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Targeted next-generation sequencing characterization of respiratory pathogens in children with acute respiratory infection

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Abstract

Background Acute respiratory infections (ARIs) pose a significant global health burden, particularly affecting infants and young children with high morbidity and mortality rates. Targeted next-generation sequencing (tNGS) has emerged as a rapid and cost-effective diagnostic tool capable of identifying a broad range of respiratory tract infections.

Methods Oropharyngeal swabs and sputum samples were collected from patients and subjected to tNGS and sputum culture, respectively, for diagnosing ARIs. A retrospective analysis was conducted on clinical data to explore the clinical diagnosis and therapeutic application of tNGS.

Results This study included 336 pediatric patients with confirmed ARIs. tNGS detected 38 potential pathogens, comprising 25 species (15 bacteria and 10 viruses) and 13 viral subtypes. The overall microbial detection rate using tNGS was 100%. The leading bacterial pathogens identified were *Streptococcus pneumoniae* (36.0%), *Stenotrophomonas maltophilia* (30.4%), *Streptococcus intermedius* (29.5%), *Moraxella catarrhalis* (27.1%), and *Hemophilus influenzae* (20.2%). The predominant viral pathogens included human adenovirus (31.3%), human rhinovirus (26.5%), human parainfluenza virus (25.0%), cytomegalovirus (19.0%), and human bocavirus (11.0%). Among the 94 patients who underwent simultaneous sputum culture and Gram staining, tNGS exhibited a superior detection rate compared to sputum culture (100% vs. 53.2%). Among the 50 patients with concordant positive results for both tNGS and sputum culture, 80% (40/50) demonstrated full or partial agreement. Additionally, tNGS revealed age-specific heterogeneity in pathogen distribution across different age groups.

Conclusion Traditional diagnostic methods often fall short of meeting the diagnostic demands of ARIs. This study underscores the potential of tNGS in oropharyngeal swabs for enhancing pathogen detection, thereby improving the diagnosis, treatment, and prevention of ARIs.

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Importance This study represents the first investigation utilizing oropharyngeal swabs for tNGS in diagnosing and treating ARIs. By analyzing surveillance data from a local hospital's patients with ARIs, we have identified the spectrum of bacterial and viral pathogens and explored demographic differences among patients. These findings underscore the potential of tNGS in ARI surveillance, diagnosis, pathogen detection, and prevention.

Keywords Acute respiratory infections, Targeted next-generation sequencing, Pharyngeal swabs, Pathogen spectrum, Diagnosis

Introduction

Acute respiratory infections (ARIs) represent a significant global health burden, particularly impacting infants and young children with high morbidity and mortality rates [1–3]. Timely and accurate pathogen detection is crucial for the clinical diagnosis and treatment of ARIs. Traditional testing methods, such as culture, are hampered by limitations, including time consumption and the inability to detect unculturable bacteria. Viruses are also just detectable by molecular techniques, with the exception of highly time-consuming methods such as cell culture [4]. Antigen-antibody detection also faces challenges such as testing times and accuracy issues due to prolonged target generation windows [5]. Additionally, antibody detection necessitates the performance of tests at a minimum of two distinct time points. Furthermore, molecular diagnostic methods like multiplex polymerase chain reaction (PCR) can detect numerous pathogens within two hours but are limited in their capacity for pathogen diversity and genotyping [6].

On the other hand, next-generation sequencing (NGS) has emerged as a promising technology for pathogen detection, offering higher yields, shorter turnaround times (TAT), and more comprehensive genomic information [7]. Among these methods, metagenomic NGS (mNGS) has shown high efficiency in detecting pathogens associated with ARIs [8–10]. However, mNGS faces inherent challenges such as significant amounts of host nucleic acid, high costs, considerations regarding sequencing depth, external contamination and internal or cross contamination, and a lack of standardized interpretation protocols, which limit its widespread clinical applications [11]. Considering the sensitivity and cost-effectiveness of mNGS in pathogen detection, targeted NGS (tNGS), which combines PCR and NGS technologies, has the potential to enhance pathogen signals while reducing the amount of sequencing data. This dual optimization aims to improve detection performance while managing costs. Studies have demonstrated that tNGS performs comparably to mNGS in detecting respiratory pathogens from bronchoalveolar lavage fluid specimens (BALF) [12]. Furthermore, tNGS costs only a fraction of mNGS expenses while covering > 95% of respiratory infections [13]. Recent research from our study indicates that tNGS achieves a significantly higher microbial detection rate compared to conventional microbiological tests (CMTs) (96.7% vs. 36.8%) in pulmonary infections. Moreover, there was an 86.8% full or partial agreement in concordant positive results between tNGS and CMTs [14].

While current tNGS studies predominantly focus on BALF [12] and sputum [14], there are limited investigations into the clinical diagnostic potential of tNGS using pharyngeal swabs for ARIs. Therefore, our study aimed to assess the performance of tNGS in diagnosing pathogens associated with ARIs. Using a tNGS assay targeting 95 pathogens (Supplementary Table S1), we explored its clinical diagnosis and therapeutic application in children with ARIs. Additionally, we sought to explore the heterogeneity in pathogen distribution within the study cohort based on tNGS results.

Methods

Study design

This retrospective case series analyzed 336 oropharyngeal swabs (OPS) and 94 sputum samples collected between July and December 2022 at Binyang Women and Children's Hospital in China. OPS and sputum samples were subjected to tNGS and sputum culture, respectively. Researchers comprehensively reviewed clinical data from each patient diagnosed with ARIs who had undergone tNGS. The study received approval from the local Ethics Committee and adhered to the principles outlined in the 1990 Declaration of Helsinki and its subsequent amendments. All the data used in this study were anonymized and exclusively utilized for analysis in this paper. Strict measures were implemented to maintain patient confidentiality, including obtaining informed consent from all participants.

The inclusion criteria for the study were: (i) patients diagnosed with ARIs; (ii) use of tNGS for pathogen diagnosis; (iii) availability of complete clinical data; and (iv) patients who provided informed consent to participate. The exclusion criteria included: (i) patients who declined sample collection for tNGS; (ii) OPS that did not meet tNGS quality standards; and (iii) patients with incomplete clinical data.

Sample collection

A flocked swab was recommended for the collection of OPS specimens. Patients were instructed to gargle with normal saline. If mouth-rinsing was unsuccessful,

patients were asked to either spit out or swallow a small amount of water. To expose the oropharynx, patients were instructed to open their mouth and make the "ah" sound, or a tongue depressor was used to hold down the tongue. The swab is then guided across the base of the tongue to the posterior pharyngeal wall, or the posterior uvula. The posterior pharyngeal wall and both sides of the tonsils were wiped moderately, with the swab rotated appropriately to increase contact area while avoiding the tongue. OPS were placed in viral transport medium (VTM) or other buffers. For infants or young children who are unable to expectorate sputum through coughing, a disposable suction catheter was utilized to aspirate the sputum using negative pressure. It is emphasized that all these procedures were conducted exclusively by physicians trained according to specific collection protocols. Approximately one to two OPS were collected and stored at − 20 °C within 48 h for subsequent tNGS analysis. Approximately 1–3 mL of sputum was collected for smear microscopy and culture.

tNGS

Sample Preparation

A 1.3-mL volume of the sample was collected into a 1.5-mL centrifuge tube. The mixture was homogenized for 15 s using a vortex mixer and then centrifuged at 12,000 rpm for five minutes to enrich the pathogens. Concurrently, positive, negative, and empty controls from the Respiratory Pathogen Detection Kit (KS608-50SHXD96, KingCreate, Guangzhou, China) were included to monitor the entire experimental process of tNGS.

