

RESEARCH ARTICLE

Open Access



The diagnostic value of metagenomic next-generation sequencing for identifying *Streptococcus pneumoniae* in paediatric bacterial meningitis

Xi-xi Zhang^{1†}, Ling-yun Guo^{1†}, Lin-lin Liu¹, Ao Shen², Wen-ya Feng¹, Wen-hua Huang³, Hui-li Hu¹, Bing Hu¹, Xin Guo¹, Tian-ming Chen¹, He-ying Chen¹, Yong-qiang Jiang³ and Gang Liu^{1*}

Abstract

Background: There is currently no research on the diagnostic value of metagenomic next-generation sequencing (mNGS) for a single pathogens in CSF. The aim of this study was to analyse the value of mNGS for identifying *Streptococcus pneumoniae* (*S. pneumoniae*) in paediatric bacterial meningitis.

Methods: Bacterial meningitis (BM) cases from October 23, 2014, to December 31, 2016, and December 1, 2017, to July 31, 2018 at Beijing Children's Hospital were reviewed. Clinical features and pathogens were analysed.

Results: We diagnosed 135 patients with BM in this study. A total of 43 *S. pneumoniae* were identified by combination methods. 26/135 (19.3%) patients had positive results in *S. pneumoniae* by blood and/or cerebrospinal fluid (CSF) culture. Alere BinaxNow®*Streptococcus pneumoniae* Antigen test was positive in 35/135(25.9%) cases. 32/135 (23.7%) *S. pneumoniae* were identified by mNGS. Six CSF samples were identified as *S. pneumoniae* only by mNGS technology. Taking culture as the gold standard, the sensitivity and specificity of mNGS for diagnosing *S. pneumoniae* meningitis were 73.1 and 88.1%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) of diagnosing *S. pneumoniae* meningitis by mNGS were 59.4 and 93.2%, respectively. When comparison between mNGS and combined tests (culture and Alere BinaxNow®*Streptococcus pneumoniae* Antigen test), the sensitivity and specificity of mNGS for *S. pneumoniae* identification were 70.3 and 93.9%, the PPV and NPV in the identification of *S. pneumoniae* by mNGS were 81.4 and 89.3%, respectively. The difference in number of unique reads of *S. pneumoniae* in from CSF sample (< 14 days onset) and CSF sample (> 14 days from onset) was statistically significant (170.5 VS. 13, $P = 0.019$). The difference in the collected time of CSF for culture and mNGS was statistically significant (4 days VS. 14 days, $P < 0.001$).

Conclusions: mNGS has high sensitivity and specificity for *S. pneumoniae* identification. The pathogen load (number of unique reads) of *S. pneumoniae* is related to the CSF collection time. mNGS was less affected than culture by the use of antibiotics before CSF collection.

Keywords: Metagenomic next-generation sequencing (mNGS), Sensitivity, Specificity, *Streptococcus pneumoniae*, Meningitis, Children

* Correspondence: liugangbch@sina.com

[†]Xi-xi Zhang and Ling-yun Guo contributed equally to this work.

¹Key Laboratory of Major Diseases in Children, Ministry of Education, Department of Infectious Diseases, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, No. 56 Nan Lishi Road, Beijing 100045, China

Full list of author information is available at the end of the article



Highlights

Metagenomic Next-generation sequencing (mNGS) had high sensitivity and specificity for *S. pneumoniae* identification in CSF specimens.

mNGS was less affected than culture by the use of antibiotics before CSF collection.

The number of unique reads of *S. pneumoniae* detected by mNGS was related to the duration from onset to the sample collection time.

Background

Bacterial Meningitis (BM) is an infection of the meninges and subarachnoid space that can sometimes present as an infection of the brain cortex and parenchyma [1]. BM had an annual incidence of 16 million people worldwide in 2013, of which 1.6 million had sequelae [2]. BM causes significant morbidity and mortality in both developed and developing countries [3–5]. The fatality rate of *Streptococcus pneumoniae* (*S. pneumoniae*) meningitis can reach 20–37% in high-income countries and up to 51% in low-income countries [6]. Neurological sequelae are common among survivors [7–10]. Therefore, prompt pathogen diagnosis and accurate antibiotic treatment are essential to improve the prognosis of BM.

