International Journal of General Medicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Evaluation of Diagnostic Efficacy of Targeted Next-Generation Sequencing on Nasopharyngeal Swabs in Pediatric Community-Acquired Pneumonia

Cheng Shi[1,2], Juan Zhang[3]

[1]Department of Pediatrics, Tongling Maternal and Child Health Hospital, Tongling City, Anhui Province, 244000, People's Republic of China; [2]The Fourth People's Hospital of Tongling City, The Fourth People's Hospital of Tongling City, Tongling City, Anhui Province, 244000, People's Republic of China; [3]Department of Child Health Care, The Matemity and Child Health Hospital of Ji'an, Ji'an City, Jiangxi Province, 343000, People's Republic of China

Correspondence: Juan Zhang, Email juanzhang232@163.com

Objective: The objective of this study is to evaluate diagnostic efficacy of targeted next-generation sequencing (tNGS) on nasopharyngeal (NP) swabs in pediatric community-acquired pneumonia (CAP).

Methods: We conducted a retrospective analysis, compiling nasal and bronchoalveolar lavage fluid (BALF) specimens from a cohort of 147 children diagnosed with CAP at Tongling Maternal and Child Health Hospital from May 2023 to October 2024. The diagnostic accuracy of nasal tNGS was evaluated and compared against BALF tNGS and conventional microbiological tests (CMT), which included microbial culture and targeted polymerase chain reaction assays.

Results: Nasal tNGS detected a broader range of 25 pathogens, while CMT identified 17, and BALF tNGS identified 21, with significant differences in detection rates favoring tNGS methods ($P<0.001$). Single pathogen detection rates were 35.6% for nasal tNGS and 54.4% for BALF tNGS, with *Mycoplasma pneumoniae* being the most prevalent. Concordance in pathogen detection between nasal swabs and BALF using tNGS was highest for *Human respiratory syncytial virus* (Cohen's kappa, 0.92; 95% CI, 0.81–1), indicating almost perfect agreement.

Conclusion: NP swab-based tNGS technique offers valuable insights for the early etiological diagnosis and treatment of pneumonia, particularly in identifying viral pneumonia.

Keywords: pediatric community-acquired pneumonia, targeted next-generation sequencing, nasopharyngeal swabs, diagnostic efficacy, pathogens

Introduction

Globally, community-acquired pneumonia (CAP) is a widespread and severe acute respiratory infection in children, significantly endangering their health and lives.[1] Identifying CAP early and administering appropriate antibiotics promptly are crucial for enhancing the prognosis in children.[2] Misdiagnosing CAP often leads to the use of unsuitable antibiotics, posing a significant issue in clinical settings.[3] A major contributor to the growth of antimicrobial resistance is the misuse of these antimicrobial agents.[4] Not only does this phenomenon complicate infection treatment, but it also results in a chain of negative effects, including rising healthcare costs and direct negative health impacts on children, which is a crucial issue.

The gold-standard method for diagnosing microbiological issues is through bacteria culture.[5] The traditional fungal culture method, considered the gold standard, is used to diagnose fungal infections.[6] These methods are limited by their inapplicability to noncultivable bacteria and their selectivity for easily cultivable bacteria.[7] In the realm of molecular diagnostics for virus detection, nucleic acid amplification is regarded as the gold standard.[8] Conventional nucleic acid extraction techniques are challenged by multi-step processes, significant time demands, and the need for substantial

Received: 2 April 2025
Accepted: 9 August 2025
Published: 27 August 2025

sample volumes. Despite their effectiveness, commercial kits are expensive. Studies indicate that even with combined techniques, these tests do not significantly increase pathogen detection in children with lower respiratory tract infections.[9–12] Metagenomic Next-Generation Sequencing (mNGS) is the application of NGS technology in clinical microbiological diagnostics to detect nucleic acids.[13] Targeted next-generation sequencing (tNGS) has made significant strides in overcoming the limitations of mNGS, such as the high background of human host nucleic acids, challenges in discriminating colonization, and the associated high costs, by expanding its target spectrum from singleplex to high-multiplex assays.[14–16]

Bronchoalveolar lavage has traditionally been viewed as the definitive method for diagnosing lower respiratory tract infections in children.[17,18] However, collecting BALF in children might require anesthesia and heavily depends on the physician's expertise, which can hinder early and quick pathogen diagnosis. The procedure of nasopharyngeal (NP) swab sampling is simpler, and its use instead of BALF would considerably ease the diagnostic process.[19] However, there are only a handful of case reports documenting the success of tNGS on NP swabs in diagnosing pediatric CAP. Thus, the goal of this research was to examine the diagnostic capability of tNGS in identifying pathogens from NP swabs of children with CAP.

