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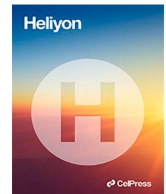

Research article

The impact of neutrophil count on the results of metagenomic next-generation sequencing in immunocompromised febrile children

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A R T I C L E I N F O

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A B S T R A C T

Metagenomic next-generation sequencing (mNGS) has revolutionized the detection of pathogens, particularly in immunocompromised individuals such as pediatric patients undergoing intensive chemotherapy and hematopoietic stem cell transplantation. This study aims to explore the impact of neutrophil count on the diagnostic efficacy of mNGS in diagnosing infections in pediatric patients with febrile diseases. We conducted a retrospective analysis of pediatric patients with febrile diseases in the hematology/oncology department from January 2019 to September 2022. The study included 387 patients with 516 febrile episodes. Analyzing data from 516 pediatric cases, our study found that 70.7 % had febrile neutropenia (FN) and 29.3 % had febrile without neutropenia (FWN). mNGS demonstrated a high positive detection rate of 84.9 %, compared to 29.7 % for conventional microbiological tests (CMT). While the positive detection rates of mNGS were similar in both FN and FWN groups, bacterial pathogens were more frequently detected in FN patients. Furthermore, the rate of identifying a "probable" microbial etiology was lower in the FN group (46.8 %) compared to the FWN group (65.6 %, $p < 0.001$). When analyzing the types of organisms and specimens, the "probable" identification rates were particularly lower for viruses and fungi detected by mNGS, as well as in blood and nasopharyngeal swab samples. These findings underscore the significant influence of neutrophil counts on mNGS results in pediatric febrile patients and highlight the necessity for tailored diagnostic approaches in this population.

1. Introduction

1.1. Background

Infections are a primary cause of death among children with hematologic/oncologic diseases and those undergoing hematopoietic

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stem cell transplantation (HSCT) because of the intensive chemotherapy and immunosuppression [1]. Therefore, early pathogen identification and targeted anti-infection therapy are crucial for significantly reducing complications and improving the long-term survival of these patients [2]. However, traditional microbiological detection methods, such as blood cultures, serum immunological tests, and polymerase chain reaction (PCR) techniques, are often time-consuming and yield low positive detection rates [3].

Metagenomic next-generation sequencing (mNGS) helps overcome these challenges by acquiring genetic information through high-throughput sequencing of gene fragments from pathogenic microorganisms [3,4]. mNGS has the capability to detect a wide range of pathogens in clinical samples, including conventional bacteria, as well as atypical, rare, and viral pathogens, fungal infections, and clinically significant polymicrobial infections that conventional microbiological tests (CMT) may miss [1,5–8]. In addition, the duration of pathogen diagnosis is generally shorter when using mNGS, and the results of mNGS have fewer interference factors when compared to traditional microbiological detection techniques [9]. Previous studies have documented the effectiveness of mNGS in various infectious diseases, including infections of the central nervous system, respiratory tract, focal sites, bloodstream, urinary tract, and periprosthetic joints [10–12]. Additionally, the results of mNGS influence antibiotic stewardship and help minimize unnecessary antimicrobial exposure, particularly in critically ill patients and febrile neutropenic children with hematological diseases, where CMT fails to provide early-stage infection diagnosis [13–15].

1.2. Research significance

Patients with hematological diseases, particularly pediatric patients, face a significant risk of developing neutropenia, often as a result of their underlying conditions or treatments [16]. This leads to increased susceptibility to a diverse range of infections [17,18]. The severity and characteristics of these infections are influenced by a variety of factors, including immunologic deficits, antineoplastic therapy, antimicrobial prophylaxis, medical devices, and local epidemiology. Neutropenia, a state of decreased neutrophil count, is a key indicator of a patient's immunocompromised status and their susceptibility to infections. Given that mNGS is increasingly used for pathogen detection in such patients, understanding how neutrophil count influences mNGS results is crucial. This investigation is key for improving diagnostic workflows in clinical practice as mNGS gains broader application, aiming to optimize care for immunocompromised individuals by ensuring accurate and tailored pathogen detection.