Nucleic acid extraction

After removing the supernatant, 500 μL of the homogenate was used for total nucleic acid extraction and purification using the MagPure Pathogen DNA/RNA Kit (R6672-01B, Magen, Guangzhou, China), following the manufacturer's protocol.

Library construction, sequencing, and bioinformatics analysis

The library was constructed using the Respiratory Pathogen Detection Kit (KS608-50SHXD96, KingCreate, Guangzhou, China). This process involved cDNA synthesis and two rounds of PCR amplification. The sample nucleic acids and cDNA served as templates, and a set of 95 microorganism-specific primers were selected for ultra-multiplex PCR amplification to enrich the target pathogen sequences, spanning bacteria, viruses, mycoplasma, and chlamydia. After the first round of amplification, PCR products underwent purification with magnetic beads to screen target fragments, followed by the second round of amplification using primers containing sequencing adapters. After the purification, generated libraries were quantified using Qubit dsDNA HS Assay Kit (Q32854, Thermo Fisher Scientific, Massachusetts, United States) with Qubit 4.0 fluorometer (Thermo Fisher Scientific, Massachusetts, United States) to ensure all samples with library concentration ≥ 0.5 ng/μL. The quality of the mixed library was evaluated using the Qsep100 Bio-Fragment Analyzer (Bioptic, Taiwan, China). Generally, the mixed library fragments were approximately 250–350 bp in size. The concentration of the mixed library was requantified and then diluted to a final concentration of 1 nmol/L. Subsequently, 5 μL of the mixed library was combined with 5 μL of freshly prepared NaOH (0.1 mol/L). After briefly vortexing and centrifuging, the library was incubated at room temperature for 5 min. After the mixed library was diluted to a final loading concentration of 1.0 pM, a single-end 100 bp sequencing was carried out on the Illumina MiniSeq Platform using the universal sequencing reagent kit (KS107-CXR, KingCreate, Guangzhou, China), with an average of 0.1 million sequencing reads per sample.

Sequencing data underwent analysis utilizing the data management and analysis system (v3.7.2, KingCreate). Initially, the raw data were identified by the adapter, retaining reads with a single-end length exceeding 50 bp. Subsequently, low-quality filtering was performed to retain reads with Q30 > 75% as high-quality data. The aligned single-ended reads were then compared with the Self-Building clinical pathogen database to ascertain the read count of the specific amplification target present in each sample. The reference sequences employed for read mapping were derived from a meticulously curated database, which integrates data from various sources, including GenBank database, RefSeq database, and Nucleotide database from NCBI.

Interpretation of tNGS results

The interpretation of the tNGS results adhered to the experimental principle of amplifying microbial sequences using specific primers. Key indicators for interpretation included amplicon coverage and normalized read counts of detected microorganisms within the sample. Reporting criteria for infectious pathogens identified by tNGS were as follows: (i) for bacteria and atypical pathogens, amplicon coverage ≥ 50% and normalized read count ≥ 10; (ii) for viruses, amplicon coverage ≥ 50% and normalized read count ≥ 3, or alternatively normalized read count ≥ 10. Following this, two experienced clinicians independently assessed the clinical data of the patients to determine the presence of a respiratory tract infection and the clinical relevance of potential pathogens. This assessment incorporated the medical history, symptoms, imaging findings, and tNGS results of the patients. In

cases where interpretations differed, consultation with a senior physician was sought to reach consensus.

Data management and statistical analyses

Data management and statistical analyses involved defining three age groups: infants and toddlers (0–3 years old), preschool-age children (4–6 years old), and school-age children (7–11 years old). Quantitative variables were described using medians and ranges, while categorical variables were presented as numbers and percentages. Statistical analyses were conducted using IBM SPSS Statistics for Windows (version 20.0; IBM Corp., Armonk, NY, USA). A significance level of $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 372 patients diagnosed with ARIs and undergoing tNGS were initially considered for review and potential enrollment in this study. Of these, 36 patients were excluded for various reasons, including repetition ($n = 10$), non-respiratory infectious diseases ($n = 5$), and incomplete data ($n = 21$). Consequently, a definitive cohort of 336 patients met the enrollment criteria and underwent further analysis (Fig. 1). The median age of the cohort was two years, with 209 (62.2%) patients being male. Of 336 patients in the retrospective study, 13 (3.9%) patients were identified as immunocompromised. A majority of patients ($n = 301$, 89.6%) had been exposed to antibiotics before sample collection. Cough was reported in 303 (90.2%) patients. Among 336 patients, 154 (45.8%) patients were diagnosed with upper respiratory tract infections, while a majority of patients ($n = 314$, 93.5%) presented with lower respiratory tract (LRT) infections. Additionally, there were 262 (78.0%) patients confirmed cases of pneumonia. Notably, all infections were classified as community-acquired (Table 1).

Pathogen positive rate

Among the 336 enrolled patients, tNGS detected a total of 38 potential pathogens, comprising 25 species (15 bacteria and 10 viruses) and 13 viral subtypes (Figs. 2 and 3). The overall microbial detection rate for tNGS was 100%.

In total, 88.1% (296 out of 336) of the patients with ARI had at least one positive bacterium detection, with the highest rate observed in school-age children (89.5%, 17 out of 19). This rate decreased to 89.2% (224 out of 251) in infants and toddlers and 83.3% (55 out of 66) in preschool-age children (Table 2). The positive rates were comparable between genders (89.0% in males vs. 86.6% in females; $P > 0.05$).

Similarly, 87.5% (294 out of 336) of patients with ARI had at least one positive virus detection, with the highest rate found in infants and toddlers (89.6%; 225 out of 251). This rate decreased to 84.8% (56 out of 66) in preschool-age children and 68.4% (13 out of 19) in school-age children (Table 2). The gender comparison showed comparable rates (87.1% in males vs. 88.2% in females; $P > 0.05$). Statistically significant differences were observed between infants, toddlers, and school-age children (89.6% vs. 68.4%, $P < 0.05$). Among the 294 viral-positive samples, single, double, triple, quadruple, quintuple, and sextuple viral co-detection were observed in 47.6% ($n = 160$), 28.0% ($n = 94$), 9.2% ($n = 31$), 2.1% ($n = 7$), 0.3% ($n = 1$), and 0.3% ($n = 1$) of patients, respectively (Supplementary Table S2).

