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Original Article

Diagnostic efficacy of targeted high-throughput sequencing for lower respiratory infection in preterm infants

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Abstract: Objective: To examine the pathogen diagnostic performance of targeted high-throughput next-gen sequencing (tNGS) in respiratory infectious diseases in preterm infants using dynamic follow-up. Methods: Clinical samples of respiratory secretions were consecutively collected from 20 preterm infants weekly for 5 weeks, during which 10 developed bronchopulmonary dysplasia. Pathogen identification from these collected specimens was performed by both conventional cultivation and tNGS. Results: We found that targeted next-generation sequencing shared a 90.9% full or partial consistency for lower respiratory pathogen detection with the traditional culture-based approach, and increased the detection rate by 105.9%. Moreover, most of the pathogens identified by tNGS were diminished in patients after treatment. Conclusion: This study reveals the high sensitivity and performance of targeted high-throughput sequencing for respiratory infectious disease diagnosis and pathogen identification. The trial registry number is NCT03850457, and the trial URL is https://clinicaltrials.gov/ct2/show/NCT03850457.

Keywords: Targeted next-generation sequencing, multiplex PCR, respiratory infectious disease, preterm infant, pathogen detection, diagnosis

Introduction

Infectious diseases cause more than 17 million deaths annually, which account for over 25% of total mortality in the world [1]. Among these infectious diseases, acute lower respiratory infection, especially pneumonia, kills approximately 4.4 million people each year, over 90% of whom are children [2]. Bronchopulmonary dysplasia (BPD) is a chronic lung disease most commonly detected in premature infants, especially those less than 30 weeks of gestational age [3]. BPD patients usually require oxygen therapy and mechanical ventilation [4]. The pulmonary morbidity of BPD can induce significant chronic lung injury in the long term, with a considerable burden on the health system [5].

Notably, the efficacy of life-saving drugs, especially antibiotics, against infectious diseases has been compromised, due to the rapid development of drug resistance in various pathogens [6, 7]. Therefore, countering global antimicrobial resistance by restricting the widespread overuse of antibiotics has been pledged as a part of a national action plan in China [8]. The accurate detection and rapid characterization of pathogenic microorganisms is a prerequisite for precise diagnosis of infectious diseases and is critical for determining treatment strategy [9, 10]. However, the conventional methodologies for microbial identification and diagnosis of infections, represented by specimen culture, are usually time-consuming and associated with low sensitivity [11]. Moreover, several types of microorganisms such as fastidious bacteria, anaerobes, and viruses are extremely difficult culture [12]. Therefore, the traditional culture-based approaches fail to fulfill the requirement for clinical diagnosis of pathogenic infections, both in terms of accuracy and timeliness.

Recently, the rapid development of molecular diagnostic technology and increased understanding of the microbiome have substantially

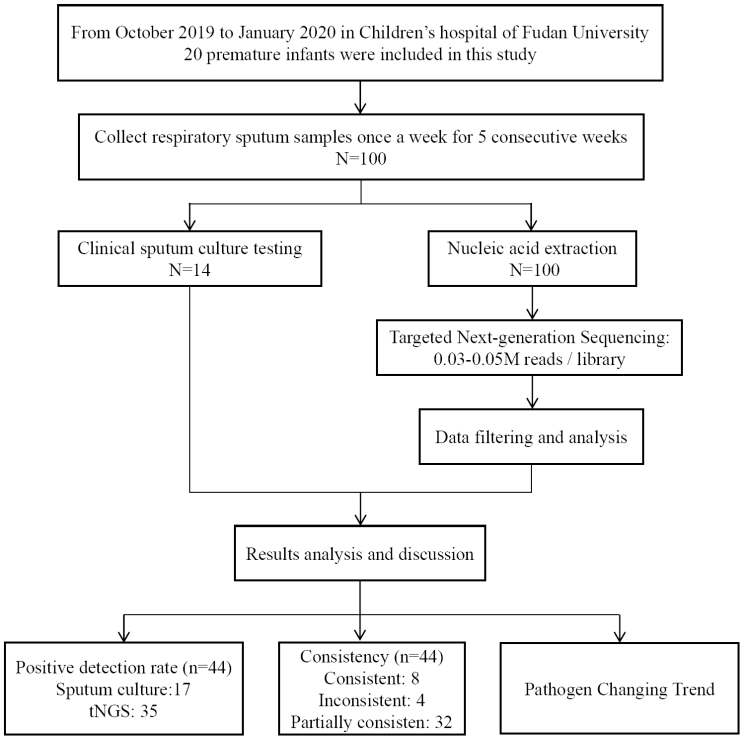


Figure 1. Flow diagram displaying the step-wise design of the study.