The focus of this study is to elucidate the correlation between neutrophil counts and the diagnostic efficacy of mNGS in identifying pathogens in pediatric patients with hematology/oncology diseases or those undergoing HSCT. We hypothesize that variations in neutrophil counts, especially in cases of neutropenia, significantly affect the detection capability of mNGS. This research aims to address a critical gap in our understanding of how varying neutrophil levels affect mNGS's ability to accurately diagnose infections, with the goal of enhancing infection management strategies for these immunocompromised children.

2. Material and methods

2.1. Subjects recruitment

From January 2019 to September 2022, all pediatric patients under 18 years with febrile diseases from the hematology/oncology department at Children's Hospital of Zhejiang University School of Medicine (ZCH) were comprehensively retrospectively recruited for this study. The study involved 516 episodes of febrile diseases among 387 patients. The sample size was determined by considering the incidence rates of febrile episodes among pediatric patients undergoing treatment for cancer and other hematological diseases at ZCH. The inclusion criteria for further analysis of these patients were described as follows: 1) Patients diagnosed with leukemia, lymphoma, or other types of malignancies, as well as conditions requiring HSCT, who developed febrile illnesses during chemotherapy or the HSCT process. 2) Fever was characterized by an ear temperature exceeding 38.5 °C or by two or more readings between 38.0 °C and 38.4 °C within a 24-h period. 3) All enrolled patients had their specimens analyzed using both standard clinical CMT and mNGS tests performed concurrently. 4) The mNGS samples were collected directly from the locations of infectious sites. 5) Episodes from the same patient were included only if they occurred more than one month after the resolution of the previous fever.

Specimens were collected from potential pathogenic sites, such as peripheral blood in cases of bloodstream infection (BSI), and nasopharyngeal swab (NPS), sputum, or bronchoalveolar lavage fluid (BALF) in instances of respiratory tract infections (RTI) or pneumonia. Details on the sample collection and testing methodologies are provided in the appendix. The study protocol received approval from the Ethics Committee of ZCH and adhered to the Declaration of Helsinki (IRB number 2023-IRB-0040). Informed consent was secured from all authorized family members for their participation in the study.

2.2. Neutropenia definition

Neutropenia is characterized by an absolute neutrophil count (ANC) below 0.5×10^9/L [19]. We categorized febrile patients into two groups based on their neutrophil counts: those with an ANC of 0.5×10^9/L or higher (defined as febrile without neutropenia, FWN) and those with an ANC below 0.5×10^9/L (classified as febrile neutropenia, FN). We then examined their microbial distribution and compared the consistency of mNGS results with CMT.

2.3. Etiology definition

In our study, we employed a structured approach to classify the results reported by mNGS, aiming for objectivity and consistency.

Each episode was individually evaluated by two independent investigators to ascertain whether the microorganisms identified by mNGS were responsible for the febrile diseases. This assessment was based on several criteria:1) The microorganism's inherent pathogenicity and its capacity to cause diseases in immunosuppressed children. 2) Whether the patient's medical history and characteristics could be associated with the identified microorganism. 3) The potential activity of empirical antimicrobial treatments administered before mNGS against these microorganisms, and the effectiveness of antimicrobial therapy adjustments made according to mNGS results.

Pathogens identified by mNGS were classified as causative agents if their detection was consistent with CMT results [20–22]. Mixed infection was defined as a polypathogenic infection. Febrile diseases in which the specific lesions and infectious pathogens could not be confirmed by CMT were considered as fever of unknown origin (FUO). Patients were further categorized into "Probable", "Possible", and "Unlikely" groups based on comprehensive analysis, including clinical presentations, imaging results, CMT outcomes, and mNGS findings.

1) "Probable" cases corresponded with clinical symptoms and showed a positive response to revised antimicrobial treatments, and the microorganism was considered pathogenic in immunocompromised children. It also encompassed cases where mNGS-identified organisms matched those from CMT conducted within 7 days of sample collection.
2) "Possible" cases included organisms suspected of being pathogenic, but they were likely either suppressed by prior antimicrobial treatment or did not respond to adjustments in antimicrobial regimen informed by mNGS results.
3) "Unlikely" cases were those where the organisms identified did not match clinical indications or treatment responses.

2.4. mNGS analysis

Samples were collected from patients, placed on dry ice immediately after collection, and delivered to a commercial laboratory for analysis within an 8-h window. The mNGS workflow comprised the steps of preparing libraries, conducting metagenomic sequencing, and analyzing the data through a bioinformatics pipeline. The construction of DNA libraries included steps such as nucleic acid extraction, sample processing, enzymatic fragmentation, end repair, terminal adenylation, and adapter ligation [1,23].