Viral-bacterial co-detection was observed in 75.6% (254 out of 336) of patients, with the highest co-detection

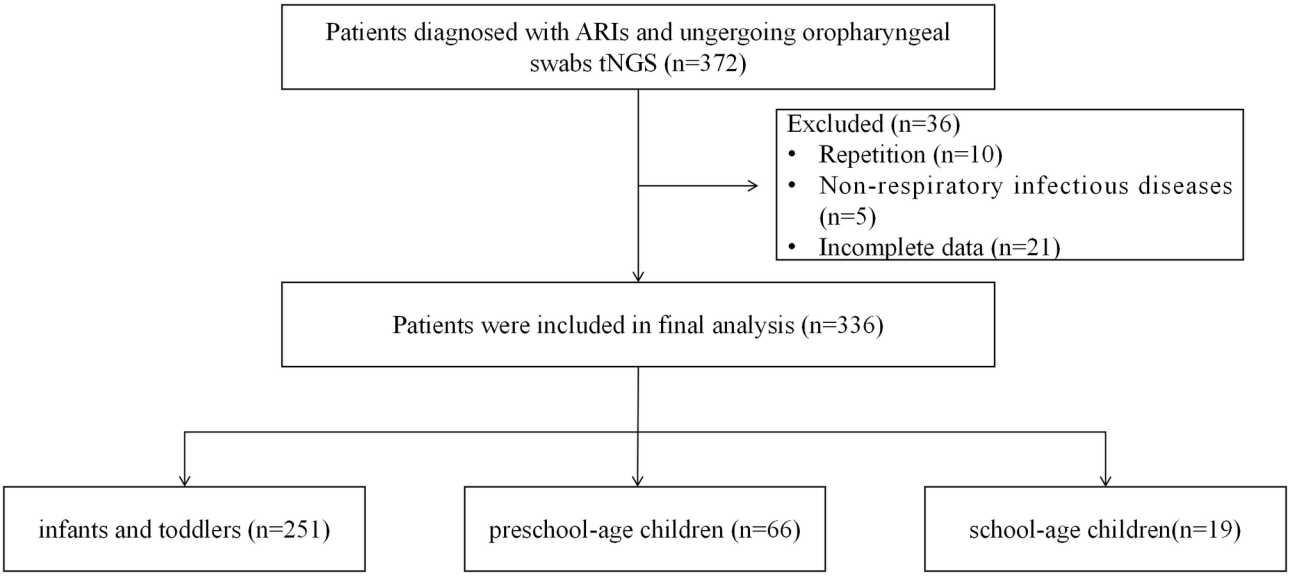

Fig. 1 Flow diagram of the study

Table 1 Baseline characteristics of the 336 patients enrolled

Characteristic	Value (*n* = 336)
Median age, years	2 (0–11)
Age group, n(%)	
infants and toddlers (0–3 years of age)	251 (74.7%)
Preschool children (4–6 years)	66 (19.6%)
School-age chlidren (7–11 years)	19 (5.7%)
Gender, n(%)	
Male	209 (62.2%)
Female	127 (37.8%)
Immunocompromised, n(%)	13 (3.9%)
Antibiotic exposure, n(%)	
Yes	301 (89.6%)
No	35 (10.4%)
Acute respiratory infections symptom, n(%)[a]	
cough	303 (90.2%)
runny nose	244 (72.6%)
fever	213 (63.4%)
nasal obstruction	198 (58.9%)
expectoration	177 (52.7%)
sore throat	35 (10.4%)
Categorization of respiratory tract infections, n(%)	
upper respiratory tract infections	154 (45.8%)
lower respiratory tract infections	314 (93.5%)
pneumonia	262 (78.0%)
non-pneumonia	52 (15.5%)
Differentiation between community-acquired and hospital-acquired infections, n(%)[b]	
community-acquired	336 (100%)
hospital-acquired	0 (100%)

[a] Individual symptom percentages do not add up to 100 because patients often had multiple symptoms. Acute respiratory infections are defined as having at least one of the following symptoms: cough, runny nose, fever, nasal obstruction, expectoration, and sore throat.

[b]Community-acquired infections are defined as infections contracted outside of a healthcare setting, including those with a defined incubation period that present during this period after admission. Hospital-acquired infections, on the other hand, are infections that become apparent 48 h or more after hospital admission, assuming the patient was not in the incubation phase upon admission.

rate seen in infants and toddlers (78.9%), followed by preschool-age children (68.2%) and school-age children (57.9%; Table 2). co-detection comparable rates (76.6% in males vs. 74.8% in females; $P > 0.05$).

Pathogen detection and age-specific pathogen spectrum

Based on the proportion of viral positive detections, the most frequently identified viral pathogen was human adenovirus (HAdV), accounting for 31.3% of all positive detections, followed by human rhinovirus (RV, 26.5%), human parainfluenza virus (HPIV, 25.0%), cytomegalovirus (CMV, 19.0%), human bocavirus (HBoV, 11.0%), respiratory syncytial virus (RSV, 8.9%), Epstein-Barr virus (EBV, 8.0%), human coronavirus (HCoV, 7.1%), enterovirus (EV, 4.2%), and Herpes simplex virus 1 (HSV1, 1.8%). Further genotyping analysis for HAdV revealed HAdV-B3 as predominant, accounting for 76.2% of genotyped patients, with HAdV-C2 and HAdV-C1 accounting for 8.6% and 1.0%, respectively. Among genotyped RV cases, RV-C was predominant at 55.1%, followed by RV-A (31.5%) and RV-B (3.4%). Among genotyped HPIV cases, HPIV-3 was most prevalent (67.9%), followed by HPIV-4 (25.0%) and HPIV-1 (7.1%). For HCoV, HCoV-OC43 and HCoV-HKU1 accounted for 62.5% and 37.5% of genotyped cases, respectively. Age-specific analysis revealed different patterns of viral pathogens. Among infants and toddlers, HPIV was the most common pathogen (31.1%), followed by RV (28.3%), HAdV (26.7%), CMV (24.7%), and HBoV (13.1%). In preschool-age children, HAdV surpassed RV and HPIV as the leading pathogens (HAdV > RV > HPIV > HCoV > RSV). Among school-age children, EBV emerges as the most detected pathogen, followed by HAdV, RV, EV, and HBoV (EBV > HAdV = RV > EV = HBoV; Fig. 2).

Based on the proportion of positive detections for bacteria, *Streptococcus pneumoniae* was the most frequently detected bacterium, accounting for 36.0% of all positive detections, followed by *Stenotrophomonas maltophilia* (30.4%), *Streptococcus intermedius* (29.5%), *Moraxella catarrhalis* (27.1%), *Haemophilus influenzae* (20.2%), *Acinetobacter baumannii* (19.0%), *Streptococcus anginosus* (13.7%), *Klebsiella pneumoniae* (7.1%), and *Staphylococcus aureus* (6.8%). *Mycoplasma pneumoniae* was detected in 1.8% of patients ($n = 6$; Fig. 2). Age-specific differences were observed in pathogen distribution. Among infants and toddlers, the top five bacterial pathogens were *S. pneumoniae* (38.6%), *S. maltophilia* (32.3%), *M. catarrhalis* (29.9%), *S. intermedius* (29.5%), and *A. baumannii* (23.9%). In preschool-age children, *S. pneumoniae* remained predominant, followed by *S. intermedius*, *S. anginosus*, *S. maltophilia*, and *H. influenzae*. Among school-age children, *S. anginosus* emerged as the most detected pathogen, with *H. influenzae* moving up significantly from seventh place in infants and toddlers to second place. The ranking of other pathogens showed minor variations (*S. anginosus* > *H. influenzae* > *S. intermedius* > *S. maltophilia* = *M. catarrhalis*; Fig. 2).