reduced the duration for diagnosis, which also allows the detection and characterization of viable but non-culturable bacteria [13]. Current microbial diagnostic techniques, such as real time-PCR [14], FilmArray [15], or GeneXpert [16], can target only a single or a few types of pathogen for each examination. The microbial detection based on these techniques is highly dependent on the accurate prediction of specific pathogens, but they remain restricted for detecting unknown microorganisms [17].

Next-generation sequencing (NGS) has been developed in recent years, and can rapidly provide high-throughput data for DNA sequencing [18]. Metagenomic sequencing possesses the capacity to detect clinical pathogens, but it is largely limited by the compromised sensitivity caused by the high DNA background of the human host (e.g., tissue specimen) [19]. Furthermore, difficulties in defining the targeted pathogens for specific infections from a complex microbiome (e.g., respiratory secretions) also prevent metagenomic sequencing from replacing culturing-based approaches for diagnosis of infectious diseases [19]. On the contrary, the combination of multiplex pathogen-targeted PCR amplification and high-throughput sequencing can rapidly identify pathogens and their antimicrobial resistance or virulence genes within 24 hours [3]. Its follow-up species determination and antibiotic resistance gene analysis can be completed within merely two hours, which significantly reduces diagnostic time [20, 21].

We aimed to investigate the accuracy and sensitivity of targeted next-generation sequencing (tNGS) for pathogen detection by comparing it to the traditional culture-based method, to further improve the entire diagnostic procedure including specimen collection, DNA isolation, library preparation, sequencing, and data analysis (**Figure 1**). Through dynamic follow-up, the outcomes of treatment in BPD patients were recorded, which allowed us to determine whether tNGS can provide guidance for BPD treatment strategy. Overall, this study reveals a high sensitivity and accuracy of tNGS for pathogen detection, which establishes a foundation for its diagnostic application for acute infectious diseases.

Methods

Patient enrollment

In this prospective study, the diagnosis of neonatal respiratory distress syndrome in participants was based on clinical and radiographic results. Diagnosis of BPD was according to the standard of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development [22]. Diagnosis of pneumonia was based on clinical, radiographic, and etiological findings. Diagnosis of sepsis was based on clinical results and positive blood culture outcome. During the study period (28 days), 10 individuals were diagnosed with BPD, defined as a persistent need for supplemental oxygen for the first 28 days after birth. Based on their levels of oxygen demands at 36 weeks post-

menstrual age (PMA), these patients were further categorized as having: Mild BPD, breathing room air at 36 weeks PMA or discharge, whichever came first; Moderate BPD, need for < 30% oxygen at 36 weeks PMA or discharge, whichever came first; Severe BPD, need for ≥ 30% oxygen and/or positive pressure at 36 weeks PMA or discharge, whichever came first. All the infants' parents were informed about the risks and benefits of the free microbial test based on targeted high-throughput sequencing.

Inclusion criteria were as follows: Individuals who had a gestational age < 30 weeks or a birth weight < 1,250 g; individuals who were admitted to the neonatal ICU (NICU) within 12 hours after birth; individuals whose parents agreed to participate in this study and provide the informed consent.

Exclusion criteria were as follows: Individuals whose parents refused to provide informed consent; individuals who had congenital abnormalities such as congenital diaphragmatic hernia or alveolar-capillary dysplasia that lead to breathing difficulty; individuals who were discharged or passed away before the completion of five consecutive specimen collections.

According to the above criteria, 20 patients were included in the analysis (**Figure 1**) and the study was approved by the Ethics Commission of Children's Hospital of Fudan University. The trial registry number is NCT03850457, and the trial URL is https://clinicaltrials.gov/ct2/show/NCT03850457.

Sample collection

Sputum samples were weekly collected from each patient for five consecutive times, starting from the first day of hospitalization (namely 0 week) and ending at the 4th week of hospitalization (at 7, 14, and 28 days). For infants with invasive mechanical ventilation, closed suction tubes were used to collect sputum. For other infants, a sterile suction tube was used to suck sputum through the nose. A total of 100 sputum samples were collected and further processed for pathogen identification by both culturing-based method and targeted next-generation sequencing.