With the application of the meningococcal vaccine, *S. pneumoniae* conjugate vaccine and Hib vaccine, the incidence of BM caused by these pathogens has decreased in developed countries [11]. However, the pneumococcal conjugate vaccine is not universally used in China. A multicentre study of BM in Chinese children showed that *S. pneumoniae* was still the most common pathogen, occurring in up to 46.5% of cases [12]. The conventional pathogen detection method (culture) may take a long time (3 to 5 days) and have a low positive rate. Metagenomic next-generation sequencing (mNGS) is a high-throughput sequencing method that can directly detect the nucleic acids of pathogens in clinical specimens and then analyse the nucleic acid sequences by bioinformatics methods. As a novel diagnostic tool, mNGS has been used for the identification of various pathogens such as bacteria, viruses, fungi and parasites from clinical samples (tissues, CSF or plasma) in an unbiased, simultaneous and direct manner [13–20].

However, at present, there are few studies on pathogen detection by mNGS in bacterial meningitis. Our team previously identified pathogens from cerebrospinal fluid specimens in children with bacterial meningitis by mNGS. The results showed that mNGS is of great value for the identification of pathogens in cerebrospinal fluid (CSF), and *S. pneumoniae* is still the most common pathogen [17]. However, there is currently no research on the diagnostic value of mNGS for a single pathogen. Hence, this study aimed to investigate the diagnostic value of mNGS for identifying *S. pneumoniae* in children with BM.

Methods

Study population and specimen collection

All BM patients aged 29 days to 18 years were admitted to the Department of Infectious Diseases at Beijing Children's Hospital from October 23, 2014, to December 31, 2016, and December 1, 2017, to July 31, 2018. The diagnosis criteria for BM were consistent with the World Health Organization (WHO), as follows: (1) acute fever (> 38.5 C rectal or > 38.0 C axillary); (2) headache, meningeal irritation, or altered consciousness; (3) at least one of the following in the cerebrospinal fluid: leukocytes > 100 cells/mm [3] or leukocytes 10–100 cells/mm [3] with elevated protein (> 100 mg/dL) or decreased glucose (< 40 mg/dL); and (4) positive culture, positive Gram stain, or positive bacterial antigen in the CSF. A case meeting diagnostic criteria 1, 2, and 3 at the same time was considered a probable case. A probable case meeting criterion 4 was considered a confirmed case. Exclusion criteria were as follows: cerebrospinal fluid < 1 ml and bloody CSF. The clinical data of all included cases including demographic characteristics, antibiotic use, specimen collection and clinical microbiology tests results [culture and Alere BinaxNow® *Streptococcus pneumoniae* Antigen test (Alere, USA)] were recorded.

One millilitre of CSF was collected for mNGS when the lumbar puncture was performed after admission. This study was approved by the Ethics Committee of Beijing Children's Hospital affiliated to Capital Medical University (No. 2017–74). Written informed consent was obtained from the patient's parents or other legal representatives.

DNA extraction

DNA was extracted directly from the 300ul CSF sample (each patient and negative “no-template” control) using the TIANamp Micro DNA Kit (DP316, Tiangen Biotech, Beijing, China). The sample was added proteinase K (10 ml) and 300 ml buffer GB (with carrier RNA) and then incubated at 56 °C (10 min). After 300 ul cold absolute ethyl alcohol was added and the tube was incubated at room temperature (5 min). Transferring the liquid to a new adsorption column and the liquid was washed with buffer GD and buffer PW. The DNA was dissolved in 40ul of Tris–ethylenediaminetetraacetic acid buffer.

Library generation, and sequencing

The extracted DNA was sonicated with a Bioruptor Pico device to generate 200–300 bp fragments. According to the standard protocol of the BGISEQ-500 sequencing platform (BGI-Tianjin, Tianjin, China), DNA libraries were constructed through end repaired, adapter added overnight and polymerase chain reaction amplification to the extracted DNA. Quality control was carried out using a bioanalyser (Agilent 2100, Agilent Technologies, Santa Clara, CA, USA) combined with quantitative PCR

to measure the adapters before sequencing. DNA sequencing was then performed using the BGISEQ-500 platform (BGI-Tianjin, Tianjin, China) [21].

Data processing and analysis

High quality sequencing data was generated after removal of short (< 35 bp) reads, low quality and low complexity reads. The readings were then mapped to the human reference genome (hg19 and YH sequences) using the Burrows-Wheeler Aligner [22]. The remaining data were aligned with the NCBI microbial genome database (<ftp://ftp.ncbi.nlm.nih.gov/genomes/>), which included the genome sequences of 3446 bacterial species (104 species of *Mycobacterium tuberculosis* and 45 species of mycoplasma/chlamydia), 1515 viral species, 206 fungal species and 140 parasites connected to human diseases. The mapped data were used for further analysis. The depth and coverage of each species was calculated using Soap Coverage on the SOAP website (<http://soap.genomics.org.cn/>).