To evaluate the quality of DNA libraries, the KAPA Library Quant Kit (Illumina) Universal qPCR Mix was utilized. The qPCR master mix was mixed with samples or standards over a concentration range spanning from 20 pM to 0.002 pM. PCR reactions were conducted using the Gentier 96R real-time qPCR system. The quality of the DNA library was assessed by evaluating qPCR counts, standard fragment sizes, and the mean fragment size of the DNA library.

Shotgun sequencing, utilizing 50 bp single-end reads, was performed on the Illumina NextSeq platform. After excluding low-quality, low-complexity, and short reads (less than 35 bp), along with removing human-origin sequences (GRCh38.p13), each library produced roughly 20 million reads. The remaining reads were then matched against an extensive microbial reference database, encompassing NCBI nt, GenBank, and a proprietary curated genomic database. This database contained 11,162 bacterial genomes or scaffolds, 11,704 complete viral genomes, 1324 fungal genomes, and 229 parasite genomes. The quantity of aligned reads and their relative abundance were determined.

Microbial reads from the library were included in the analysis if they fulfilled the following conditions: 1) The sequencing data passed rigorous quality control measures, which required a library concentration above 50 pM, Q20 scores above 85 %, and Q30 scores above 80 %. 2) The species in question were either absent in the negative control samples from the same sequencing run or exhibited a reads per million total reads (QPM) to reads per million (RPM) ratio of $\geq$5 in comparison to the negative control. This empirically determined threshold, based on prior research (references 23 and 24), was employed to distinguish genuine positives from background contaminations [23,24].

2.5. Statistical analysis

In our research, statistical analyses were carried out utilizing SPSS version 22.0 (IBM, Armonk, NY, United States), tailored to the data types and research objectives. For categorical variables, the decision to use either the chi-square test or Fisher's exact test was based on the expected frequencies. Fisher's exact test was utilized for smaller samples where the chi-square test may not provide reliable results. The Kruskal-Wallis test was employed to analyze continuous variables that did not follow a normal distribution across multiple groups. For continuous variables with normal distributions, the independent sample *t*-test was used to compare means between two groups, particularly in cases where the sample size was greater than 30. The Mann-Whitney *U* test was utilized for continuous variables without a normal distribution, acting as a non-parametric alternative to the *t*-test, irrespective of sample size. Effect sizes for significant findings were quantified using Cohen's h, with values interpreted as indicating small (0.2), medium (0.5), or large (0.8) effects, thus providing a meaningful gauge of the magnitude of observed associations. Data visualization was carried out using the R package ggplot2 (Version 4.2.3). A p-value of less than 0.05 was considered statistically significant throughout the analysis.

3. Results

3.1. Patients' characteristics

3.1.1. Demographics and underlying conditions

The study involved 516 episodes of febrile diseases among 387 patients. Episodes from the same patient were included only if they

occurred more than one month after the resolution of the previous fever. There were 297 patients with 1 episode, 63 patients with 2 episodes, 20 patients with 3 episodes, and 7 patients with 4 or more episodes. For each febrile episode, only one specimen was collected from the most clinically relevant potential pathogenic location. No multiple specimens were submitted for the same febrile episode. The patients had a median age of 5.9 years, with ages spanning from 0.2 to 17.3 years, and a male-to-female ratio of 1.4:1. The predominant underlying conditions within this cohort were acute lymphoblastic leukemia (n = 308), acute myeloid leukemia (n = 91), lymphoma (n = 26), hemophagocytic lymphohistiocytosis (HLH) (n = 10), and patients who had undergone HSCT (n = 48).

3.1.2. Types of infections and neutrophil counts

Regarding infection types, 140 cases were identified as having RTI or pneumonia, 168 as having FUO, 92 as having bloodstream infections (BSI), 31 as having other specific site infections, and 85 as having multiple site infections. In terms of neutrophil counts, neutropenia was observed in 365 patients (70.7 %), with a median absolute neutrophil count (ANC) of 0.07×10^9/L (95 % CI: 0.06, 0.09). The remaining 151 (29.3 %) patients had an ANC of more than 0.5×10^9/L, with a median ANC of 4.23 (95 % CI: 3.596, 4.866) $\times 10^9$/L ($p < 0.001$) (Table 1). The Cohen's d value is 0.951. Out of the 516 specimens collected for febrile diseases, 358 were peripheral blood, 81 NPS, 45 BALF, 6 sputum, and 26 were other specimens such as pus, cerebrospinal fluid, hydrothorax, and ascites.