Specimen processing and DNA extraction

An aliquot of 200 μL sputum sample was transferred into a 1.5 mL centrifuge tube and mixed with an equal volume of sputum digestive buffer (R30144; Yuanye Biotechnology, Shanghai, China). The mixture was incubated at 37°C for 30 min and vortexed every 5-10 min for homogenization. Then, an aliquot of 250 μL digested sputum was transferred into a DNA/RNA Shield Lysis & Collection Tube (R1103; ZymoBIOMICS, Irvine, CA, USA) and lysed by bead milling on a grinder (Jingxin Industrial Development Co., Ltd, Shanghai, China) for 5 min at 70 Hz. The DNA was subsequently extracted from the lysed sputum sample by using the DNA Miniprep Kit (D4304; ZymoBIOMICS).

Library preparation and sequencing

The DNA library for targeted sequencing was prepared by two rounds of PCR amplification using the Respiratory Pathogen Detection Kit v1.11 (090116; MorGene Biotech, Shanghai, China). Targeted enrichment amplification was conducted in the first round of PCR based on GenSeizer, which is the core patent of Shanghai Morgene Biotechnology Co. LTD. Sequencing adapters were added to the amplicons in the second round of PCR. The constructed library was inspected by the Qsep100 automatic nucleic acid analysis system and quantified using Qubit 4.0 fluorometer. The diluted and denatured library was further sequenced by the Illumina MiSeq Reagent Nano Kit on an Illumina MiSeq platform.

Dataset processing and quality control

The raw sequencing data including 0.03-0.05 million reads on average for each DNA library were first subjected to adapter trimming. Initial quality control was performed to retain the reads with double-ended length > 60 bp. A second quality control was performed to retain the reads with Q30 > 50% to generate high-quality data. Then, primer identification was performed, retaining the reads with correct alignment at both ends. The final reads were compared against the pathogen database (MorGene Biotech, Shanghai, China) for pathogen identification.

Effectiveness evaluation and follow-up

The pathogen detection ability and diagnostic performance of targeted high-throughput sequencing were compared to those of the traditional culturing-dependent methods based on clinical observations. Culture was consid-

Table 1. Characteristics of newborns with bronchopulmonary dysplasia admitted to NICU

Patient ID	Gestational age (d)	Birth weight (g)	Gender	Antibiotic treatment	Respiratory support	WBC 10^9/L	Lymphocytes %	Neutrophils %
P1	207	900	female	Unasyn + Fortum	NIPPV	3.9	41.4	46.1
P2	202	1240	female	Unasyn + Fortum	BIPAP	19.9	13.6	66.5
P3	202	1300	male	Unasyn + Fortum	HFO	19.1	26.3	55
P4	200	930	male	-	NIPPV	7	48.1	42.5
P5	199	1070	male	Unasyn	PC	27.5	13.7	80.2
P6	213	1150	female	Penicillin	BIPAP	9.3	56.7	29
P7	195	1110	male	Unasyn + Fortum	PC	50.9	15.4	78
P8	192	1390	male	Unasyn + Fortum	BIPAP	48.2	10	75
P9	197	690	female	Unasyn + Fortum	PC	11.7	37.5	50.1
P10	198	1385	female	Unasyn + Fortum	BIPAP	70.1	12	63
P11	211	1350	male	Unasyn	CPAP	19.1	21	62.6
P12	219	1120	male	Unasyn + Fortum + Metronidazole	NIPPV	10.9	47.6	43.3
P13	193	985	male	Unasyn	HPO	7.1	41.2	42
P14	193	885	female	Unasyn	PC	6.7	45.4	41.7
P15	193	955	male	Unasyn	NIPPV	6.4	27.9	51.2
P16	207	1450	male	Unasyn + Fortum	BIPAP	18.1	22.8	65
P17	207	1315	female	Unasyn	BIPAP	12.3	37.2	52.7
P18	194	1260	male	Unasyn	NIPPV	16.4	14.3	72.8
P19	216	1280	female	Unasyn	BIPAP	6.9	30.2	57.3
P20	200	1000	female	Unasyn + Fortum	BIPAP	11.8	70.1	19.3

ered the gold standard method in the bacteria detection in this study. The efficacy of the treatment was followed up to evaluate the overall effectiveness of targeted high-throughput sequencing in infectious disease diagnosis and treatment guidance.