Quantitative real-time PCR (qPCR) validation

We performed *S. pneumoniae* qPCR to validate the mNGS results. The DNA was extracted using the QIAamp cadior Pathogen Mini Kit (Qiagen, 54,106). qPCR was carried out on the ViiATM 7 real-time PCR system using cycling conditions comprising 2 min at 50 °C and 10 min at 95 °C followed by 45 two-step cycles of 15 min at 95 °C and 1 min at 60 °C. The sequences of the primers and probes are as follows: F: ACGCAATC-TAGCAGATGAAGCA, R: TCGTGCGTTTAAATTC-CAGCT and P: FAM-AACGCTTGATACAGGGAG-MGB [23].

Statistical methods

Continuous variables were expressed as the mean \pm standard deviation or as the median. Two groups were compared using the independent t-test for parametric data and the Mann-Whitney *U* test for non-parametric data. Continuous variables with *P*-values < 0.05 were considered statistically significant, and all tests were 2-tailed. Based on the extracted data, a 2 \times 2 contingency table was used to determine sensitivity, specificity, PPV, and NPV. All of the statistical analyses were conducted using SPSS 23.0 software (SPSS Inc. USA).

Results

Patient demographics and microbiology results

A total of 135 children with BM were included in this study. *S. pneumoniae* was identified in 43/135 (31.9%) patients by all microbiology tests. A total of 26/43 (60.5%) cases were male, and the median age was 11.5 (8.5, 48.9) months. A total of 37/135 (27.4%) cases were identified as *S. pneumoniae* infection by clinical microbiology tests

(culture and/or Alere BinaxNow® *Streptococcus pneumoniae* Antigen test). *S. pneumoniae* was identified by blood and/or CSF culture in 26/135 (19.3%) cases. A total of 35/135 (25.9%) patients had positive results on the Alere BinaxNow® *Streptococcus pneumoniae* Antigen test. *S. pneumoniae* was identified in 32/135 (23.7%) patient CSF specimens by mNGS. Six CSF samples were identified as *S. pneumoniae* only by mNGS.

mNGS information and the related influencing factors

Among 32 patients who were diagnosed with *S. pneumoniae* meningitis by mNGS, the number of unique reads of *S. pneumoniae* ranged from 4 to 341,303. The coverage of *S. pneumoniae* ranged from 0.011 to 86.68%, with a depth value of 1–36.57 (Table 1). The collection time of CSF specimens for mNGS was 4 to 121 days after disease onset, and the median collection time was 14 days. The difference in the number of unique reads of *S. pneumoniae* in the different groups (CSF collection time < 14 days and > 14 days from onset) was statistically significant (170.5 vs. 13, *P* = 0.019) (Fig. 1). All 32 mNGS-positive children were treated with antibiotics prior to mNGS sample collection.

Based on the number of unique reads of *S. pneumoniae*, 32 patients were divided into two groups. The unique reads \leq 100 group comprised 17 (41.4%) patients, while the unique reads > 100 group comprised 15 patients. Comparing the two groups, patients in the unique reads > 100 group had a generally shorter duration from disease onset to sample collection than patients in the mNGS unique reads \leq 100 group (11 days vs. 20 days, *P* = 0.027). There was a significant difference in the simultaneous CSF white blood cell count and protein level between the two groups ($10 \times 10^9/L$ vs. $32 \times 10^9/L$, *P* = 0.001; 1280 mg/L vs. 855 mg/L, *P* = 0.016) (Table 2).

Comparison of mNGS and clinical microbiology methods

The median collection time of CSF specimens for culture that had positive *S. pneumoniae* results was 4 (3, 5) days. The median collection time of CSF specimens for mNGS that had positive *S. pneumoniae* results was 14 (8, 23) days from onset. The difference in the collected time of CSF for culture and mNGS was statistically significant (4 days vs. 14 days, *P* < 0.001).

Among the 135 BM patients, mNGS and clinical microbiology tests (culture and/or Alere BinaxNow® *Streptococcus pneumoniae* Antigen test) were both positive for *S. pneumoniae* in 26 (19.3%) samples and were both negative for *S. pneumoniae* in 92 (68.1%) samples. The accordance rate of mNGS and clinical microbiology tests (culture and/or Alere BinaxNow® *Streptococcus pneumoniae* Antigen test) for the identification of *S. pneumoniae* was 87.4% (118/135).