3.2. Distribution of microorganisms

3.2.1. Overall detection rates and microorganism diversity

In total, mNGS yielded positive results in 438 tests (84.9 %, 95 % CI: 81.5 %, 87.7 %), detecting 158 bacterial strains, 45 fungi, and 29 viruses. The most frequently identified bacteria included *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus parainfluenzae*, *Streptococcus pneumoniae*, and *Escherichia coli*. The most common viruses were *Cytomegalovirus*, *Epstein-Barr virus*, *human herpesvirus* 7, *human herpesvirus* 1, and *human parvovirus B19*. Among fungi, *Aspergillus fumigatus*, *Candida parapsilosis*, and *Pneumocystis carinii* were the most prevalent. Conversely, CMT identified positive results in 153 tests (29.7 %, 95 % CI: 25.9 %, 33.7 %), detecting 36 bacterial strains, 4 fungi, 11 viruses, and 1 mycoplasma. The most identified organisms by CMT included *Pseudomonas aeruginosa*, coagulase-negative *Staphylococcus*, *Klebsiella pneumoniae*, *Escherichia coli*, *parainfluenza virus*, *cytomegalovirus*, and *Epstein-Barr virus*. Fig. 1A displays the main organisms identified by both mNGS and CMT.

3.2.2. Co-infection analysis

mNGS identified two or more pathogens in a single specimen in many cases, with only 52.5 % of the tests detecting a single organism. Out of the 438 tests, mNGS found one type of organism in 230 tests, two types in 163 tests, and three or more types in 45 tests. According to mNGS results, co-infections involving both bacteria and viruses made up 35.2 % (154 out of 438) of the positive tests. In

Table 1
Clinical characteristics of enrolled children.

Parameters	Total	FN group	FWN group	*P*-value
Characteristics				
Male (n)	301	211 (70.1 %)	90 (29.9 %)	0.707
Female (n)	215	154 (71.6 %)	61 (28.4 %)	
Median age (year)	5.9 (0.2–17.3)	5.9 (0.3–16.9)	5.9 (0.2–17.3)	0.128
Underlying diseases (n)				
ALL	308	221 (71.8 %)	87 (28.2 %)	0.005
AML	91	73 (80.2 %)	18 (19.8 %)	
Lymphoma	26	19 (73.1 %)	7 (26.9 %)	
HSCT	48	24 (50.0 %)	24 (50.0 %)	
Other hematological disorders	43	28 (65.1 %)	15 (34.9 %)	
Infection site (n)				
Bloodstream	92	69 (75.0 %)	23 (25.0 %)	0.016
Respiratory tract	140	91 (65.0 %)	49 (35.9 %)	
Multiple sites	85	59 (69.4 %)	26 (30.6 %)	
Others	31	16 (51.6 %)	15 (48.4 %)	
Fever of unknown origin	168	130 (77.4 %)	38 (22.6 %)	
Organisms (n)				
Bacteria	158	129 (81.6 %)	55 (34.8 %)	0.144
Virus	29	26 (89.7 %)	21 (72.4 %)	
Fungus	45	39 (86.7 %)	17 (37.8 %)	
Specimen type				
Blood	358	270 (75.4 %)	88 (24.6 %)	<0.001
BALF	45	19 (42.2 %)	26 (57.8 %)	
NPS	81	60 (74.1 %)	21 (25.9 %)	
Other	32	16 (50.0 %)	16 (50.0 %)	
Reads etiology median	43	45	38.4	0.304

FUO: fever of unknown origin, BALF: bronchoalveolar lavage fluid, NPS: nasopharyngeal swab, AML: acute myeloid leukemia, HSCT: hematopoietic stem cell transplantation, ALL: acute lymphoblastic leukemia. P values obtained from comparing the composition ratios of all subgroups within this category using the chi-square or Fisher's exact test (two-sided).