Data analysis

The traditional cultivation method was used as the standard for pathogen identification (culture medium: bacteria: blood agar plates/chocolate agar plates/MacConkey agar; fungi: Sabouraud Dextrose Agar; Mycobacterium: Roche medium. Condition: 35°C, with 5% CO_2). The effectiveness of targeted high-throughput sequencing was compared to the standard based on a four-column comparison table, and the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of pathogen detection by targeted next-generation sequencing were further calculated.

Results

Demographic and baseline clinical characteristics

A total of 20 pediatric preterm infants were enrolled from October 2019 to January 2020. Demographic and baseline clinical characteristics of these infants are summarized in **Table 1**, and during the study period, 10 of them developed BPD.

Targeted next-generation sequencing showed stronger pathogen detection ability

Among the 100 sputum samples, 56 were both negative and 44 were positive by either traditional culture or tNGS methods, so we further analyzed the data using these 44 samples. Pathogens were detected in 17 samples by the culture-based method, providing a positive detection rate of 38.6% (**Figure 2A**). In comparison, targeted next-generation sequencing could detect pathogens from 35 samples with a positive detection rate of 79.5% (**Figure 2A**). These results indicate that tNGS has a stronger ability to detect pathogens from sputum specimens.

Pathogen identification results of targeted next-generation sequencing were partially consistent with those of culturing-based method

We further evaluated the consistency of pathogen identification between the traditional culturing and targeted next-generation sequencing methods. Based on the results of pathogen

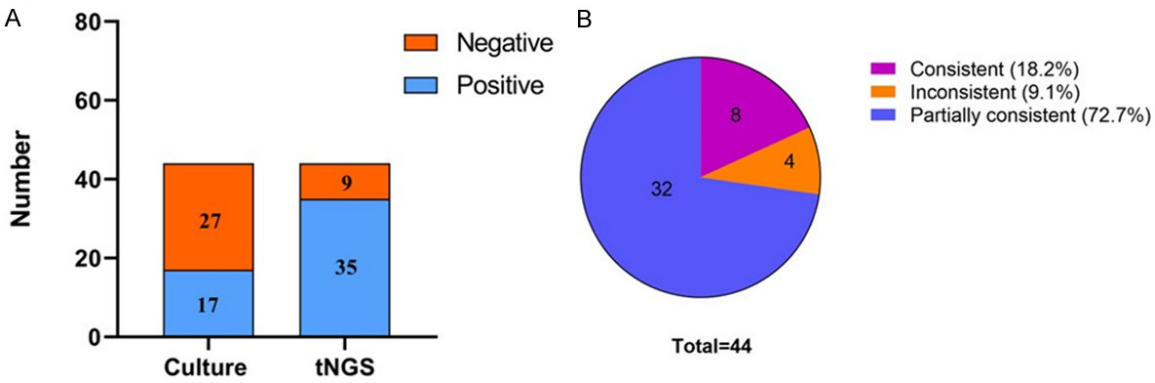


Figure 2. Comparison of (A) the positive rate and (B) consistency of pathogen detection between the culture-based method and targeted next generation sequencing.

Table 2. Distribution of microbes detected in Targeted next-generation sequencing