The comparison between mNGS and clinical microbiology tests (culture and Alere BinaxNow® *Streptococcus*

Table 1 Clinical microbiology results and sequencing information for patients who were positive for *S. pneumoniae* by mNGS

Patient	Clinical microbiology tests			mNGS				qPCR
	Pathogen	Culture (blood/CSF)	Antigen test	Pathogen	Reads	Coverage%	Depth	
P1	SP	–	+	SP	262	1.1	1	ND
P2	SP	+	+	SP	1105	11	1.1	ND
P3	SP	+	+	SP	573	2.6	1	+
P4	SP	+	+	SP	429	1.9	1	+
P5	SP	–	+	SP	425	1.9	1	+
P6	SP	–	+	SP	176	1.8	1	ND
P7	SP	+	ND	SP	162	0.75	1	+
P8	SP	+	+	SP	38	0.16	1	ND
P9	SP	–	+	SP	21	0.12	1	ND
P10	SP	+	+	SP	13	0.066	1	ND
P11	SP	+	+	SP	11	0.052	1	ND
P12	SP	+	+	SP	7	0.048	1	–
P13	SP	+	+	SP	6	0.034	1	–
P14	SP	+	+	SP	4	0.041	1	ND
P15	SP	–	+	SP	4	0.016	1	–
P16	SP	–	+	SP	3189	28	1.3	ND
P17	SP	–	+	SP	23,247	75	3.9	ND
P18	SP	+	+	SP	25	0.19	1	+
P19	SP	+	+	SP	2603	7.25	1.06	ND
P20	SP	+	+	SP	341,303	86.68	36.57	+
P21	SP	+	+	SP	165	0.7413	1.01	+
P22	SP	+	+	SP	1335	12.03	1.09	+
P23	SP	+	+	SP	456	5.41	1.04	+
P24	SP	+	+	SP	32	0.0744	1	ND
P25	SP	+	+	SP	218	1.04	1	ND
P26	SP	+	+	SP	20	0.1488	1	–
P27	–	–	–	SP	33	0.31	1	ND
P28	SA	SA	ND	SP	27	0.15	1	–
P29	–	–	–	SP	24	0.12	1	ND
P30	–	–	ND	SP	12	0.95	1	ND
P31	–	–	–	SP	6	0.055	1	ND
P32	–	–	–	SP	4	0.011	1	–

Note: mNGS metagenomic next-generation sequencing, CSF Cerebrospinal fluid, qPCR quantitative real-time PCR, SP *Streptococcus pneumoniae*, SA *Streptococcus aureus*, E. coli *Escherichia coli*, + positive, – negative, ND Not done

pneumoniae Antigen test) was shown in Table 3. Taking culture as the gold standard, the sensitivity and specificity of *S. pneumoniae* identification by mNGS were 73.1 and 88.1%, respectively. The PPV and NPV of diagnosing *S. pneumoniae* meningitis by mNGS were 59.4 and 93.2%, respectively. Comparing mNGS and the combined tests (culture and Alere BinaxNow® *Streptococcus pneumoniae* Antigen test), the sensitivity and specificity of mNGS for *S. pneumoniae* identification were 70.3 and 93.9%, respectively, and the PPV and NPV for the identification of *S. pneumoniae* by mNGS were 81.4 and 89.3%, respectively.

In total, we performed qPCR validation for 15 CSF specimens, while insufficient CSF sample was available for qPCR validation in the other cases. These 15 CSF specimens were collected for mNGS and qPCR at the same time. The qPCR results were positive in 9 (9/15, 60%) cases [(mNGS unique reads: 429 (25–341,303)] and negative in 6 (6/15, 40%) cases. The latter 6 cases had fewer unique reads of *S. pneumoniae*, and the number of unique reads was 4, 4, 6, 7, 20 and 27. The clinical microbiology tests (culture and/or Alere BinaxNow® *Streptococcus pneumoniae* Antigen test) were positive