Pathogen detection using tNGS, sputum culture and antibody detection

Among the 336 patients enrolled in the study, only 94 patients (28.0%) were able to provide sputum and then underwent simultaneous sputum culture and Gram staining. Within this cohort, the overall microbial detection rates for tNGS and sputum culture were 100% (94/94) and 53.2% (50/94), respectively. A total of 11 potential pathogens were identified through tNGS, while sputum culture detected 7 pathogens. Notably, the detection rate of tNGS significantly exceeded that of sputum

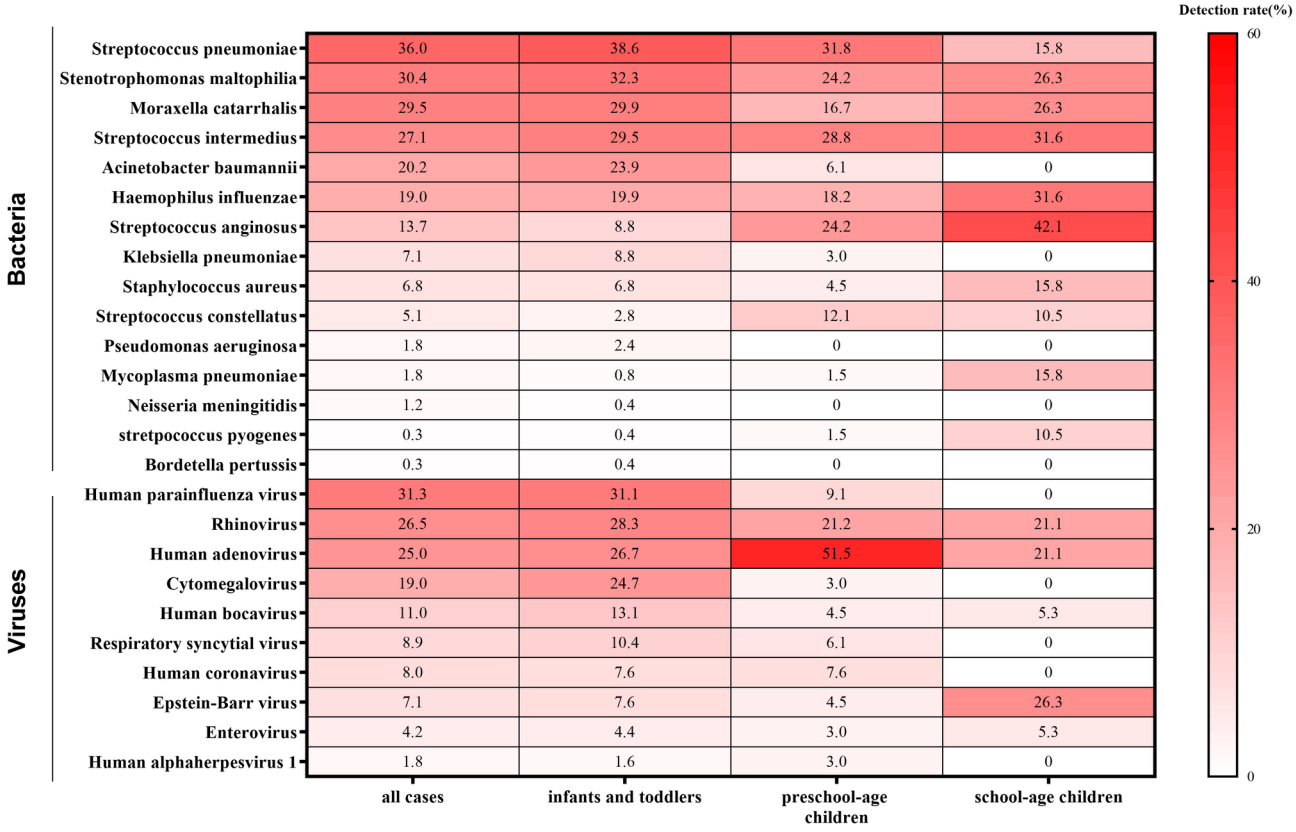

Fig. 2 Distribution of potential pathogens in the study cohort and heterogeneity of the pathogen spectrum among different age groups of children

culture ($P<0.0001$). Among the 94 patients, 50 exhibited positive results for both tNGS and sputum culture. The pathogens identified by both methods included *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, and *P. aeruginosa*, with detection rates of 18.1%, 14.9%, 8.5%, and 1.1%, respectively (Fig. 4). Among the 50-double positive patients, 6 patients (6.4%, 6/94)) displayed completely consistency between tNGS and sputum culture, whereas 34 patients (36.2%, 34/94) exhibited partial consistency, and 10 patients (10.6%, 20/94) showed complete inconsistency. Additionally, two microorganisms were solely identified by sputum culture: *S. mitis* and *Staphylococcus xylosus*, which fell outside the detection range of tNGS. Moreover, only 18 patients (5.4%) underwent *Mycoplasma pneumoniae* (MP) and *Chlamydia pneumoniae* (CP) IgM/IgG assays. All patients exhibited negative results for MP and CP antibody detection, which was consistent with the tNGS results.

Clinical impact of tNGS

It is important to note that this study lacked standard reference results for pathogen diagnosis due to the absence of bacterial culture and the real-time PCR method for multiple virus detection. Despite this limitation, treatment records for all 336 patients showed that treatment adjustments were directly made for 33 patients based on tNGS results, while 303 continued with their existing treatment regimens to assess efficacy. The majority of those who did not have treatment adjustments had previously received empirical medications that effectively targeted the detected pathogens. Additionally, the average TAT for tNGS was 16.9 h, demonstrating rapid and consistent TAT. The cost of a single tNGS assay ranges from 400 to 800 RMB, presenting a significant cost reduction of approximately 75–90% when compared to a mNGS assay.

Discussion

To the best of our knowledge, this study represents the first investigation utilizing OPS for tNGS in diagnosing and treating ARIs. By analyzing surveillance data from a local hospital's patients with ARIs, we have identified the spectrum of bacterial and viral pathogens and explored demographic differences among patients.