Microbe	1 week (n=20)	2 week (n=20)	3 week (n=20)	4 week (n=20)	5 week (n=20)
Epstein-Barr virus	4 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cytomegalovirus	2 (10%)	2 (10%)	1 (5%)	3 (15%)	1 (5%)
Candida albicans	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Candida parapsilosis complex	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Fusarium	2 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Candida guiemont	2 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Acinetobacter baumannii	0 (0%)	2 (10%)	1 (5%)	0 (0%)	0 (0%)
Citrobacter freudii	3 (15%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter cloacae	1 (5%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)
E. coli	0 (0%)	0 (0%)	1 (5%)	1 (5%)	0 (0%)
Haemophilus influenzae	3 (15%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)
Enterobacter aerogenes	2 (10%)	2 (10%)	0 (0%)	0 (0%)	0 (0%)
Klebsiella oxytoca	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Klebsiella pneumoniae	2 (10%)	0 (0%)	2 (10%)	2 (10%)	2 (10%)
Morganella	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pseudomonas aeruginosa	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Rickettsia	0 (0%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)
Serratia marcescens	4 (20%)	1 (5%)	2 (10%)	0 (0%)	0 (0%)
Stenotrophomonas maltophilia	4 (20%)	5 (25%)	1 (5%)	0 (0%)	0 (0%)
Enterococcus faecalis	2 (10%)	3 (15%)	2 (10%)	2 (10%)	2 (10%)
Enterococcus faecium	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus aureus	1 (5%)	0 (0%)	4 (20%)	3 (15%)	5 (25%)
Staphylococcus epidermidis	2 (10%)	3 (15%)	6 (30%)	7 (35%)	5 (25%)
Staphylococcus haemolyticus	4 (20%)	8 (40%)	16 (80%)	12 (60%)	11 (55%)
Streptococcus constellus	0 (0%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)
Streptococcus pneumoniae	1 (5%)	0 (0%)	2 (10%)	3 (15%)	4 (20%)
Mycobacterium tuberculosis complex	3 (15%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Reads number > 0.

identification from the 44 sputum samples, fully consistent results were observed in 8 (18.2%) samples, and inconsistent results were observed in only 4 (9.1%) samples (**Figure 2B**). Detailed information of detection inconsistency is summarized in **Table 2**. Generally, the results of pathogen identification from most of the samples (32/44, 72.7%) exhibited partial consistency between the two methods employed, for which the pathogens detected by culturing

Table 3. Detailed information of samples with conflicting results

Patient ID	Time point	Culture results	Possible pathogens by tNGS
P1	4th week	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
			Enterococcus faecalis
			Staphylococcus aureus
			Staphylococcus haemolyticus
P2	0 week	*Negative*	*Staphylococcus epidermidis*
			Klebsiella pneumoniae
			Staphylococcus haemolyticus
			Stenotrophomonas maltophilia
	4th week	*Klebsiella pneumoniae*	*Negative*
P3	0 week	*Negative*	*Staphylococcus haemolyticus*
	1st week	*Negative*	*Staphylococcus haemolyticus*
	3rd week	*Negative*	*Staphylococcus haemolyticus*
	4th week	*Negative*	*Staphylococcus haemolyticus*
P4	4th week	*Escherichia coli*	*Enterococcus faecalis*
			Staphylococcus haemolyticus
P5	3rd week	*Citrobacter freudii*	*Staphylococcus epidermidis*
P5	4th week	*Negative*	*Staphylococcus haemolyticus*
P6	0 week	*Negative*	*Streptococcus pneumoniae*
			Staphylococcus haemolyticus
			Staphylococcus epidermidis
P6	4th week	*Negative*	*Staphylococcus haemolyticus*
P7	0 week	*Negative*	*Serratia marcescens*
P7	4th week	*Staphylococcus aureus*	*Streptococcus pneumoniae*
P8	0 week	*Negative*	*Haemophilus influenzae*
P8	3rd week	*Klebsiella pneumoniae*	*Staphylococcus haemolyticus*
P9	0 week	*Staphylococcus aureus*	*Haemophilus influenzae*
	1st week	*Coagulase negative staphylococcus*	*Serratia marcescens*
	2nd week	*Staphylococcus aureus*	*Staphylococcus aureus*
			Staphylococcus haemolyticus
P10	2nd week	*Negative*	*Staphylococcus haemolyticus*
P11	1st week	*Negative*	*Staphylococcus haemolyticus*
P12	0 week	*Negative*	*Serratia marcescens*
	2nd week	*Negative*	*Staphylococcus haemolyticus*
			Staphylococcus aureus
	3rd week	*Negative*	*Staphylococcus haemolyticus*
P13	0 week	*Negative*	*Enterococcus faecium*
	2nd week	*Staphylococcus epidermidis*	*Negative*
	4th week	*Negative*	*Staphylococcus aureus*
			Staphylococcus haemolyticus
P14	0 week	*Negative*	*Klebsiella oxytoca*
P15	0 week	*Negative*	*Citrobacter freundii*
			Serratia marcescens
P16	3rd week	*Klebsiella pneumoniae*	*Staphylococcus epidermidis*
			Klebsiella pneumoniae
			Staphylococcus haemolyticus
P17	2nd week	*Coagulase negative staphylococcus*	*Staphylococcus haemolyticus*
			Staphylococcus aureus
			Staphylococcus epidermidis
	3rd week	*Coagulase negative staphylococcus*	*Staphylococcus haemolyticus*