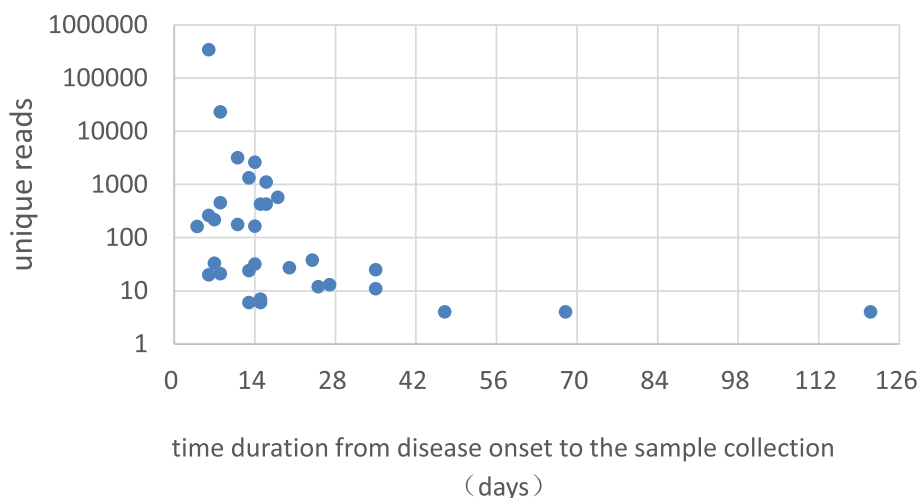


Fig. 1 The relationship between unique reads of *Streptococcus pneumoniae* and sample collection time

Table 2 Comparison of different mNGS unique read groups

Items	mNGS unique reads ≤ 100 (N = 17)	mNGS unique reads > 100 (N = 15)	P
Sex (female/male)	12/5	7/8	0.169
Age (months)	11.2(5.9,33.5)	13.9(9.2,48.9)	0.290
Duration from onset to CSF collection (days)	20(13,35)	11(7,16)	0.027
Fever [n (%)]	17(100)	15(100)	
Seizure [n (%)]	8(47.1)	10(66.7)	0.265
Vomiting [n (%)]	10(58.8)	9(60)	0.946
Meningeal irritation [n (%)]	9(52.9)	12(80)	0.103
Altered mental status [n (%)]	7(41.2)	8(53.3)	0.492
Peripheral blood			
WBC (×10 ⁹ /L)	16.98(15.10,23.69)	17.3(11.9,24.2)	0.895
Neutrophils (%)	78.2(60.7,86.3)	77.8(62.7,85.6)	0.985
Haemoglobin (g/L)	104.4 ± 19.0	105.5 ± 18.7	0.767
C-reactive protein (mg/L)	63.5(19.6,104)	109.5(49,157.4)	0.281
1st CSF ^a			
WBC (×10 ⁶ /L)	1714(495,2635)	1886(910,3600)	0.597
Protein (mg/L)	1280(779,2250)	1650(1161,3033)	0.180
Glucose (mmol/L)	1.84(1.00,3.34)	0.67(0.19,1.92)	0.081
Simultaneous CSF ^b			
WBC (×10 ⁶ /L)	10 (2,12)	32(14,76)	0.001
Protein (mg/L)	1280(779,2250)	855(527,1488)	0.016
Glucose (mmol/L)	3.42 ± 0.99	3.39 ± 1.12	0.928

Note: ^aThe first CSF refers to the first cerebrospinal fluid examination in the early stage of the disease
^bSimultaneous CSF refers to the CSF results by clinical testing at which time CSF was also tested by mNGS
P-values in bold italic shows variables with evidence of association in univariate analysis

Table 3 Diagnostic performance of mNGS compared with culture and Alere BinaxNow® *Streptococcus pneumoniae* Antigen test for the detection of *Streptococcus pneumoniae*

Clinical microbiology tests	mNGS			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture ^a	73.1	88.1	59.4	93.2
Combined test ^b	70.3	93.9	81.3	89.3

Note: ^arefers to the blood and/or CSF culture; ^brefers to culture and or Alere BinaxNow® *Streptococcus pneumoniae* Antigen test. PPV positive predictive value, NPV negative predictive value

for *S. pneumoniae* in 4 (4/6) cases (unique reads: 4, 6, 7 and 20). One (1/6) case (unique reads: 4) was negative for *S. pneumoniae* in the clinical microbiology tests (blood and/or CSF culture and Alere BinaxNow® *Streptococcus pneumoniae* Antigen test). Another case (1/6) (P28, unique reads: 27) was positive for *Staphylococcus aureus* (*S. aureus*) in the blood culture. However, the result of specific PCR from CSF specimens was negative for both *S. pneumoniae* and *S. aureus* in P28.

In addition, we identified multiple pathogens in the CSF sample of P14 by mNGS, including *Escherichia coli* (*E. coli*) (unique reads 30) and *S. pneumoniae* (unique reads 4). In terms of clinical microbiology, P14 had a positive result for *S. pneumoniae* in the CSF culture and a positive Alere BinaxNow® *Streptococcus pneumoniae* Antigen test in the CSF. However, the Sanger validation of the CSF sample was negative for both *S. pneumoniae* and *E. coli*.