In this study, we utilized a tNGS assay targeting 95 pathogens to assess its performance in detecting microbial pathogens in OPS, addressing concerns regarding sample collection, testing costs, TAT, and accessibility. Among the enrolled patients, tNGS identified 38 pathogens, comprising 25 species (15 bacteria and 10 viruses) and 13 viral subtypes. Our findings indicate a 100% detection rate for microbial pathogens in OPS using

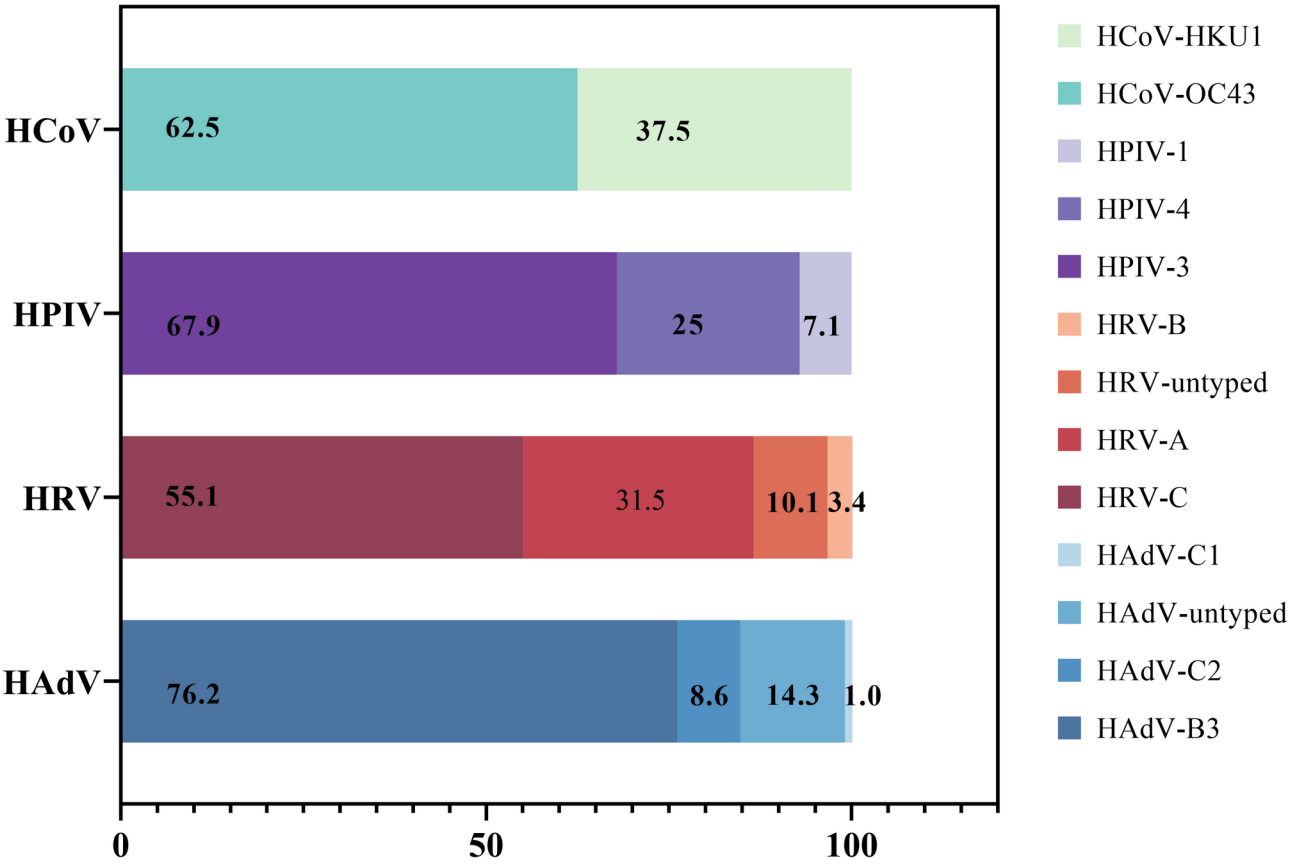

Fig. 3 Distribution of genotyped viruses in the study cohort

Table 2 Positive rates of viral and bacterial pathogens and viral-bacterial co-detection rates among patients with ARI

	Viral pathogens tested	Bacterial pathogens tested	Viral-bacterial co-detection tested[a]
All	294 (87.5%)	296 (88.1%)	254 (75.6%)
Gender, n(%)			
Male	182 (87.1%)	186 (89.0%)	159 (76.1%)
Female	112 (88.2%)	110 (86.6%)	95 (74.8%)
Age group, n(%)			
infants and toddlers (0–3 years of age)	225 (89.6%)	224 (89.2%)	198 (78.9%)
Preschool children (4–6 years)	56 (84.8%)	55 (83.3%)	45 (68.2%)
School-age chlidren (7–11 years)	13 (68.4%)	17 (89.5%)	11 (57.9%)

The number and proportion of at least one positive viral or bacterial pathogen are shown in the table.

[a]The viral-bacterial co-detection referred to simultaneous identification of viral and bacterial pathogens.

ARI, acute respiratory infection.

tNGS, which aligns with the 100% detection rate observed using mNGS on 35 BALF samples from 32 patients [15]. Specifically, the viral detection rate of 87.8% is consistent with the 91.9% reported by Graf et al. [16] albeit lower than the 97.6% reported by Ogunbayo et al. [10] and higher than the 43% reported by Thorburn et al. [17]. Heterogeneity in reported detection rates may be attributed to factors such as sampling techniques, sample size, geographical location, and season.

In this study, HAdV, RV, HPIV, CMV, HBov, and RSV were the most frequently detected pathogens in ARIs. Among these, HPIV was identified as the predominant pathogen in infants and toddlers, while HAdV was the leading viral pathogen in preschool-age children. Contrastingly, EBV was most frequently detected in school-age children, with RV being the second most common across all age groups. EBV can intermittently be identified in the saliva of healthy individuals due to the continuous shedding of the virus throughout the lifetime, which remains asymptomatic or manifests with nonspecific symptoms. However, primary EBV infection may results in infectious mononucleosis (IM) during childhood or adolescence when it infects an immune-naive individual. The typical clinical manifestations of IM encompass pharyngitis, fever, and lymphadenopathy, which may