Methods

Study Population

We retrospectively analyzed BALF specimens from 147 children with CAP at Tongling Maternal and Child Health Hospital between May 2023 and October 2024. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Tongling Maternal and Child Health Hospital Ethics Committee (Ethics Approval No. TLFYBJY-2023026). Prior to the commencement of the study, written informed consent was obtained from the parents or legal guardians of all participating subjects, ensuring their comprehensive understanding of the study's purpose, procedures, potential risks, and benefits.

Definitions

Pediatric CAP is an infection that starts outside the hospital, even if caused by pathogens with a specific incubation period that show symptoms after admission, according to the "Guidelines for the Diagnosis and Treatment of Pediatric Community-Acquired Pneumonia (2019 Edition)".[20] The definition of severe CAP includes having at least one major criterion or three minor criteria, as stipulated by the Infectious Diseases Society of America/American Thoracic Society guidelines.[21]

Specimen Acquisition and Examinations

Nasal specimens were collected by inserting the swab tip until resistance was encountered, followed by gentle rotation of the swab several times.[22] BALF specimens were obtained from all patients who underwent bronchoscopic examination, following standardized protocols.[23] Nasal and BALF samples were preserved in the biological specimen storage cabinets at Tongling Fourth People's Hospital. The specimens were subsequently utilized for the detection of pathogens employing both tNGS technique and CMT. DNA/RNA extraction from these samples was performed using an automated nucleic acid extractor, the KingFisher Flex (Thermo Fisher Scientific Inc., MA, USA), following the manufacturer's protocol. The preparation of polymerase chain reaction (PCR) libraries was carried out using the Respiration100TMPlus system (KingCreate, Guangzhou, China). The tNGS detection workflow adhered to the methods described in previous studies.

Statistical Analysis

Categorical data were depicted using percentages. Comparative analyses of categorical and numerical variables were performed using the Chi-square test and the Student's *t*-test, respectively. Statistical significance was defined as a p-value less than 0.05. Interrater agreement between Nasal tNGS and BALF tNGS-detected pathogens was computed utilizing Cohen's Kappa. The statistical analysis was conducted utilizing R software (version 4.4.1), and graphical representations were created with GraphPad software (version 8.1).

Results

Clinical Characteristics

Our research encompassed a total of 147 pediatric patients hospitalized due to CAP. Table 1 shows the clinical characteristics of the study population. The distribution of pediatric patients illustrated infants under 1 year of age accounted for 10 (6.80%) cases, toddlers between 1 and 3 years of age comprised 12 (8.16%) cases, children aged 3 to 5 years made up 43 (29.25%) cases, and children over 5 years of age constituted the majority with 82 (55.78%) cases. There are 83 (56.46%) males and 64 (43.54%) females in the study population. The median length of hospital stay was 6.00 days, with an interquartile range of 5.00 to 7.00 days. Regarding the severity of CAP, 81 (55.10%) patients were classified as having mild CAP, while 66 (44.90%) patients had severe CAP. In terms of signs and symptoms, the majority presented with wet cough (138 patients, 93.88%) and fever (138 patients, 93.88%). Other symptoms included dry cough in 9 (6.12%) patients, chest pain in 4 (2.72%) patients, shortness of breath or chest tightness in 22 (14.97%) patients, hemoptysis or bloody sputum in 9 (6.12%) patients, and fatigue in 17 (11.56%) patients. Radiological findings revealed multi-lobe involvement in 71 (48.30%) patients, multifocal consolidation in 46 (31.29%) patients, airway patency in 72 (48.98%) patients, atelectasis in 35 (23.81%) patients, pleural effusion in 13 (8.84%) patients, and interstitial edema in 5 (3.40%) patients.

