%)
Disease onset time (days)	
> 3 days	88 (67%)
≤ 3 days	44 (33%)
CRP (mg/L)	
> 20	52 (39%)
≤ 20	80 (61%)
WBC ($\times 10^9$/L)	
> 10	56 (42%)
≤ 10	76 (58%)
PCT (ng/mL)	
> 0.5	42 (32%)
≤ 0.5	90 (68%)

CRP, C-reactive protein; N, no; Y, yes; WBC, White blood cell count; PCT, Procalcitonin

(42%) children, and 76 (58%) children had WBC counts of 10×10^9/L or less. PCT levels were above 0.5 ng/mL in 42 (32%) children, and 90 (68%) children had PCT levels of 0.5 ng/mL or lower. The general characteristics of the patients are shown in Table 1.

Comparison of tNGS with traditional detection methods

A total of 267 pathogen diagnoses by senior clinicians were made for 132 patients, based on clinical symptoms, signs, and auxiliary tests, including tNGS (BALF) and traditional diagnostic methods (sputum culture, respiratory viral antigen tests, Mycoplasma/Chlamydia antibody tests, and urinary pneumococcal antigen detection). These diagnoses included 76 bacterial infections, 110 viral infections, and 81 atypical pathogen infections. tNGS (BALF) identified 68 bacterial infections (89.5%), 108 viral infections (98.2%), and 63 atypical pathogen infections (77.8%), while conventional methods detected 18 bacterial infections (23.7%), 10 viral infections (9.1%), and 66 atypical pathogen infections (81.5%). The detection rates for bacteria and viruses were significantly higher with tNGS compared to conventional methods ($P < 0.001$ for both), with tNGS identifying 56 bacterial and 99 viral infections that conventional methods missed. However, there was no significant difference in the detection rates for atypical pathogens between the two methods ($P = 0.59$), with both methods detecting similar numbers of infections (tNGS: 63, conventional: 66). These findings indicate that tNGS is more sensitive than conventional methods for detecting bacterial and viral infections; however, both methods show comparable performance in detecting atypical pathogens. Detailed detection results are presented in Table 2.

Impact of antibiotic use on tNGS bacterial detection

The impact of prior antibiotic use on bacterial detection using tNGS was assessed by comparing the detection rates in samples from patients administered antibiotics prior to testing versus those who had not. Of the 62 samples for which antibiotics had been used prior to testing, 60 (96.77%) tested positive for bacterial pathogens, while 2 (3.23%) tested negative. In contrast, all 6 samples (100%) from patients who had not received antibiotics tested positive for bacterial pathogens. The difference in bacterial detection rates between these two groups was not statistically significant ($P = 0.237$). Detailed results are presented in Table 3.

Analysis of consistency of pathogen detection between throat swabs and BALF

Using the results of BALF pathogen detection as the "gold standard," the sensitivity, specificity, positive predictive value, and negative predictive value for pathogen detection in throat swabs were calculated, as shown in Table 4. For bacterial detection, bacteria were detected in throat swab+/BALF- in 37 cases (28.0%), throat swab+/BALF + in 46 cases (34.8%), throat swab-/BALF + in 2 cases (1.5%), and throat swab-/BALF- in 47 cases (35.6%). The sensitivity was 95.83%, specificity was 55.95%, positive predictive value was 55.42%, and negative predictive value was 95.92%. For viral detection, viruses were

Table 2 Differential detection of pathogens by tNGS and conventional methods

Pathogen Type	tNGS+ / Conv+	tNGS+ / Conv-	tNGS- / Conv+	tNGS- / Conv-	Total	tNGS Detection	Conv Detection	*P*
Bacteria	12	56	6	2	76	68 (89.5%)	18 (23.7%)	< 0.001
Virus	9	99	1	1	110	108 (98.2%)	10 (9.1%)	< 0.001
Atypical Pathogen	49	14	17	1	81	63 (77.8%)	66 (81.5%)	0.59
Total	70	169	24	4	267	239 (89.5%)	94 (37.1%)	--

tNGS, targeted next-generation sequencing, Conv, Conventional Methods, including bacterial culture, respiratory viral antigen detection, Chlamydia and Mycoplasma antibody tests, and urinary pneumococcal antigen detection