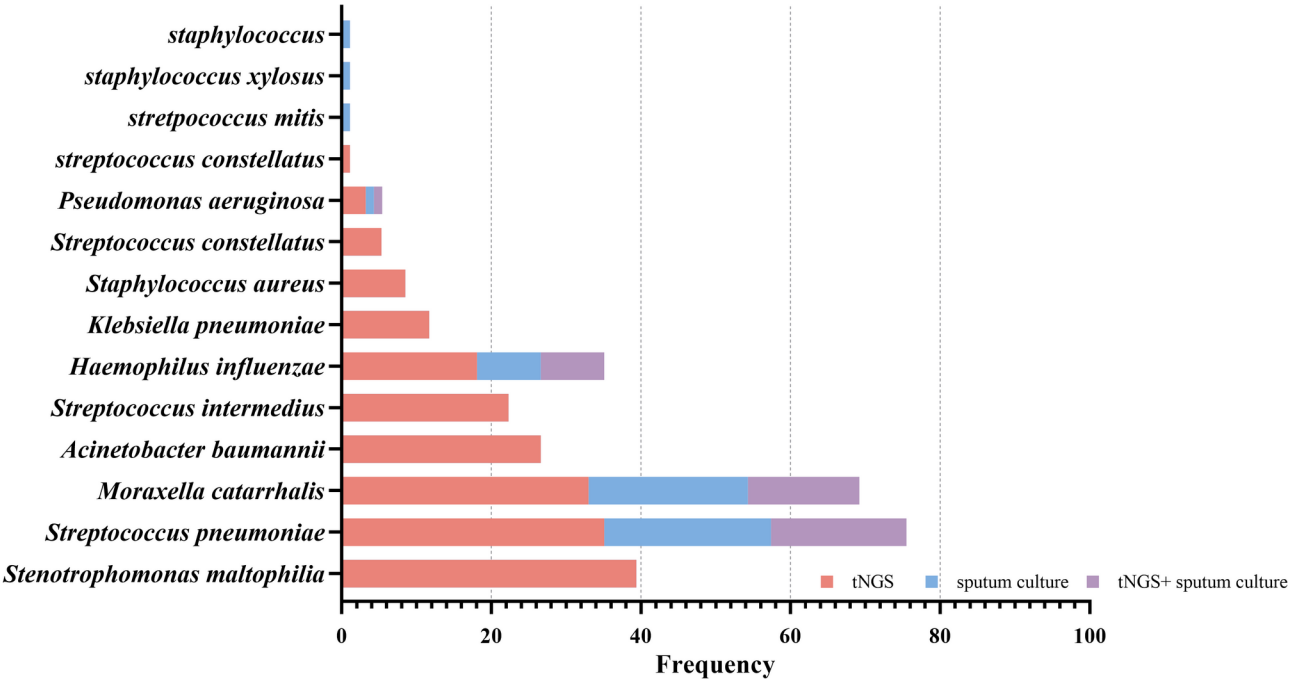

Fig. 4 Distribution of potential pathogens in the study cohort of 94 patients and the respective contributions of tNGS and sputum culture for pathogen detection. tNGS, targeted next generation sequencing

occasionally be associated with hepatic impairment, hepatosplenomegaly, and eyelid edema [18]. Moreover, CMV was most frequently detected in infants and toddlers. It has been reported that CMV may function as an immunomodulator, enhancing the transmission and pathogenicity of RSV among young children. Consequently, viral ARIs are markedly more prevalent in CMV-infected children [19]. Molecular subtyping of viral species is beneficial for identifying genotypic markers of drug resistance or pathogenicity. Our study using tNGS revealed several key findings: (1) RSV-A was the predominant pathogen among RSV-positive specimens [20], with RSV-B not detected, possibly reflecting the prevalence of RSV-A in the community; (2) 76.2% of HAdV infections belonged to HAdV-B3 and more pathogenic species C, consistent with previous reports from Huzhou, China, with HAdV-B3 accounting for 75.00% and 63.64% of typed HAdV infections in 2017 and 2019 [21]; (3) In contrast to RV-genotyping results from north-east Brazil (73% RV-A, 27% RV-C, and 0% RV-B) [22] and a study on children with community-acquired pneumonia in China during 2017–2019 (109 RV-A, 20 RV-B, and 80 RV-C) [23], our study found that 55.1% RV infections belonged to RV-C, with RV-A at 31.5% and RV-B at 3.4%; (4) HPIV-3 was more prevalent subtype (67.9%), followed by HPIV-4 (25.0%) and HPIV-1 (7.1%), consistent with findings from Beijing [24]; (5) HCoV-OC 43 was more prevalent than HCoV-HKU 1, as previously reported by Ye et al. [25]. Notably, the influenza virus was not detected in our study. This could be attributed to the small sample size and strict coronavirus disease 2019 (COVID-19) control measures during the pandemic, including mask usage, which likely reduced influenza transmission.

In this study, our aim was to investigate variations in pathogen spectra across patients of different ages. We observed a diverse age-related pattern of bacterial detections. Numerous respiratory bacteria colonize the healthy human respiratory tract, occasionally leading to respiratory infections. Species such as *S. pneumoniae*, *S. maltophilia*, *S. intermedius*, *M. catarrhalis*, *H. influenza*, *A. baumannii*, *S. anginosus*, *K. pneumoniae*, and *S. aureus* were frequently found in this cohort. Specifically, *S. pneumoniae*, *M. catarrhalis*, and *H. influenza* findings align with a previous study involving 226 asymptomatic children across various settings [26]. Moreover, *S. pneumoniae* was most prevalent in infants, toddlers, and preschool-age children, whereas *S. anginosus* predominated in school-age children, consistent with age-specific trends observed in a comprehensive 11-year surveillance study [2]. *S. maltophilia* is typically regarded as an opportunistic pathogen, primarily affecting immunocompromised patients [27]. Similarly, *S. intermedius* is predominantly associated with complex and severe infections rather than ARI in healthy pediatric populations [28]. *S. anginosus* is associated with acute complicated sinusitis and may lead to increased morbidity and complications [29]. It has been reported that *A. baumannii* is commonly associated with hospital-acquired infections

[30]. *H. influenzae* and *M. catarrhalis* are more prevalent in children who develop ARIs and may be associated with an increased frequency or severity of ARIs [31]. Furthermore, previous studies have demonstrated that *Moraxella* constitutes the largest proportion of the nasal microbiome in healthy children, potentially playing a role in protecting them from COVID-19 infection, as well as being involved in amino acid and lipid metabolism [32]. In addition, the evidence for *S. maltophilia*, *S. intermedius*, *S. anginosus*, and *A. baumannii* causing ARIs in healthy children is limited, whereas *H. influenzae* and *M. catarrhalis* may play a more significant role in ARIs. Commensal flora such as *S. aureus*, *S. pneumoniae*, *S. maltophilia*, and *A. baumannii* typically colonize the upper respiratory tract and can become pathogenic under conditions of lowered immunity or other external factors. Furthermore, a study comparing microbiological detections between OPS and LRT samples from 103 patients using a syndromic PCR-based respiratory panel indicated high positive and excellent negative percent agreements for common community-acquired pneumonia pathogens *S. pneumoniae* and *H. influenzae* between OPS and LRT samples. This suggests that OPS could serve as a convenient alternative to LRT samples [33].

In the study cohort of 94 patients who underwent concurrent sputum culture and Gram staining, tNGS identified a greater number of potential pathogens (11 vs. 7) and demonstrated an enhanced detection rate compared to sputum culture (100% vs. 53.2%). These findings highlight the fact that a considerable portion of patients may carry potential pathogens that remain undetected despite having undergone sputum culture. Additionally, among the 50 patients who tested positive for both tNGS and sputum culture, a notable 80% exhibited either complete or partial consistency between the two methods. This evidence emphasizes the promising capabilities of tNGS in these patients.

Nevertheless, when considering the clinical impact of tNGS in treating ARIs, antibiotic adjustments were made for 9.8% (33 out of 336) of patients. tNGS proves beneficial, particularly for patients with unidentified pathogens like *M. pneumoniae* and those with mixed infection. However, large-scale clinical studies are needed to fully understand the role of tNGS in diagnosing and treating ARIs.