Comparison of the Diagnostic Performances of CMT, Nasal tNGS, and BALF tNGS

In our study focused on pediatric patients with CAP, we utilized three distinct diagnostic approaches to detect a spectrum of pathogens (Figure 1). The CMT method identified 17 different pathogens. Nasal tNGS, on the other hand, detected a broader

Table 1 Baseline Characteristics of Hospitalized Children with Community-Acquired Pneumonia

Clinical Characteristics	
Age	
<1y	10 (6.80%)
1-3y	12 (8.16%)
3-5y	43 (29.25%)
>5y	82 (55.78%)
Gender	
Male	83 (56.46%)
Female	64 (43.54%)
Length of stay, days	6.00 (5.00–7.00)
Severity	
Mild CAP	81 (55.10%)
Severe CAP	66 (44.90%)
Signs and symptoms	
Dry cough	9 (6.12%)
Wet cough	138 (93.88%)
Chest pain	4 (2.72%)
Shortness of breath or chest tightness	22 (14.97%)
Hemoptysis or bloody sputum	9 (6.12%)
Fever	138 (93.88%)
Fatigue	17 (11.56%)
Imaging features	
Multi-lobe involvement	71 (48.30%)
Multifocal consolidation	46 (31.29%)
Airway patency	72 (48.98%)
Atelectasis	35 (23.81%)
Pleural effusion	13 (8.84%)
Interstitial edema	5 (3.40%)

Abbreviations: CAP, community-acquired pneumonia; tNGS, targeted next-generation sequencing.

https://doi.org/10.2147/IJGM.S531908

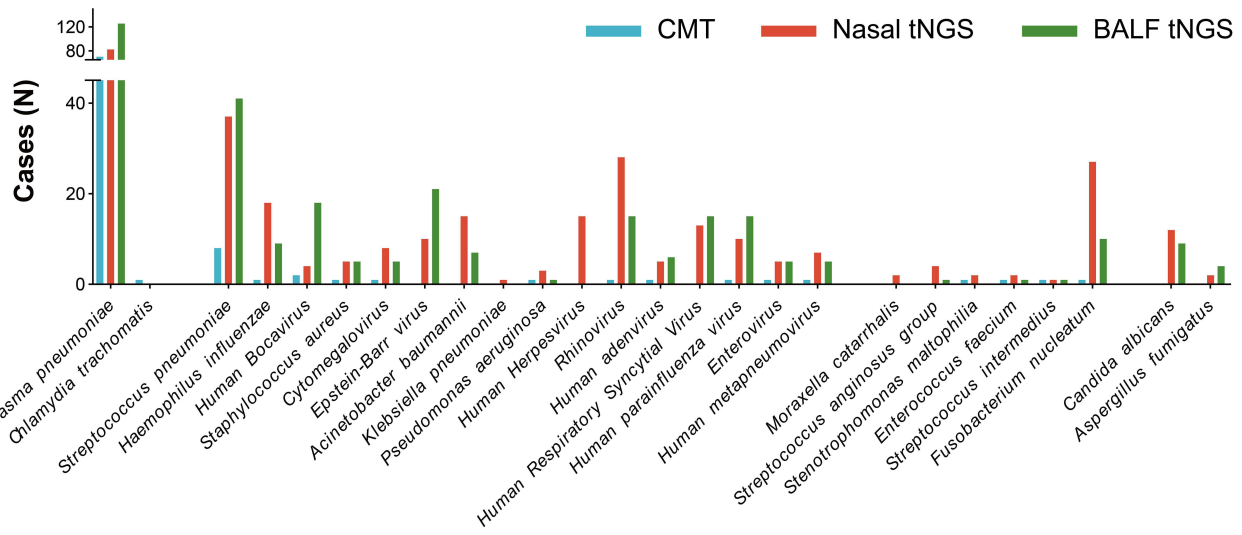

Figure 1 Pathogen distribution among CMT, nasal tNGS, and BALF tNGS.
Abbreviations: CMT, conventional microbiological tests; tNGS, targeted next-generation sequencing; BALF, bronchoalveolar lavage fluid.

range of 25 distinct pathogens, and BALF tNGS identified 21 unique pathogens. There was an overlap, with 15 pathogens being detected by all three methods (Figure S1). CMT uniquely identified Chlamydia trachomatis, while nasal tNGS was the only method to detect Klebsiella pneumoniae, Human herpesvirus, and Moraxella catarrhalis, as detailed in Figure S1. In the study, CMT identified pathogens in 91 cases, which accounts for 61.90% of the participants. For those who underwent both nasal tNGS and BALF tNGS testing, pathogens were detected in 144 (98.00%) cases and 147 (100.00%) cases, respectively. The detection rates for both Nasal tNGS and BALF tNGS were significantly higher than those for CMT, with the differences being statistically significant (P<0.001) (Table S1).