P18	0 week	*Negative*	*Pseudomonas aeruginosa*
			Citrobacter freundi
			Candida guiemont
			Enterobacter cloacae
P19	1st week	*Staphylococcus epidermidis*	*Enterococcus faecalis*
		Coagulase negative staphylococcus	*Stenotrophomonas maltophilia*
	3rd week	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
			Streptococcus pneumoniae
P20	0 week	*Staphylococcus epidermidis*	*Cytomegalovirus*

approach were also detected by targeted sequencing (**Figure 2B**). The distribution of identified pathogens by tNGS during the 5-week examination is summarized in **Table 3**.

Most pathogens detected by targeted next-generation sequencing were decreased in patients after treatment

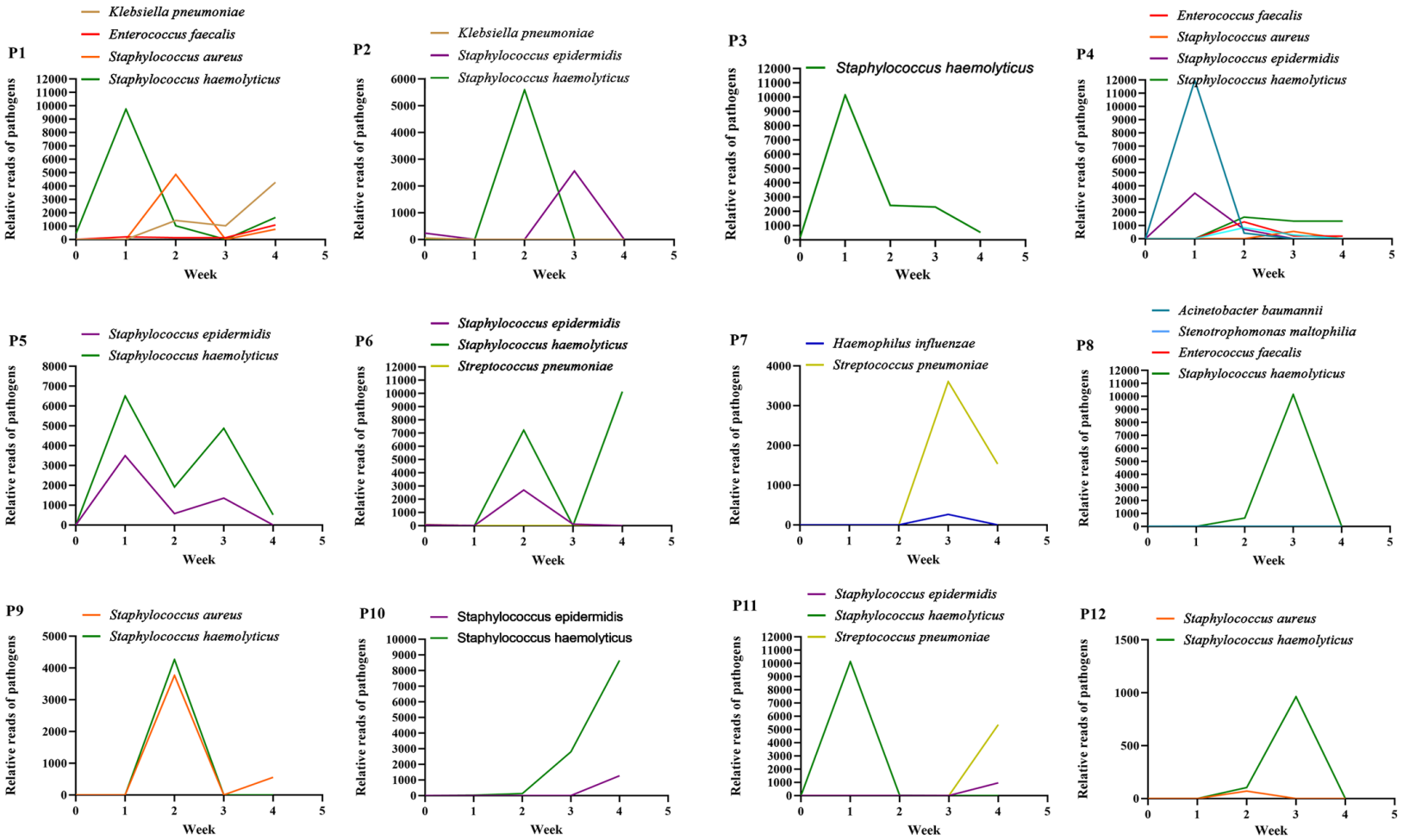
Sputum samples from the same 20 patients were collected again after corresponding treatment for pathogen detection by tNGS. We compared the number of individual pathogens before and after treatment and found that most of them were reduced or excluded after treatment (**Figure 3**). Only a few pathogens were unchanged or elevated after treatment (**Figure 3**), including *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, and *Streptococcus pneumoniae*.