The multiplex PCR technique enables the simultaneous detection of several to dozens of pathogens, with a TAT of approximately 1.5 to 3 h, which is crucial for clinical scenarios requiring rapid diagnosis and treatment. Additionally, the technology of multiplex PCR is relatively advanced, with a straightforward operational process, and it imposes relatively low demands on laboratory equipment and personnel, facilitating its widespread adoption and application in clinical laboratories [34, 35]. Conversely, tNGS integrates ultra-multiplex PCR amplification with high-throughput sequencing to concurrently identify dozens to hundreds of known pathogenic microorganisms, along with their virulence and/or resistance gene. The temporal efficiency of tNGS is constrained, often requiring several hours to a full day, with significant financial costs. Nonetheless, its capacity to identify intricate and polymicrobial infections provides a notable benefit in specific clinical contexts [36]. Multiplex PCR's detection is confined to known targets via pre-designed primers, with sensitivity limited in samples with high human background. In contrast, tNGS enhances sensitivity through next-generation sequencing. Multiplex PCR only identifies target presence or provides approximate quantification, lacking detailed sequence data such as drug resistance mutations or single nucleotide polymorphisms. Its scalability is limited, as adding targets necessitates primer redesign and reaction optimization. Conversely, tNGS allows rapid detection scope expansion through amplicon design, offering superior adaptability. In conclusion, the two methods are not entirely mutually exclusive. In practical applications, selections can be customized to meet particular diagnostic needs and clinical settings.

Unlike mNGS, which identifies all microorganisms without bias, tNGS enhances detection sensitivity for specific microorganisms but may miss casual pathogens not included in the target panel. In clinical settings, it is crucial to comprehensively understand and judiciously select appropriate tNGS technologies. For different infection sites, it is advisable to utilize tNGS panels that cover the pertinent pathogen spectrum, such as those for pulmonary or central nervous system infections. The targeted pathogen spectrum should incorporate pathogens that are challenging to culture and rare pathogens associated with the syndrome, while excluding microorganisms lacking clinical relevance for the specific site. Furthermore, the primer sets and databases used in tNGS should be regularly updated to accommodate emerging pathogens, as exemplified by the SARS-CoV-2 pandemic in 2019. The application contexts of tNGS should be distinctly differentiated and prioritized from those of mNGS. For patients with non-severe conditions, tNGS is recommended when conventional microbiological assays fail to identify pathogens. Conversely, in cases of critical illness or novel infectious diseases, mNGS may be prioritized over tNGS [34, 35]. Taken together, the two NGS methods provide complementary insights, thereby enhancing the breadth of molecular diagnostics within clinical laboratory settings.

Nonetheless, there are notable limitations. Firstly, The absence of healthy control group and standardized reference results for pathogenic diagnosis hindered the assessment of sensitivity and specificity for tNGS,

thereby impeding a comprehensive evaluation of its diagnostic performance. Secondly, the small sample size and short study duration may affect tNGS accuracy and performance. Thirdly, distinguishing between microbial colonization and infection is challenging due to the lack of widely accepted quantitative cutoffs for tNGS in diagnosing causative pathogens. Fourthly, prior use of antibiotics or antiviral drugs before sample collection may impact pathogen detection rates with tNGS. Fifthly, tNGS positive detections of pathogens do not establish causal relationships, particularly for bacterial pathogens where culture results from nasopharyngeal aspirates or sputum may indicate colonization rather than invasive infection. Conversely, evidence suggests a correlation between bacterial colonization levels and future ARI incidence [37]. Notably, a recent multicenter prospective cohort study found nasopharyngeal reverse transcriptase PCR detected the most abundant virus identified by metagenomic next-generation RNA sequencing in 92.4% of tracheal aspirates, suggesting nasopharyngeal samples could serve as a viable substitute for critically ill pediatric patients suspected of LRT infection [38].

In conclusion, our study underscores the clinical utility of OPS for tNGS in diagnosing pathogens in ARIs. The findings highlight the high sensitivity of tNGS in detecting ARI pathogens and its ability to provide precise treatment guidance for pediatric patients. Moreover, tNGS shows promise for rapid and reliable monitoring of the emergence and spread of respiratory tract pathogens. These findings underscore the potential of tNGS in ARI surveillance, diagnosis, pathogen detection, and prevention. However, future research should include larger sample sizes and more prospective studies to further evaluate these findings.

Abbreviations

ARI	Acute respiratory tract infection
tNGS	Targeted next-generation sequencing
OPS	Oropharyngeal swabs
LRT	Lower respiratory tract

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

CL: Conceptualization, Formal analysis, Software, Visualization, Writing– original draft. XZ: Project administration, Formal analysis, Validation, Writing– original draft. PL, ML: Data curation, Validation, Writing– review & editing. LX, FO: Data curation, Investigation, Writing– review & editing. HD, WZ: Data curation, Writing– review & editing. ZD: Methodology, Investigation, Resources, Writing review & editing. SL: Conceptualization, Investigation, Project administration, Supervision, Writing– review & editing.

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Data availability

The data that support the conclusions of this article are included in this published article. The tNGS sequence results have been deposited in the NCBI database under BioProject accession number SRA: PRJNA1124340.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Binyang Women and Children's Hospital and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients' parents or their legal representatives.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors have no competing interests to declare.

Clinical trial number

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References

1. Huai Y, Guan X, Liu S, Uyeki TM, Jiang H, Klena J, et al. Clinical characteristics and factors associated with severe acute respiratory infection and influenza among children in Jingzhou, China. Influenza Other Respir Viruses. 2017;11:148–56.
2. Li Z-J, Zhang H-Y, Ren L-L, Lu Q-B, Ren X, Zhang C-H, et al. Etiological and epidemiological features of acute respiratory infections in China. Nat Commun. 2021;12:5026.
3. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019. Lancet Lond Engl. 2020;396:1204–22.
4. Shao J, Hassouna A, Wang Y, Zhang R, Zhen L, Li R, et al. Next-generation sequencing as an advanced supplementary tool for the diagnosis of pathogens in lower respiratory tract infections: an observational trial in Xi'an, China. Biomed Rep. 2022;16:14.
5. Ma Y, Luo Y, Feng X, Huang C, Shen X. Smartphone-controlled biosensor for viral respiratory infectious diseases: screening and response. Talanta. 2023;254:124167.
6. Mattila S, Paalanne N, Honkila M, Pokka T, Tapiainen T. Effect of Point-of-Care testing for respiratory pathogens on antibiotic use in children: A randomized clinical trial. JAMA Netw Open. 2022;5:e2216162.
7. Wang X, Stelzer-Braid S, Scotch M, Rawlinson WD. Detection of respiratory viruses directly from clinical samples using next-generation sequencing: A