Table 3 Effect of antibiotics on bacterial detection using tNGS in samples submitted for examination

Bacterial detection status	Were antibiotics used before testing?		Total	*P*
	Yes	No		
Positive	60	6	66	-
Negative	2	0	2	
Total	62	6	-	
Correct detection rate (%)	96.77	100.00	-	0.237

tNGS, targeted next-generation sequencing

detected in throat swab+/BALF- in 9 cases (6.8%), throat swab+/BALF + in 67 cases (50.8%), throat swab-/BALF + in 9 cases (6.8%), and throat swab-/BALF- in 47 cases (35.6%). The sensitivity was 88.16%, specificity was 83.93%, positive predictive value was 88.16%, and negative predictive value was 83.93%. For atypical pathogen detection, Mycoplasmas were detected in throat swab+/BALF- in 0 cases (0%), throat swab+/BALF + in 58 cases (20.8%), throat swab-/BALF + in 5 cases (6.0%), and throat swab-/BALF- in 69 cases (51.6%). The sensitivity was 92.06%, specificity was 100%, positive predictive value was 100%, and negative predictive value was 93%. Detailed results are presented in Table 4; Fig. 1.

Economic cost and invasiveness analysis

Table 5 presents a comparison of the sampling costs between BALF and throat swabs. Throat swab sampling was significantly less costly than BALF collection ($P < 0.05$), and is simple, convenient, and requires fewer materials and reagents, thereby reducing sampling costs. Additionally, swab sampling causes less pain and discomfort for patients, requiring no anesthesia or sedation, as reflected in the significantly lower pain and complexity scores ($P < 0.05$). Moreover, the complication rate for throat swabs was 0%, compared with 12.5% for BALF sampling ($P < 0.05$). These factors contributed to shorter hospital stays and recovery times, thereby reducing overall healthcare costs.

Discussion

ALRTIs represent a leading cause of pediatric hospitalizations and mortality, posing a significant global health challenge. Childrens' immune systems are underdeveloped, making them particularly susceptible to infections [14]. Traditional diagnostic methods, such as bacterial culture, antigen detection, antibody detection, and nucleic acid testing, often fall short in terms of sensitivity, specificity, and comprehensiveness. While these methods may detect specific pathogens, they often fail to simultaneously identify a broad range of bacterial, viral, and atypical pathogens, thus limiting their diagnostic utility in cases of complex or mixed infections [15]. Furthermore, collecting samples from the lower respiratory tract can be invasive and pose risks to patients [16]. These limitations further underscore the urgent need for more accurate, comprehensive, and less invasive diagnostic techniques to improve clinical outcomes in pediatric ALRTIs [17].

In the present study, tNGS detected 68 and 108 cases of bacterial and viral infections, respectively, from bronchoalveolar lavage fluid (BALF) samples, significantly exceeding the 18 and 10 cases detected by traditional methods ($P < 0.001$). This highlights the significant advantage of tNGS over traditional methods, particularly in detecting bacterial and viral pathogens. This result is consistent with the clinical application assessments of other metagenomic next-generation sequencing (mNGS) detection technologies [18, 19]. This is mainly because, compared to traditional microbiological detection methods, tNGS or mNGS, which are based on high-throughput sequencing technology, have the technical advantages of high sensitivity and broad coverage in detecting pathogens in samples; traditional bacterial detection relies on culture methods, which have a low positivity rate and are easily affected by antibiotic use [20]. Our study further found that the bacterial detection rate using tNGS showed no significant difference ($P = 0.237$) in patients receiving antibiotic treatment. This indicates that tNGS is not easily influenced by prior antibiotic use, and provides a strong advantage even in the presence of antibiotics. In terms of viral detection, the sensitivity of antigen testing is low. Additionally, it is limited by the provided antigen kits; in this study, our viral antigens could only detect influenza A, influenza B, respiratory syncytial virus, adenovirus, and parainfluenza virus (types I, II, and III), while common viruses such as rhinovirus and metapneumovirus could not be detected, resulting in a viral detection rate of only 9.1%. Notably, in the present study, we found no statistical difference in the detection of atypical pathogens between the two methods ($P = 0.59$), which is inconsistent with the study by He et al. [21], likely because 67% of the children in this study had a disease duration exceeding 3 days, while corresponding IgM may have already been produced, leading to no significant difference.