A

Enterococcus faecium
Stenotrophomonas maltophilia
Escherichia coli
Staphylococcus aureus
Staphylococcus epidermidis
Acinetobacter baumannii
Streptococcus pneumoniae
Klebsiella pneumoniae
Pseudomonas aeruginosa
Haemophilus parainfluenzae
Parainfluenza
Respiratory syncytial virus
Human adenovirus
Human herpesvirus 6
Torque teno virus
Polyomavirus
Human parvovirus B19
Human herpesvirus 1
Human herpesvirus 7
Epstein-barr virus
Cytomegalovirus
Candida tropicalis
Candida parapsilosis
Aspergillus fumigatus
Pneumocystis carinii
0
20
40
60
80
CMT
mNGS-FWN
mNGS-FN

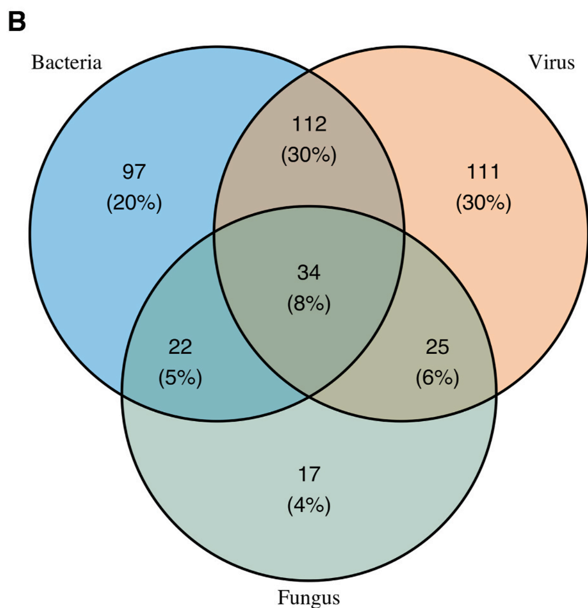

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Fig. 1. Distribution of the organisms detected by CMT and mNGS. **A)** The main organisms (including 10 bacteria, 11 viruses, and 4 fungi) detected by CMT and mNGS in patients suffering FN and FWN for various pathogens. **B)** The Venn diagram illustrates the overlap of bactetia, viruses and fungi detected by mNGS.

tests where mNGS confirmed the presence of bacteria, 46.5 % (147 out of 275) showed two or more bacterial strains. Similarly, 43.1 % (168 out of 295) of tests that confirmed the presence of viruses via mNGS detected two or more viral strains, and 14.4 % (15 out of 104) of tests that identified fungi via mNGS reported two or more fungal strains (Fig. 1B).

3.2.3. Detection rates comparison between mNGS and CMT

Of all 516 specimens, 303 were mNGS positive but CMT negative, 18 were mNGS negative but CMT positive, 60 were both mNGS and CMT negative, 135 were both mNGS and CMT positive, of which 81 were the same organism tested by mNGS and CMT, a total of 54 different organisms were tested using both mNGS and CMT. The positive detection rate of mNGS was higher compared to that of CMT (84.9 % *vs* 29.7 %, $p < 0.001$). (Fig. 2).

3.3. mNGS performance in patients with FN and FWN

3.3.1. mNGS diagnostic yield and organism load in FN and FWN patients

Among patients with FN, mNGS returned positive results in 310 tests, identifying 129 bacterial strains, 39 fungi, and 26 viruses. In contrast, for those with FWN, mNGS was positive in 128 tests, detecting 55 bacterial strains, 17 fungi, and 21 viruses. The median number of reads of organisms identified by mNGS in patients with neutropenia was 45 (range: 1–2,305,266), whereas in non-neutropenic patients, it was 38.5 (range: 1-1,762,102) ($p = 0.304$) (Fig. 3).

3.3.2. Comparative positive rates of mNGS across sample types in FN and FWN patients

The detection rates of mNGS across various sample types were examined. The positive rates of different organisms in blood samples were comparable between FN and FWN patients (bacteria, 66.1 % *vs.* 58.9 %, $p = 0.268$; viruses, 60.6 % *vs.* 67.1 %, $p = 0.321$; fungi, 24.0 % *vs.* 17.8 %, $p = 0.273$; respectively). Viral infection was detected more frequently in FN group than in the FWN group in