Among these cases, the proportion of patients with a single pathogen detected by nasal tNGS and BALF tNGS was 35.6% and 54.4%, respectively. For patients with two pathogens, the proportions were 41.1% and 14.3%, respectively. For three pathogens, the proportions were 15.1% and 23.1%, and for four pathogens, they were 6.8% and 8.1% (Figure 2A). Among the single pathogen cases, *Mycoplasma pneumoniae* had the highest prevalence, with 71.1% detected by nasal tNGS and 81.3% detected by BALF tNGS (Figure 2B).

Concordance in Pathogen Detection Between NP Swabs and BALF Utilizing tNGS

Table 2 illustrates the comparative analysis of the detection of various pathogens using nasal tNGS and BALF tNGS. The two diagnostic methods exhibited almost perfect agreement in the detection of *Human respiratory syncytial virus*, with a Cohen's kappa of 0.92 (95% confidence interval, CI, 0.81–1). Substantial agreement was observed between the tests for *Human metapneumovirus* (Cohen's kappa, 0.83; 95% CI, 0.59–1), *Aspergillus fumigatus* (Cohen's kappa, 0.66; 95% CI, 0.22–1), and *Human parainfluenza virus* (Cohen's kappa, 0.61; 95% CI, 0.38–0.84). Moderate agreement was noted for *Rhinovirus* (Cohen's kappa, 0.6; 95% CI, 0.42–0.78), *Cytomegalovirus* (Cohen's kappa, 0.6; 95% CI, 0.28–0.92), *Haemophilus influenzae* (Cohen's kappa, 0.56; 95% CI, 0.33–0.78), *Epstein-Barr virus* (Cohen's kappa, 0.55; 95% CI, 0.32–0.79), and *Human adenovirus* (Cohen's kappa, 0.53; 95% CI, 0.16–0.89). Fair agreement was found between the two tests for *Candida albicans* (Cohen's kappa, 0.39; 95% CI, 0.12–0.65), *Streptococcus pneumoniae* (Cohen's kappa, 0.38; 95% CI, 0.1–0.67), and *Mycoplasma pneumoniae* (Cohen's kappa, 0.33; 95% CI, 0.21–0.46).

Discussion

In children, respiratory illnesses are the most common, with pneumonia being a major reason for hospitalization, particularly in those under five, where it is a significant cause of illness and death.[24] CAP accounts for the majority of these cases, with varying incidence and mortality rates across countries, often higher in developing nations.[25] Therefore,