A key finding of this study is the promising performance of throat swabs for pathogen detection as compared with BALF samples. Throat swabs demonstrated high sensitivity (95.83%) and moderate specificity (55.95%) in the detection of bacteria, high sensitivity

Table 4 Analysis of the consistency of pathogen detection between throat swabs and BALF samples

	Throat Swab+/BALF+	Throat Swab+/BALF-	Throat Swab-/BALF+	Throat Swab-/BALF-	Sensitivity(%)	Specificity (%)	PPV (%)	NPV (%)
Bacterial Group	**46**	**37**	**2**	**47**	**95.83**	**55.95**	**55.42**	**95.92**
Streptococcus pneumoniae	21	20	2	89				
Haemophilus influenzae	21	13	0	98				
Moraxella catarrhalis	8	11	2	111				
Klebsiella pneumoniae	2	4	1	125				
Staphylococcus aureus	6	6	1	119				
Pseudomonas aeruginosa	1	0	0	131				
Streptococcus intermedius	1	13	1	117				
Streptococcus anginosus	0	4	0	128				
Streptococcus pyogenes	0	1	0	131				
Acinetobacter baumannii	0	16	0	116				
Escherichia coli	0	0	1	131				
Viral Group	**67**	**9**	**9**	**47**	**88.16**	**83.93**	**88.16**	**83.93**
Rhinovirus	23	7	6	96				
RSV	16	2	1	113				
Adenovirus	10	1	3	118				
Herpes simplex virus	11	13	4	104				
Human metapneumovirus	3	0	1	128				
Influenza virus	1	0	0	131				
Parainfluenza virus	7	1	2	122				
Human bocavirus	7	0	3	122				
Human coronavirus	6	2	0	124				
SARS-CoV-2 (COVID-19)	1	0	0	131				
Enterovirus	1	0	0	131				
Cytomegalovirus	1	0	2	129				
Atypical Pathogen	**58**	**0**	**5**	**69**	**92.06**	**100**	**100**	**93**
Mycoplasma pneumoniae	55	0	5	72				
Chlamydia trachomatis	3	0	0	129				

BALF, bronchoalveolar lavage fluid; COVID-19, coronavirus disease 2019; NPV, negative predictive value; PPV, positive predictive value; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