Discussion

In this study, clinical microbiology tests (culture and Alere BinaxNow® *Streptococcus pneumoniae* Antigen test) were compared with an emerging pathogen detection method (mNGS). When the combined culture and pneumococcal antigen test were regarded as the gold standard, mNGS showed high sensitivity and specificity for the diagnosis of *S. pneumoniae* meningitis. Previous studies have reported that mNGS has different sensitivities and specificities for the identification of different types of pathogens (bacterial, viral or fungal). The sensitivity and specificity of mNGS for the identification of bacteria had a wide range, with sensitivity ranging from 50.7 to 100% and specificity ranging from 76.5 to 87.5% [16, 24]. In this study, some of the CSF specimens were collected at the convalescence stage. If all CSF specimens were collected during the acute phase, the sensitivity of mNGS would be higher.

The number of unique reads of *S. pneumoniae* from CSF collected ≤ 14 days from the disease onset was significantly higher than those from CSF collected > 14 days from the disease onset. Patients in the unique reads > 100 group had a generally shorter duration from disease onset to the sample collection than those in the unique reads ≤ 100 group. These results together suggest that the number of unique

reads of *S. pneumoniae* detected by mNGS was associated with specimen collection time, which is consistent with a previous study showing that with the use of effective antibiotics and disease improvement, the number of unique reads of pathogens decreased [25].

In this study, we observed that the time from disease onset to CSF collection was longer for mNGS than for culture. All patients were given antibiotics prior to mNGS and culture, and all patients were treated with antibiotics after the onset of disease. This phenomenon suggested that after antibiotic use, the timespan for positive pathogen identification by mNGS was longer than the timespan for positive pathogen identification by culture. A previous study suggested that the DNA of the pathogen may survive in body fluids for a prolonged period of time, and mNGS was less affected than culture by the use of antibiotics before CSF collection [24, 26, 27].

Among the 6 CSF samples that were negative for *S. pneumoniae* by qPCR, *S. pneumoniae* was identified in 4 (4/6) samples by both mNGS and clinical microbiology tests (blood and/or CSF culture and Alere BinaxNow® *Streptococcus pneumoniae* Antigen test). This result showed that mNGS appears to have a higher sensitivity than qPCR in the test cases. *S. pneumoniae* (unique reads 27) was identified in P28 CSF sample by mNGS, while *S. aureus* was detected by blood culture. The P28 CSF sample was found to be negative for both *S. pneumoniae* and *S. aureus* by qPCR. In many mNGS systems, short read sequences lead to difficulties in assembling and matching to the reference sequence, especially in repeat regions, resulting in difficulties defining the final pathogen. Therefore, when the number of unique reads is very small, the interpretation of pathogen data needs to be very careful and cautious [28].

We compared groups with ≤ 100 unique reads and groups with > 100 unique reads and analysed factors that could affect the number of unique reads of *S. pneumoniae*. There were significant differences in the simultaneous CSF white blood cell counts and protein levels between the two groups. These results suggested that the inflammation status during simultaneous mNGS detection might determine the number of unique reads of the pathogen. The number of unique reads may indirectly monitor disease progression.

Currently, the criteria for diagnosing single pathogens by mNGS are unclear. Different studies have reported different diagnostic criteria, mainly based on the coverage rate, the relative abundance of pathogens or unique reads of pathogens [16, 29].

In this study, the minimum number of unique reads of *S. pneumoniae* was 4, and the clinical microbiology test was also positive for *S. pneumoniae*. Therefore, the positive criteria for single pathogen identification by mNGS testing require further research by a larger sample size.

There were several limitations in this study. First, the collection time of some CSF samples for mNGS and

clinical microbiology tests was different. The collection time of CSF was later for mNGS than for the clinical microbiology tests, which may affect the positive identification rate of mNGS. Some of the CSF specimens were collected at the convalescence stage. Second, this study was retrospective and had a limited number of BM cases, and thus large-scale research is needed. Third, mNGS still has low sequencing depth, and the pathogen database is imperfect [30]. However, sequencing technology is developing rapidly, and it is believed that the depth of sequencing will improve within a short period of time. mNGS can further improve the sensitivity and specificity of pathogen identification [31].