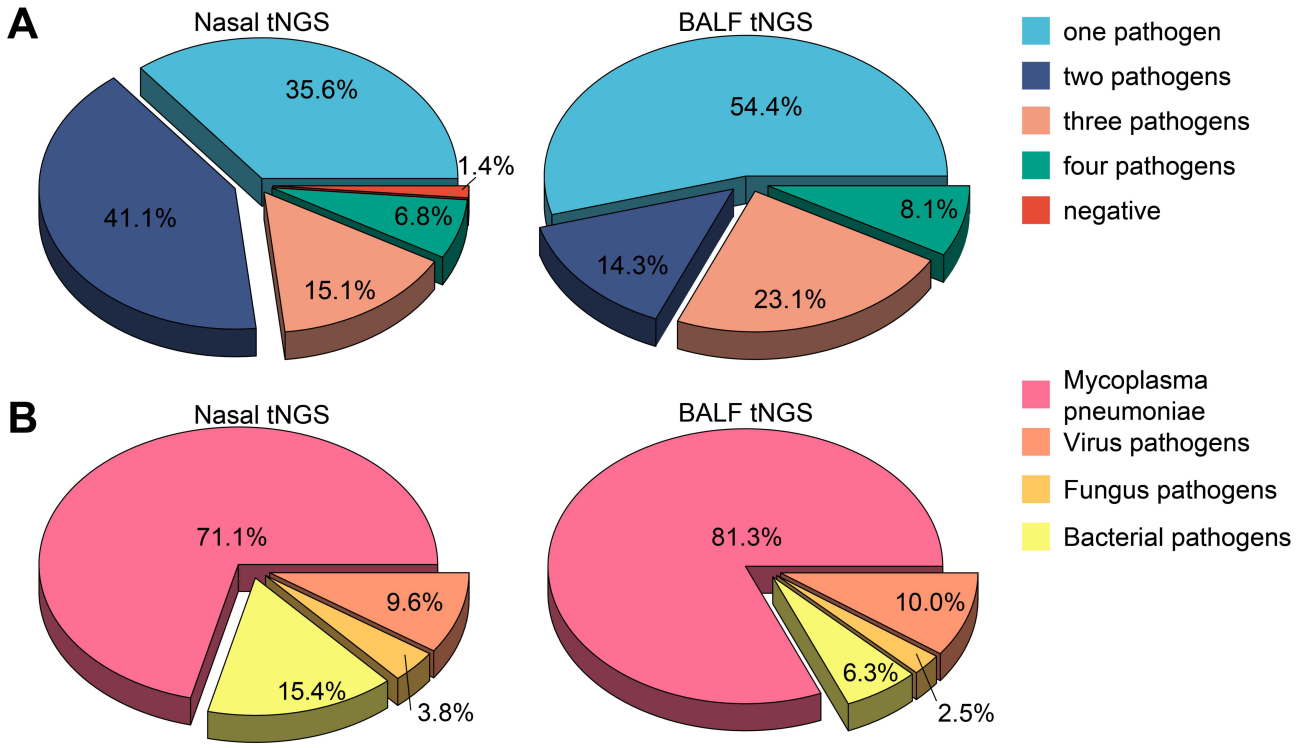

Figure 2 (A) The number of pathogens detected through nasal tNGS and BALF tNGS, (**B**) In the context of single-pathogen cases, the distribution of various pathogens detected by nasal tNGS and BALF tNGS.
Abbreviations: tNGS, targeted next-generation sequencing; BALF, bronchoalveolar lavage fluid.

rapid and accurate detection of pneumonia pathogens is of significant value in improving the prognosis of pediatric patients with pneumonia.

Specimens such as NP swabs, oropharyngeal swabs, sputum, peripheral blood, and BALF are typically used in testing for the etiology of pediatric pneumonia. Known for their simple collection and non-invasiveness, NP swabs have shown considerable diagnostic effectiveness in detection of Severe Acute Respiratory Syndrome Coronavirus 2 and influenza virus pneumonia.[26,27] BALF serves as an essential diagnostic tool, offering precise insights into the causative agents of pneumonia.[28] The invasive nature of BALF requires skilled practitioners, and it is often performed on children under anesthesia, complicating the early identification of disease causes.[29] Simultaneously, using empiric antibiotic therapy, which is both harmless and affordable, can be a practical alternative to invasive methods.[30]

The human nasopharynx hosts a diverse microbial ecosystem of normal flora, which typically includes Streptococcus, Haemophilus, Staphylococcus, Moraxella, and Corynebacterium species.[31,32] The body's defense against pathogenic and opportunistic infections is supported by these resident microbiota, which function as a vital microbial barrier.[33] NP swabs are commonly used for viral detection,[34] but there are few studies on their effectiveness in diagnosing pathogens in children with lower respiratory tract infections. The PERCH (Pneumonia Etiology Research for Child Health) study primarily utilized nasopharyngeal and oropharyngeal (NP-OP) swabs analyzed via multiplex PCR as a cornerstone for etiological inference.[35] Critically, PERCH demonstrated that while NP-OP detection alone has limitations—particularly for bacteria like *Streptococcus pneumoniae* and *Haemophilus influenzae* where colonization complicates interpretation. PERCH also highlighted the necessity of supplementary specimens to augment NP-OP findings for bacterial pathogens, as NP-OP data alone lacked sufficient discriminatory power for bacteria without density thresholds and adjustment for antibiotic exposure. Thus, while PERCH validated NP swabs as a pragmatic tool for viral identification in resource-limited settings, its design emphasized that robust etiology assessment requires contextualizing NP-OP data within a multi-specimen, analytically advanced framework to delineate pathogen-specific contributions accurately. Our study found that nasal tNGS had a significantly higher pathogen detection rate compared to conventional microbiological testing (CMT) methods (98.00% vs 61.90%, P<0.001). Using Cohen's Kappa to

Table 2 Comparative Analysis of Detection and Concordance of Various Pathogens by Nasopharyngeal Swabs and BALF tNGS