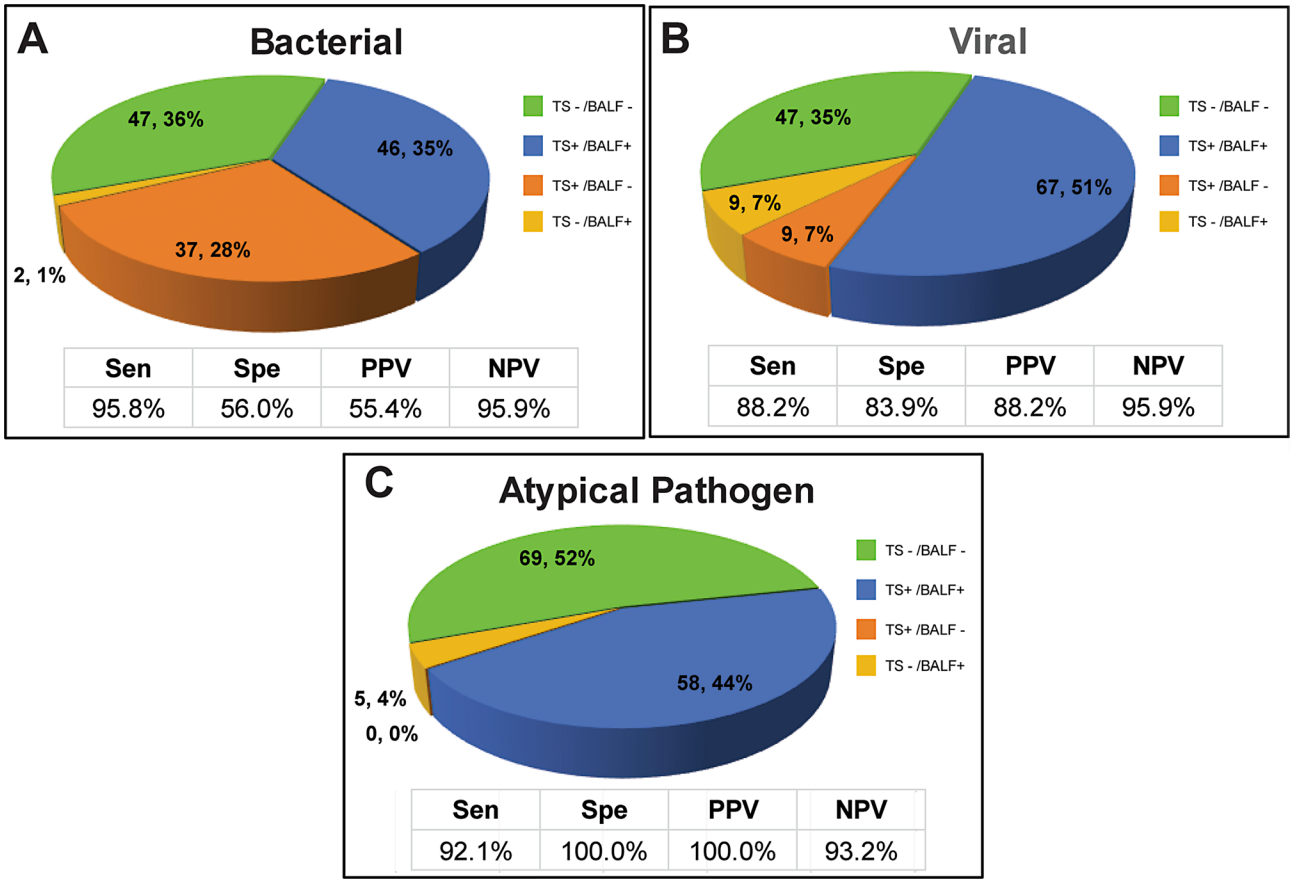

Fig. 1 Comparison of the consistency of pathogen detection between throat swab and BALF samples ($n=132$). BALF, bronchoalveolar lavage fluid; TS, throat swab

Table 5 Economic indicators and trauma comparison between throat swab samples and BALF collection

Index	Pharyngeal Swab	Alveolar Lavage Fluid	*P*
Sampling total cost (yuan)	10.54 ± 5.2	904 ± 622.1	< 0.001
Anesthesia/sedation costs (yuan)	-	443 ± 136.5	-
Sampling cost (yuan)	10.54 ± 5.2	294 ± 29.8	< 0.001
Other expenses (including post-bronchoscopy monitoring, oxygen therapy, etc.)	-	210 ± 23.8	-
Sampling complexity score	1 (simple)	5 (complex)	< 0.001
Pain score (VAS)	1.2 ± 0.3	4.5 ± 1.1	< 0.001
Complication rate (%)	0	12.5	< 0.001

BALF, bronchoalveolar lavage fluid; VAS, visual analog scale

(88.16%) and good specificity (83.93%) in the detection of viruses, and high sensitivity (92.06%) and perfect specificity (100%) in the detection of atypical pathogens. These results suggest that throat swabs, a non-invasive method, provide reliable pathogen detection and serve as a practical alternative to BALF sampling. Our results are consistent with the findings of Ma et al. [22]. It is important to note that tNGS with throat swabs showed moderate specificity (55.95%) for bacterial detection, which may be influenced by bacterial contamination in the upper respiratory tract [23, 24]. Throat swabs are also susceptible to interference from the normal bacterial flora in the oral and upper respiratory tract, resulting in potential false positives. This highlights a key limitation of tNGS, primarily that it may detect pathogens that are not necessarily responsible for ALRTI. While the high sensitivity of tNGS makes it an effective tool for ruling out ALRTI in cases with negative results, positive results should be interpreted cautiously. In clinical applications, the sensitivity and specificity of throat swabs should be considered in conjunction with the patient's clinical presentation and other diagnostic findings for a comprehensive assessment.