Conclusion

This study is the first to analyse the diagnostic value of mNGS for the identification of a single pathogen (*S. pneumoniae*). mNGS has high sensitivity and specificity for *S. pneumoniae* identification. Our study also demonstrated the relationship between the pathogen load (unique reads) and CSF collection time. Although there are no uniform criteria for pathogen identification by mNGS, there are many difficulties in interpreting the mNGS results. With the development of mNGS technology, mNGS could be a promising alternative diagnostic tool for pathogen detection.

Abbreviations

BM: bacterial meningitis; CSF: cerebrospinal fluid; mNGS: metagenomic next-generation sequencing; NPV: negative predictive value; PPV: positive predictive value; qPCR: Quantitative real-time PCR; *S. pneumoniae*: *Streptococcus pneumoniae*; *SA*: *Staphylococcus aureus*

Acknowledgements

We would like to thank the Bio bank for Diseases in Children at Beijing Children's Hospital, Capital Medical University for their contribution of storing the CSF samples with standardized procedures.

Author's contributions

All of the authors had access to the full dataset (including the statistical reports and tables) and take responsibility for the integrity of the data and the accuracy of the data analysis. XXZ, LYG and GL conceived and designed the study. XXZ, LYG, LLL, WYF, BH, HLH, TMC, XG, and HYC were involved in the case and sample collection, analysis, and interpretation of the data. XXZ, LYG, AS, WHH and YQJ participated in the laboratory analysis of the CSF. XXZ wrote the first draft of the paper. XXZ, LYG and GL reviewed and approved the final report. All authors have read and approved the final manuscript.

Funding

This work was supported by The Special Fund of The Pediatric Medical Coordinated Development Center of Beijing Municipal Administration (No. XTZD20180501), Beijing Hospital Authority "Dengfeng" Talent Training Plan (DFL 20181201), and the National Science and Technology Major Project of China (No.2018ZX10305409). The funders had no role in study design, data collection and analysis, or preparation of the manuscript.

Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University (2017-k-83). Informed

consent was waived because this was a retrospectively study. We obtained patient data from the Medical Records and Statistics Room. We analysed the data anonymously. The raw data were permitted by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Key Laboratory of Major Diseases in Children, Ministry of Education, Department of Infectious Diseases, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, No. 56 Nan Lishi Road, Beijing 100045, China. ²Tianjin Medical Laboratory, BGI-Tianjin, Tianjin, China. ³State Key Laboratory of Pathogen and Biosecurity, Institute of Microbiology and Epidemiology, Academy of Military Medical Science, Beijing 100071, China.

Received: 27 February 2019 Accepted: 24 May 2019

Published online: 04 June 2019

References

- van de Beek D, Brouwer M, Hasbun R, Koedel U, Whitney CG, Wijdicks E. Community-acquired bacterial meningitis. *Nat Rev Dis Primers*. 2016;2:16074.
- Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;386(9995):743–800.
- Lucas MJ, Brouwer MC, van de Beek D. Neurological sequelae of bacterial meningitis. *J Inf Secur*. 2016;73(1):18–27.
- McIntyre PB, O'Brien KL, Greenwood B, van de Beek D. Effect of vaccines on bacterial meningitis worldwide. *Lancet*. 2012;380(9854):1703–11.
- Li Y, Yin Z, Shao Z, Li M, Liang X, Sandhu HS, et al. Acute meningitis and encephalitis syndrome study group. Population-based surveillance for bacterial meningitis in China, September 2006–December 2009. *Emerg Infect Dis*. 2014;20:61–9.
- Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clin Microbiol Rev*. 2010;23(3):467–92.
- Hupp S, Ribes S, Seele J, Bischoff C, Förtsch C, Maier E, et al. Magnesium therapy improves outcome in *Streptococcus pneumoniae* meningitis by altering pneumolysin pore formation. *Br J Pharmacol*. 2017;174(23):4295–307.
- Grimwood K. Legacy of bacterial meningitis in infancy: many children continue to suffer functionally important deficits. *Br Med J*. 2001;323(7321):523–4.
- Libster R, Edwards KM, Levent F, Edwards MS, Rench MA, Castagnini LA, et al. Longterm outcomes of group B streptococcal meningitis. *Pediatrics*. 2012;130(1):e8.
- Stockmann C, Ampofo K, Byington CL, Filloux F, Hersh AL, Blaschke AJ, et al. Pneumococcal meningitis in children: epidemiology, serotypes, and outcomes from 1997–2010 in Utah. *Pediatrics*. 2013;132(3):421–8.
- Chávez-Bueno S, McCracken GH. Bacterial meningitis in children. *Pediatr Clin N Am*. 2005;52(3):795–810.
- Li C, Feng WY, Lin AW, Zheng G, Wang YC, Han YJ, et al. Clinical characteristics and etiology of bacterial meningitis in Chinese children >28 days of age, January 2014–December 2016: a multicenter retrospective study. *Int J Infect Dis*. 2018;74:47–53.
- Yao M, Zhou J, Zhu Y, Zhang Y, Lv X, Sun R, et al. Detection of listeria monocytogenes in CSF from three patients with meningoenzephalitis by next-generation sequencing. *J Clin Neurol*. 2016;12(4):446–51.
- Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364(16):1523–32.
- Guo LY, Feng WY, Guo X, Liu B, Liu G, Dong J. The advantages of next-generation sequencing technology in the detection of different sources of abscess. *J Inf Secur*. 2018;8 pii: S0163-4453(18)30245-7.
- Li H, Gao H, Meng H, Wang Q, Li S, Chen H, et al. detection of pulmonary infectious pathogens from lung biopsy tissues by metagenomic next-generation sequencing. *Front Cell Infect Microbiol*. 2018;8:205.