Pathogens	Nasal+ BALF+	Nasal+ BALF-	Nasal− BALF+	Nasal− BALF-	Sensitivity	Specificity	PPV	Cohen κ (95% CI)	P Value (κ)
Mycoplasma pneumoniae	81	1	44	21	0.65	0.95	0.99	0.33 (0.21–0.46)	<0.001
Haemophilus influenzae	8	10	1	128	0.89	0.93	0.44	0.56 (0.33–0.78)	<0.001
Rhinovirus	14	14	1	118	0.93	0.89	0.50	0.6 (0.42–0.78)	<0.001
Human Herpesvirus	0	15	0	132	-	-	-	-	-
Human Respiratory Syncytial Virus	13	0	2	132	0.87	1.00	1.00	0.92 (0.81–1)	<0.001
Epstein-Barr virus	8	2	9	128	0.47	0.98	0.80	0.55 (0.32–0.79)	<0.001
Human parainfluenza virus	8	2	7	130	0.53	0.98	0.80	0.61 (0.38–0.84)	<0.001
Cytomegalovirus	4	4	1	138	0.80	0.97	0.50	0.6 (0.28–0.92)	0.0002
Human metapneumovirus	5	2	0	140	1.00	0.99	0.71	0.83 (0.59–1)	<0.001
Human adenvirus	3	2	3	139	0.50	0.99	0.60	0.53 (0.16–0.89)	0.0045
Enterovirus	1	4	4	138	0.20	0.97	0.20	0.17 (−0.17–0.51)	0.3211
Human Bocavirus	2	2	13	130	0.13	0.98	0.50	0.18 (−0.07–0.42)	0.1541
Candida albicans	5	7	6	129	0.45	0.95	0.42	0.39 (0.12–0.65)	0.0046
Aspergillus fumigatus	2	0	2	143	0.50	1.00	1.00	0.66 (0.22–1)	0.0032
Acinetobacter baumannii	1	14	5	127	0.17	0.90	0.07	0.04 (−0.14–0.22)	0.6633
Streptococcus pneumoniae	4	8	3	132	0.57	0.94	0.33	0.38 (0.1–0.67)	0.0089
Fusobacterium nucleatum	2	10	1	134	0.67	0.93	0.17	0.24 (−0.05–0.53)	0.1011
Staphylococcus aureus	1	4	4	138	0.20	0.97	0.20	0.17 (−0.17–0.51)	0.3211
Streptococcus anginosus group	1	3	0	143	1.00	0.98	0.25	0.39 (−0.15–0.93)	0.1534
Pseudomonas aeruginosa	1	2	0	144	1.00	0.99	0.33	0.49 (−0.11–1)	0.106
Moraxella catarrhalis	0	2	0	145	-	-	-	-	-
Stenotrophomonas maltophilia	0	2	0	145	-	-	-	-	-
Enterococcus faecium	0	2	1	144	-	-	-	-	-
Klebsiella pneumoniae	0	1	0	146	-	-	-	-	-
Streptococcus intermedius	0	1	1	145	-	-	-	-	-

Abbreviations: BALF, bronchoalveolar lavage fluid; tNGS, targeted next-generation sequencing.

assess the diagnostic agreement between nasal tNGS and BALF tNGS for various pathogens, we observed good concordance for *Human metapneumovirus, Aspergillus fumigatus, Human parainfluenza virus, Rhinovirus, Cytomegalovirus, Haemophilus influenzae, Epstein-Barr virus, Human adenovirus, Candida albicans, Streptococcus pneumoniae*, and *Mycoplasma pneumoniae*.

Limitations

As this is a retrospective study, we are currently unable to obtain data on patient outcomes and secondhand smoke exposure. This study lacks formal turnaround time assessment and antimicrobial resistance gene detection, particularly concerning given the issue of antibiotic misuse in pediatric community-acquired pneumonia. Future investigations should evaluate TAT within real-world clinical workflows and incorporate genomic resistance markers to enhance targeted therapeutic strategies.

Conclusions

NP swab-based tNGS emerges as a highly effective diagnostic tool for pediatric CAP, demonstrating superior detection rates and strong concordance with BALF tNGS, particularly in identifying viral pathogens and providing early etiological diagnosis. This approach holds promise for streamlining the diagnostic process and informing timely treatment decisions in pediatric pneumonia management.

Data Sharing Statement

All original data in this study can be obtained from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Tongling Maternal and Child Health Hospital Ethics Committee (Ethics Approval No. TLFYBJY-2023026). Prior to the commencement of the study, written informed consent was obtained from the parents or legal guardians of all participating subjects, ensuring their comprehensive understanding of the study's purpose, procedures, potential risks, and benefits.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