Discussion

Metagenomic sequencing-based approaches, as alternative methods for traditional culturing-based approaches, have been established for microbial detection and clinical diagnosis of respiratory tract infections [23, 24]. As reported before, mNGS demonstrated higher sensitivity than traditional culture for detecting bacteria in pulmonary infections [25]. However, it has a relatively high cost [26, 27]. The targeted next-generation sequencing takes the advantage of both multiplex PCR for amplifying targeted genes of the predicted pathogens and the second-generation sequencing technology for providing high-throughput information [21, 28]. This novel approach is capable of detecting the recognized 106 types of respiratory pathogens (e.g., pathogenic bacteria and fungi) at a higher sensitivity but a lower cost, in comparison to metagenomic sequencing [28, 29]. This approach relies on microbial prediction, which cannot identify unknown pathogens [28]. Here we report a comparison study on the application of tNGS in lower respiratory infection diagnosis and pathogen identification.

Previous studies have established the application of targeted next-generation sequencing in veterinary and cancer diagnostics [20, 29, 30]. The current study demonstrated that, compared to the traditional culture-based approach, targeted high-throughput sequencing could increase the lower respiratory pathogen detection rate by 105.9% (**Figure 2**), with 90.9% full or partial consistency in the results (**Figure 2** and **Table 2**). With the combination of targeted multiplex PCR and second-generation sequencing, we successfully identified most of the clinically recognized pathogenic viruses, bacteria, and fungi (**Table 3**), indicating its promising application for diagnosis of human infectious diseases. However, considering that all the enrolled individuals in this study were newborns (192-219 days) that had a partially developed respiratory microbiome [31] and were admitted to an NICU sterile environment, the abundance of respiratory microbes (i.e. the number of sequencing reads) in these patients was detected only at a relatively low level (**Figure 3**).

According to previous studies, several pathogenic microbes can also be normal respiratory microflora [31, 32]. Therefore, the determination of commensal colonization or pathogenic infection of such microbes mainly relies on both the outcomes of sequencing detection and clinical results of the patients [33, 34]. For example, despite the fact that the abundances of most of the identified pathogens were decreased after treatment, we observed that the abundances of *E. faecalis*, *S. pneumoniae,* and *Staphylococcus* spp. Tended to increase in


P1
Klebsiella pneumoniae
Enterococcus faecalis
Staphylococcus aureus
Staphylococcus haemolyticus
P2
Klebsiella pneumoniae
Staphylococcus epidermidis
Staphylococcus haemolyticus
P3
Staphylococcus haemolyticus
P4
Enterococcus faecalis
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus haemolyticus
P5
Staphylococcus epidermidis
Staphylococcus haemolyticus
P6
Staphylococcus epidermidis
Staphylococcus haemolyticus
Streptococcus pneumoniae
P7
Haemophilus influenzae
Streptococcus pneumoniae
P8
Acinetobacter baumannii
Stenotrophomonas maltophilia
Enterococcus faecalis
Staphylococcus haemolyticus
P9
Staphylococcus aureus
Staphylococcus haemolyticus
P10
Staphylococcus epidermidis
Staphylococcus haemolyticus
P11
Staphylococcus epidermidis
Staphylococcus haemolyticus
Streptococcus pneumoniae
P12
Staphylococcus aureus
Staphylococcus haemolyticus
Relative reads of pathogens
Week