Throat swab sampling significantly reduces the invasiveness and costs compared to BALF collection [25]. Throat swab sampling does not require bronchoscopy or anesthesia, reducing patient discomfort and the risk of complications. In addition, economic analysis showed lower costs of throat swab sampling, with fewer material and procedural expenses. These advantages are consistent with the increasing emphasis on patient-centered care and cost-effective medical solutions. This, in turn, can guide more targeted and effective treatment strategies, potentially reducing hospital stays and overall medical expenses. Integrating tNGS with throat swabs into routine diagnostic protocols for pediatric ALRTIs may further lead to significant healthcare cost savings [26].

The present study has several limitations. Although the sample size of 132 pediatric patients was considered suitable for preliminary analysis, it may not be sufficiently large to ensure the generalizability of our findings. Further, this study was conducted at a single center, limiting its external validity; as such, future studies should include larger-scale multicenter cohorts to validate these results. Additionally, the study was conducted over a six-month period, which is relatively short, and thus the data does not account for potential seasonal variations in respiratory pathogens. Seasonal differences may influence the prevalence of specific infections, thereby affecting the positive and negative predictive values of the diagnostic methods. One notable limitation of this study is that we did not compare tNGS with multiplex PCR for respiratory viruses and bacteria, which is currently the standard of care in many settings in which NGS is available. This comparison would have provided more valuable insights into the relative performance of tNGS against current molecular diagnostic standards. As such, further long-term studies across different seasons, incorporating comparisons with multiplex PCR methods, are required to comprehensively evaluate the sustained effectiveness and applicability of tNGS. Furthermore, although we observed overall high consistency between throat swabs and BALF samples, the specificity of bacterial detection was low, which should be addressed in future research.

In conclusion, tNGS demonstrates higher detection rates, particularly for bacterial and viral pathogens, compared with traditional methods. The results of tNGS-based throat swabs show good consistency compared with bronchoalveolar lavage fluid samples. Combined with the lower costs, less invasiveness, and reduced patient discomfort of this technique, tNGS-based analysis of throat swabs offers a promising diagnostic tool. However, it should be noted that this study did not include a comparison with multiplex PCR for respiratory pathogens, which represents the current standard of care in many advanced clinical settings. Further large-scale, multicenter trials incorporating comparison with multiplex PCR methods are required to confirm these findings and refine the routine clinical application of tNGS.

Abbreviations

ALRTI Acute lower respiratory tract infection
BALF Bronchoalveolar lavage fluid
mNGS Metagenomic next-generation sequencing
Tngs Targeted next-generation sequencing
VAS Visual analog scale

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Investigation: Di Lian and Chenye Lin; Data analysis: Di Lian and Chenye Lin; Writing–original draft: Di Lian and Chenye Lin; Methodology: Di Lian and Chenye Lin; Validation: Di Lian and Chenye Lin; Bioinformatics analysis: Di Lian; Data curation: Di Lian, ZhiNan Zhang, and JianXing Wei; Writing–review and editing: Dong Wang; Conceptualization: QiuYu Tang; Project administration: QiuYu Tang; Supervision: QiuYu Tang. All authors read and approved the final manuscript.

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Data availability

The data of the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This retrospective study was conducted on already available data and was approved by the Medical Ethics Committee of Fujian Children's Hospital institutional review board and conducted under the Declaration of Helsinki principles (approval reference: 2024ETKLRK04001). Written informed consent was obtained from all individual participants included in the study. For participants under the age of 18, written informed consent was obtained from their legal guardians.

Consent to publish

Written informed consent was obtained from all individual participants included in the study. For participants under the age of 18, written informed consent was obtained from their legal guardians.

Competing interests

The authors declare no competing interests.

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