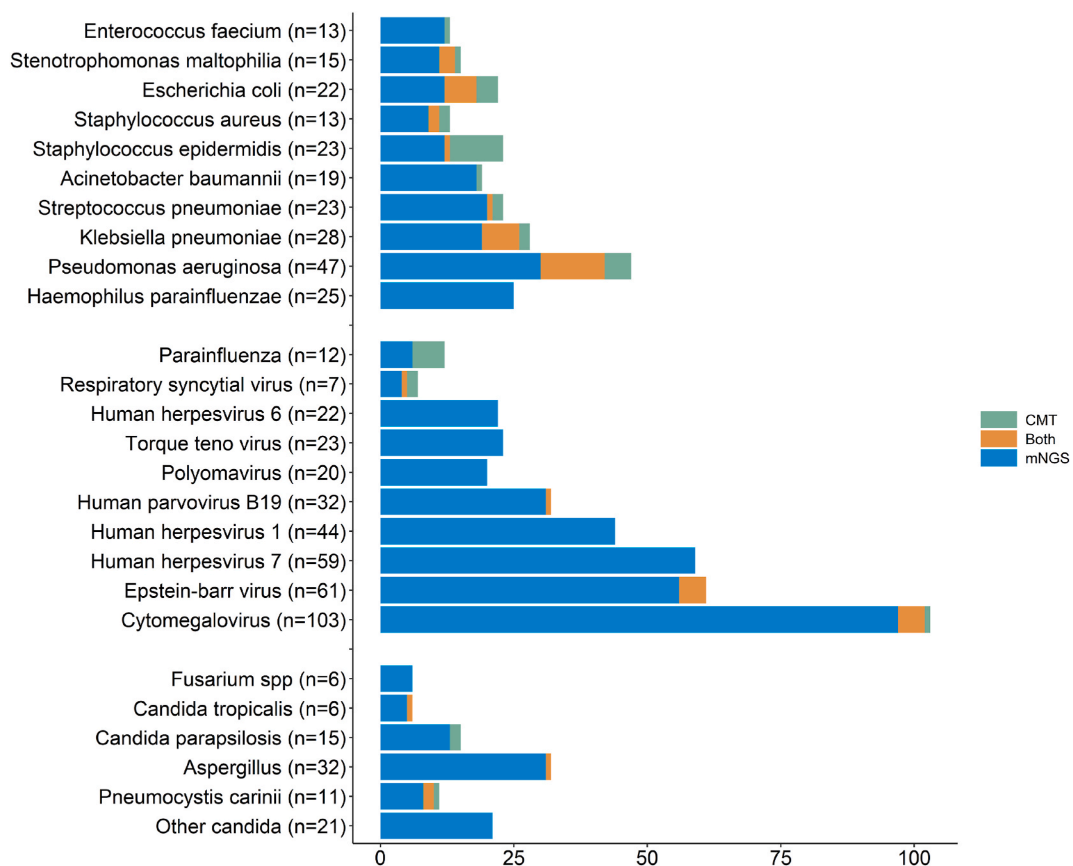

Fig. 2. Comparison of the performance of CMT and mNGS in detecting different organisms. The main organisms detected by CMT and mNGS were categorized as exclusively detected by CMT, exclusively detected by mNGS, and detected by both.

respiratory samples such as BALF and NPS (87.7 % *vs.* 63.4 %, $p = 0.002$). However, no substantial difference was observed between the two groups regarding bacterial presence (65.8 % *vs.* 51.2 %, $p = 0.128$) and fungi (24.7 % *vs.* 29.3 %, $p = 0.592$). Due to the significantly higher number of FN compared to FWN, we standardized the data by dividing the detected values by the total number of patients in each group and scaled it to 100 patients, ensuring comparability between the two groups. Additionally, we observed significant differences in the detection rates of certain pathogens between the two groups, such as *Pneumocystis carinii* and *Human herpesvirus 1* (Fig. 4).

3.4. Positive rates of different types of organisms by mNGS test in patients with FN and FWN

Of the 516 episodes of febrile illness, bacterium, virus and fungus were detected in 62.8 %, 67.4 % and 23.7 % by mNGS, respectively. The above rates were 66.1 %, 67.7 % and 24.5 % in patients with FN, while they were 54.7 %, 66.4 % and 23.7 % in patients with FWN. The incidence of bacterial positivity was higher in the FN group compared to the FWN group (66.1 % *vs.* 54.7 %, $p = 0.024$), while those of virus and fungus were comparable.