17. Guo LY, Li YJ, Liu LL, Wu HL, Zhou JL, Zhang Y, et al. Detection of pediatric bacterial meningitis pathogens from cerebrospinal fluid by next-generation sequencing technology. *J Inf Secur*. 2018;11 pii: S0163-4453(18)30357-8.
18. Fan S, Qiao X, Liu L, Wu H, Zhou J, Sun R, et al. Next-generation sequencing of cerebrospinal fluid for the diagnosis of Neurocysticercosis. *Front Neurol*. 2018;9:471.
19. Brown JR, Bharucha T, Breuer J. Encephalitis diagnosis using metagenomics: application of next generation sequencing for undiagnosed cases. *J Inf Secur*. 2018;76(3):225–40.
20. Fan S, Ren H, Wei Y, Mao C, Ma Z, Zhang L, et al. Next-generation sequencing of cerebrospinal fluid for the diagnosis of Neurobrucellosis. *Int J Infect Dis*. 2018;67:20–4.
21. Fang C, Zhong H, Lin Y, Chen B, Han M, Ren H, et al. Assessment of the cPAS-based BGISEQ-500 platform for metagenomic sequencing. *Gigascience*. 2018;7(3):1–8.
22. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*. 2009;25(14):1754–60.
23. Zhang C, Zheng X, Zhao C, Li Y, Chen S, Liu G, et al. Detection of pathogenic microorganisms from bloodstream infection specimens using TaqMan array card technology. *Sci Rep*. 2018;8(1):12828.
24. Miao Q, Ma Y, Wang Q, Pan J, Zhang Y, Jin W, et al. Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical practice. *Clin Infect Dis*. 2018;67(suppl_2):S231–40.
25. Ai JW, Zhang HC, Cui P, Xu B, Gao Y, Cheng Q, et al. Dynamic and direct pathogen load surveillance to monitor disease progression and therapeutic efficacy in central nervous system infection using a novel semiquantitative sequencing platform. *J Inf Secur*. 2018;76(3):307–10.
26. Gosiewski T, Ludwig-Galezowska AH, Huminska K, Sroka-Oleksiak A, Radkowski P, Salamon D, et al. Comprehensive detection and identification of bacterial DNA in the blood of patients with sepsis and healthy volunteers using next-generation sequencing method—the observation of DNAemia. *Eur J Clin Microbiol Infect Dis*. 2017;36(2):329–36.
27. Rhodes J, Hyder JA, Peruski LF, Fisher C, Jorakate P, Kaewpan A, et al. Antibiotic use in Thailand: quantifying impact on blood culture yield and estimates of pneumococcal bacteremia incidence. *Am J Trop Med Hyg*. 2010;83(2):301–6.
28. Hui P. Next generation sequencing: chemistry, technology and applications. *Top Curr Chem*. 2014;336:1–18.
29. Langelier C, Zinter MS, Kalantar K, et al. Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients. *Am J Respir Crit Care Med*. 2018;197(4):524–8.
30. Gargis AS, Kalman L, Lubin IM. Assuring the quality of next-generation sequencing in clinical microbiology and public health laboratories. *J Clin Microbiol*. 2016;54(12):2857–65.
31. Thorburn F, Bennett S, Modha S, Murdoch D, Gunson R, Murcia PR, et al. The use of next generation sequencing in the diagnosis and typing of respiratory infections. *J Clin Virol*. 2015;69:96–100.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