No funding was received for this research.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Torres A, Cilloniz C, Niederman MS, et al. Pneumonia. *Nat Rev Dis Primers*. 2021;7:25. doi:10.1038/s41572-021-00259-0
2. Wu J, Song X, Hu Y, Chen J, Jiang L. High-risk factors associated with refractory childhood bacterial meningitis in Southwest China. *BMC Pediatr*. 2023;23:220. doi:10.1186/s12887-023-04007-z
3. Atallah J, Mansour MK. Implications of Using Host Response-Based Molecular Diagnostics on the Management of Bacterial and Viral Infections: a Review. *Front Med Lausanne*. 2022;9:805107. doi:10.3389/fmed.2022.805107
4. Carai S, Kuttumuratova A, Boderscova L, et al. The integrated management of childhood illness (IMCI) and its potential to reduce the misuse of antibiotics. *J Glob Health*. 2021;11:04030. doi:10.7189/jogh.11.04030
5. Grąźlewska W, Ferra B, Rudzińska M, Holec-Gąsior L. Borrelia burgdorferi BmpA-BBK32 and BmpA-BBA64: new Recombinant Chimeric Proteins with Potential Diagnostic Value. *Pathogens*. 2021;10:767. doi:10.3390/pathogens10060767
6. Wang L, Xu A, Zhou P, et al. Rapid Detection of Candida tropicalis in Clinical Samples From Different Sources Using RPA-LFS. *Front Cell Infect Microbiol*. 2022;12:898186. doi:10.3389/fcimb.2022.898186
7. Xu P, Shi Y, Liu P, et al. 16S rRNA gene sequencing reveals an altered composition of the gut microbiota in chickens infected with a nephropathogenic infectious bronchitis virus. *Sci Rep*. 2020;10:3556. doi:10.1038/s41598-020-60564-8
8. Hu X, Jiang N, Li Y, et al. Rapid Nucleic Acid Extraction for Aquatic Animal DNA Virus Determination Using Chelex 100 Resin via Conventional PCR and Digital Droplet PCR Detection. *Animals*. 2022;12:1999. doi:10.3390/ani12151999
9. Montaner AE, García de Lomas J, Villa Asensi JR, et al. EPI-Strep-064 study group, Bacteria from bronchoalveolar lavage fluid from children with suspected chronic lower respiratory tract infection: results from a multi-center, cross-sectional study in Spain. *Eur J Pediatr*. 2018;177:181–192. doi:10.1007/s00431-017-3044-3
10. Granizo JJ, Giménez MJ, Barberán J, Coronel P, Gimeno M, Aguilar L. The efficacy of cefditoren pivoxil in the treatment of lower respiratory tract infections, with a focus on the per-pathogen bacteriologic response in infections caused by Streptococcus pneumoniae and Haemophilus influenzae: a pooled analysis of seven clinical trials. *Clin Ther*. 2006;28:2061–2069. doi:10.1016/j.clinthera.2006.12.010
11. Strålin K, Korsgaard J, Olcén P. Evaluation of a multiplex PCR for bacterial pathogens applied to bronchoalveolar lavage. *Eur Respir J*. 2006;28:568–575. doi:10.1183/09031936.06.00006106
12. Sakurai N, Nagayama Y, Honda A, Makuta M, Yamamoto K, Kojima S. Mycoplasma pneumoniae and other pathogens in the aetiology of lower respiratory tract infections among Japanese children. *J Infect*. 1988;16:253–261. doi:10.1016/s0163-4453(88)97604-9
13. Deng Q-M, Zhang J, Zhang -Y-Y, et al. Diagnosis and treatment of refractory infectious diseases using nanopore sequencing technology: three case reports. *World J Clin Cases*. 2024;12:5208–5216. doi:10.12998/wjcc.v12.i22.5208
14. Tan J, Chen Y, Lu J, et al. Pathogen distribution and infection patterns in pediatric severe pneumonia: a targeted next-generation sequencing study. *Clin. Chim. Acta*. 2025;565:119985. doi:10.1016/j.cca.2024.119985
15. Diao Z, Han D, Zhang R, Li J. Metagenomics next-generation sequencing tests take the stage in the diagnosis of lower respiratory tract infections. *J Adv Res*. 2022;38:201–212. doi:10.1016/j.jare.2021.09.012
16. Hong H-L, Flurin L, Thoendel MJ, et al. Targeted Versus Shotgun Metagenomic Sequencing-based Detection of Microorganisms in Sonicate Fluid for Periprosthetic Joint Infection Diagnosis. *Clin Infect Dis*. 2023;76:e1456–e1462. doi:10.1093/cid/ciac646
17. Radha S, Afroz T, Prasad S, Ravindra N. Diagnostic utility of bronchoalveolar lavage. *J Cytol*. 2014;31:136–138. doi:10.4103/0970-9371.145636