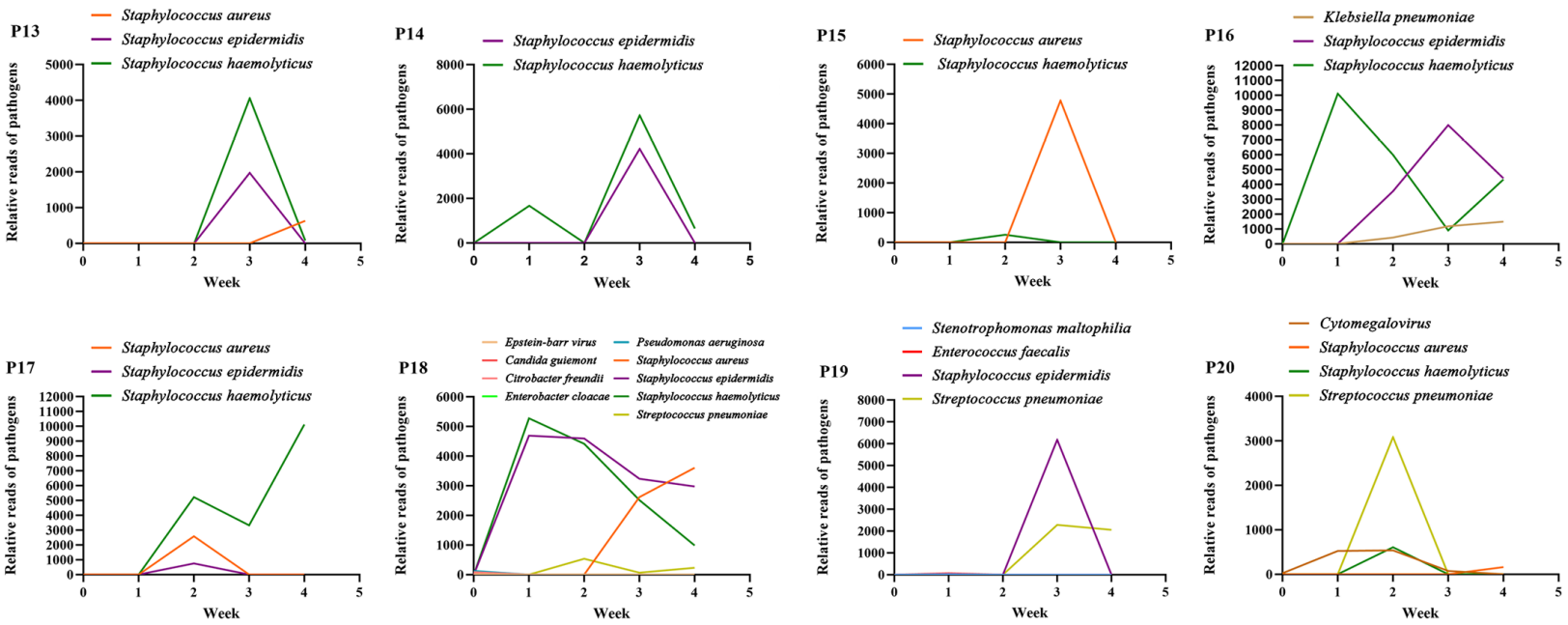


Figure 3. Trends of changes in relative abundance of respiratory pathogens from individual patients over time. The reads were generated by targeted next generation sequencing and filtered for quality control. 'Pn' represents the ID (n) of the patient (P).

the patients with an improved health condition (**Figure 3**). Therefore, we speculate that these pathogenic bacteria are respiratory commensal flora in the patients.

There are several limitations in this study: first, the sample size was limited, which may affect the accuracy and performance of tNGS. Second, false-positive and false-negative results may occur as we didn't use a more reliable diagnostic strategy such as real-time PCR. Third, a positive tNGS result in this study was the detection of pathogen reads. More attention should be paid to the diagnostic value of very few reads.

To conclude, the combined employment of targeted multiplex PCR and next-generation sequencing in respiratory pathogen detection exhibits a high sensitivity and moderate consistency with the traditional culture-based method. Meanwhile, the diagnosis of lower respiratory infection by targeted high-throughput sequencing can also provide precise guidance for treatment strategy for the preterm infant patients. These findings demonstrate the potential application of targeted next-generation sequencing in respiratory infectious disease diagnosis and pathogen detection.

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Disclosure of conflict of interest

None.

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