literature review of recent advances and potential for routine clinical use. Rev Med Virol. 2022;32:e2375.
8. Zhang H, Wang X, Xia H, Liu Z. Metagenomics next-generation sequencing assists in the diagnosis of infant pertussis encephalopathy: A case report. Med (Baltim). 2023;102:e33080.
9. Xie N, Lin Y, Li P, Zhao J, Li J, Wang K, et al. Simultaneous identification of DNA and RNA pathogens using metagenomic sequencing in cases of severe acute respiratory infection. J Med Virol. 2024;96:e29406.
10. Ogunbayo AE, Mogotsi MT, Sondlane H, Sabiu S, Nyaga MM. Metagenomics characterization of respiratory viral RNA pathogens in children under five years with severe acute respiratory infection in the free State, South Africa. J Med Virol. 2023;95:e28753.
11. 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;38:201–12.
12. Gaston DC, Miller HB, Fissel JA, Jacobs E, Gough E, Wu J, et al. Evaluation of metagenomic and targeted Next-Generation sequencing workflows for detection of respiratory pathogens from Bronchoalveolar lavage fluid specimens. J Clin Microbiol. 2022;60:e0052622.
13. Li S, Tong J, Liu Y, Shen W, Hu P. Targeted next generation sequencing is comparable with metagenomic next generation sequencing in adults with pneumonia for pathogenic microorganism detection. J Infect. 2022;85:e127–9.
14. Deng Z, Li C, Wang Y, Wu F, Liang C, Deng W, et al. Targeted next-generation sequencing for pulmonary infection diagnosis in patients unsuitable for Bronchoalveolar lavage. Front Med. 2023;10:1321515.
15. Li Y, Sun B, Tang X, Liu Y-L, He H-Y, Li X-Y, et al. Application of metagenomic next-generation sequencing for Bronchoalveolar lavage diagnostics in critically ill patients. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2020;39:369–74.
16. Graf EH, Simmon KE, Tardif KD, Hymas W, Flygare S, Eilbeck K, et al. Unbiased detection of respiratory viruses by use of RNA Sequencing-Based metagenomics: a systematic comparison to a commercial PCR panel. J Clin Microbiol. 2016;54:1000–7.
17. Thorburn F, Bennett S, Modha S, Murdoch D, Gunson R, Murcia PR. The use of next generation sequencing in the diagnosis and typing of respiratory infections. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2015;69:96–100.
18. Liu M, Wang R, Xie Z. T cell-mediated immunity during Epstein-Barr virus infections in children. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2023;112:105443.
19. Chomel JJ, Allard JP, Floret D, Honneger D, David L, Lina B, et al. Role of cytomegalovirus infection in the incidence of viral acute respiratory infections in children attending day-care centers. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2001;20:167–72.
20. Luo M, Gong C, Zhang Y, Wang X, Liu Y, Luo Q, et al. Comparison of infections with respiratory syncytial virus between children and adults: a multicenter surveillance from 2015 to 2019 in Beijing, China. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2022;41:1387–97.
21. Xu D, Chen L, Wu X, Ji L. Molecular typing and epidemiology profiles of human adenovirus infection among hospitalized patients with severe acute respiratory infection in Huzhou, China. PLoS ONE. 2022;17:e0265987.
22. Fawkner-Corbett DW, Khoo SK, Duarte CM, Bezerra PGM, Bochkov YA, Gern JE, et al. Rhinovirus-C detection in children presenting with acute respiratory infection to hospital in Brazil. J Med Virol. 2016;88:58–63.
23. Ai J, Zhu Y, Duan Y, Zhang M, Lu G, Sun Y, et al. A multicenter study on molecular epidemiology of rhinovirus isolated from children with community acquired pneumonia in China during 2017–2019. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2022;106:105384.
24. Pan Y, Zhang Y, Shi W, Peng X, Cui S, Zhang D, et al. Human parainfluenza virus infection in severe acute respiratory infection cases in Beijing, 2014–2016: A molecular epidemiological study. Influenza Other Respir Viruses. 2017;11:564–8.
25. Ye R-Z, Gong C, Cui X-M, Liu J-Y, Fan H, Xie H, et al. Continuous evolution and emerging lineage of seasonal human coronaviruses: A multicenter surveillance study. J Med Virol. 2023;95:e28861.
26. Sulikowska A, Grzesiowski P, Sadowy E, Fiett J, Hryniewicz W. Characteristics of Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis isolated from the nasopharynges of asymptomatic children and molecular analysis of S. pneumoniae and H. influenzae strain replacement in the nasopharynx. J Clin Microbiol. 2004;42:3942–9.
27. Jiang Z, Ren Y, Zhang C, Yin Y, Li C. Community-Acquired Stenotrophomonas Maltophilia infection in a child: A case report and literature review. Infect Drug Resist. 2022;15:5469–74.
28. Faden HS. Infections associated with Streptococcus intermedius in children. Pediatr Infect Dis J. 2016;35:1047–8.
29. Troy E, Sillau S, Bernard TJ, Rao S. Incidence and clinical outcomes of Streptococcus anginosus in acute complicated sinusitis: A pediatric cohort. J Pediatr Infect Dis Soc. 2021;10:168–71.
30. Ayoub Moubareck C, Hammoudi Halat D. Insights into Acinetobacter baumannii: A review of Microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. Antibiot Basel Switz. 2020;9:119.
31. Hasegawa K, Camargo CA. Airway microbiota and acute respiratory infection in children. Expert Rev Clin Immunol. 2015;11:789–92.
32. Yu X, Wang L, Zheng X, Wen Y, Zhang Z, Fan L, et al. Moraxella occupied the largest proportion in the nasal Microbiome in healthy children, which potential protect them from COVID-19. Microb Pathog. 2022;170:105685.
33. Serigstad S, Knoop ST, Markussen DL, Ulvestad E, Bjørneklett RO, Ebbesen MH, et al. Diagnostic utility of oropharyngeal swabs as an alternative to lower respiratory tract samples for PCR-based syndromic testing in patients with community-acquired pneumonia. J Clin Microbiol. 2023;61:e0050523.
34. Poritz MA, Lingenfelter B. Multiplex PCR for detection and identification of microbial pathogens. In: Tang Y-W, Stratton CW, editors. Advanced techniques in diagnostic microbiology. Cham: Springer International Publishing; 2018. pp. 475–93.
35. Islam MT, Alam ARU, Sakib N, Hasan MS, Chakrovarty T, Tawyabur M, et al. A rapid and cost-effective multiplex ARMS-PCR method for the simultaneous genotyping of the Circulating SARS-CoV-2 phylogenetic clades. J Med Virol. 2021;93:2962–70.
36. Lian D, Lin C, Zhang Z, Wei J, Wang D, Tang Q. Clinical diagnostic value of throat swabs in pediatric acute lower respiratory tract infections using targeted next-generation sequencing. BMC Pediatr. 2025;25:224.
37. Bomar L, Brugger SD, Lemon KP. Bacterial microbiota of the nasal passages across the span of human life. Curr Opin Microbiol. 2018;41:8–14.
38. Osborne CM, Langelier C, Kamm J, Williamson K, Ambroggio L, Reeder RW, et al. Viral detection by reverse transcriptase polymerase chain reaction in upper respiratory tract and metagenomic RNA sequencing in lower respiratory tract in critically ill children with suspected lower respiratory tract infection. Pediatr Crit Care Med J Soc Crit Care Med World Fed Pediatr Intensive Crit Care Soc. 2024;25:e1–11.

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