18. Siddiqui SS, Sharma T, Khurana AK, et al. Bronchoalveolar Lavage in Diagnostic Evaluation of Pulmonary Diseases- An Institutional Experience. *J Cytol*. 2023;40:68–74. doi:10.4103/joc.joc_90_22
19. Azadeh N, Sakata KK, Saeed A, et al. Comparison of Respiratory Pathogen Detection in Upper versus Lower Respiratory Tract Samples Using the BioFire FilmArray Respiratory Panel in the Immunocompromised Host. *Can Respir J*. 2018;2018:2685723. doi:10.1155/2018/2685723
20. Ni X. Guidelines for the diagnosis and treatment of community-acquired pneumonia in children (2019. *Clin Educ Gen Pract*. 2019;771–777. doi:10.13558/j.cnki.issn1672-3686.2019.09.002
21. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med*. 2019;200:e45–e67. doi:10.1164/rccm.201908-1581ST
22. Lindner AK, Nikolai O, Rohardt C, et al. Head-to-head comparison of SARS-CoV-2 antigen-detecting rapid test with professional-collected nasal versus nasopharyngeal swab. *Eur Respir J*. 2021;57:2004430. doi:10.1183/13993003.04430-2020
23. Dickson RP, Schultz MJ, van der Poll T, et al. Lung Microbiota Predict Clinical Outcomes in Critically Ill Patients. *Am J Respir Crit Care Med*. 2019d;201:555–563. doi:10.1164/rccm.201907-1487OC
24. Koenen MH, de Steenhuijsen Piters WAA, Jonge MI, et al. Salivary polyreactive antibodies and Haemophilus influenzae are associated with respiratory infection severity in young children with recurrent respiratory infections. *Eur Respir J*. 2024;64:2400317. doi:10.1183/13993003.00317-2024
25. Li F, Guo L, Li Q, et al. Changes in the epidemiology and clinical characteristics of viral gastroenteritis among hospitalized children in the Mainland of China: a retrospective study from 2016 to 2020. *BMC Pediatr*. 2024;24:303. doi:10.1186/s12887-024-04776-1
26. Li C, Wang F, Li W, et al. The diagnostic value of metagenomic next-generation sequencing in critically ill patients with sepsis: a retrospective cohort study. *Medicine (Baltimore)*. 2024;103:e39987. doi:10.1097/MD.0000000000039987
27. Deng Z, Li C, Wang Y, et al. Targeted next-generation sequencing for pulmonary infection diagnosis in patients unsuitable for bronchoalveolar lavage. *Front Med*. 2023;10:1321515. doi:10.3389/fmed.2023.1321515
28. Wu X, Tan G, Sun C, et al. *Targeted Next-Generation Sequencing - a Promising Approach in the Diagnosis of Mycobacterium Tuberculosis and Drug resistance, Infection*. 2024. doi:10.1007/s15010-024-02411-w
29. Wang Y-S, Zhou Y-L, Bai G-N, et al. Expert consensus on the diagnosis and treatment of macrolide-resistant Mycoplasma pneumoniae pneumonia in children. *World J Pediatr*. 2024;20:901–914. doi:10.1007/s12519-024-00831-0
30. Sovtic A, Grba T, Grahovac D, Minic P. Flexible Bronchoscopy in Evaluation of Persistent Wheezing in Children-Experiences from National Pediatric Center. *Medicina*. 2020;56:329. doi:10.3390/medicina56070329
31. Sakwinska O, Schmid VB, Berger B, et al. Nasopharyngeal microbiota in healthy children and pneumonia patients. *J Clin Microbiol*. 2014;52:1590–1594. doi:10.1128/JCM.03280-13
32. Dai W, Wang H, Zhou Q, et al. The concordance between upper and lower respiratory microbiota in children with Mycoplasma pneumoniae pneumonia. *Emerg Microbes Infect*. 2018;7:92. doi:10.1038/s41426-018-0097-y
33. Lu Z, Dai W, Liu Y, et al. The Alteration of Nasopharyngeal and Oropharyngeal Microbiota in Children with MPP and Non-MPP. *Genes (Basel)*. 2017;8:380. doi:10.3390/genes8120380
34. Suwaidi HA, Senok A, Varghese R, et al. Saliva for molecular detection of SARS-CoV-2 in school-age children. *Clin Microbiol Infect*. 2021;27:1330–1335. doi:10.1016/j.cmi.2021.02.009
35. O'Brien KL, Baggett HC, Brooks WA, et al. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*. 2019;394:757–779. doi:10.1016/S0140-6736(19)30721-4