3.5. Clinical concordance of different organisms reported by mNGS in patients with FN and FWN

3.5.1. Neutropenia's influence on mNGS diagnostic categories

To examine the effect of neutropenia on mNGS outcomes in clinical practice, we compared the rates of "probable," "possible," and "unlikely" classifications between patients with neutropenia and those without neutropenia. Among the 438 patients who tested positive, the "probable" rate was lower in neutropenic patients compared to non-neutropenic patients (46.8 % (41.2–52.3 %) *vs.* 65.6 % (57.4–73.9 %), $p < 0.001$), Cohen's h = 0.382 while the "possible" rates were comparable (22.3 % (17.6–26.9 %) *vs.* 14.1 % (8.0–20.1 %), $p = 0.051$), Cohen's h = 0.214. However, in neutropenic patients, the organisms identified by mNGS were significantly more likely to be classified as "unlikely" to be the cause of infection compared to non-neutropenic patients (31.0 % (25.8–36.1 %) *vs.* 20.3 % (13.3–27.3 %), $p = 0.024$), Cohen's h = 0.245 (Fig. 5).

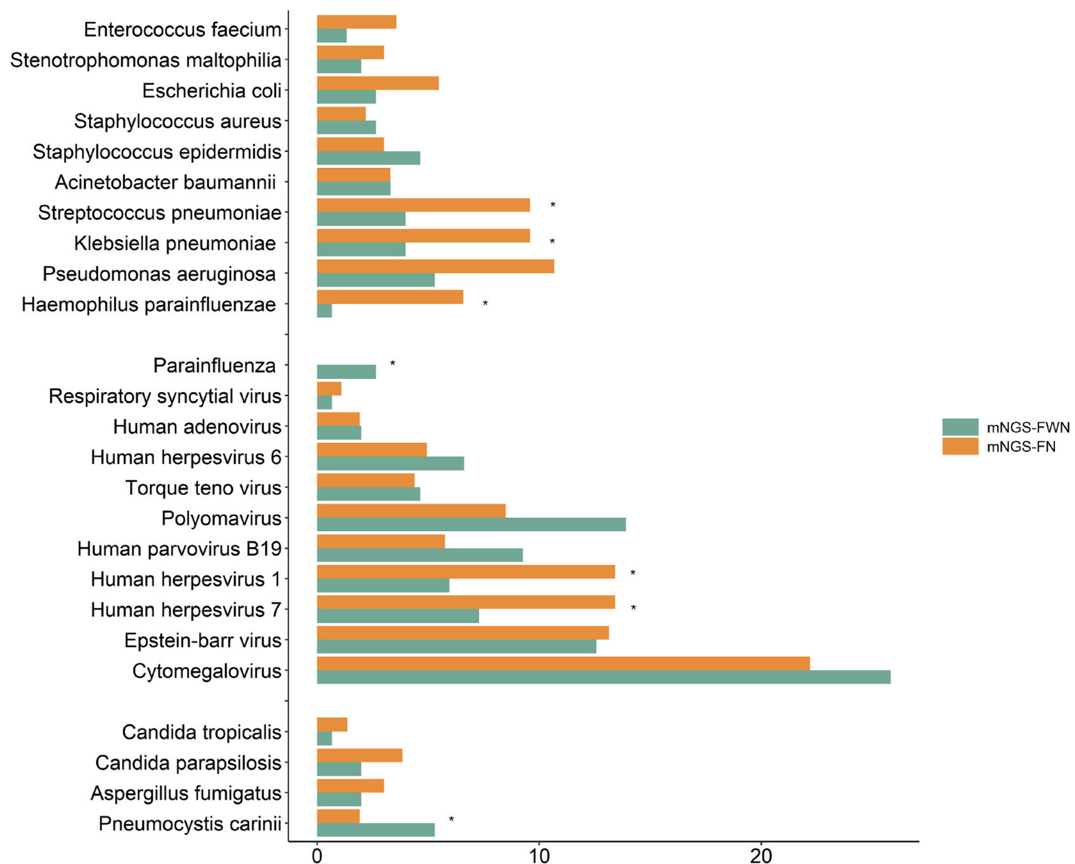

Fig. 3. The distribution of organisms detected by mNGS in the FN and FWN groups after standardizing the samples based on the total number of patients. Comparison was analyzed between FN and FWN groups. *P < 0.05.

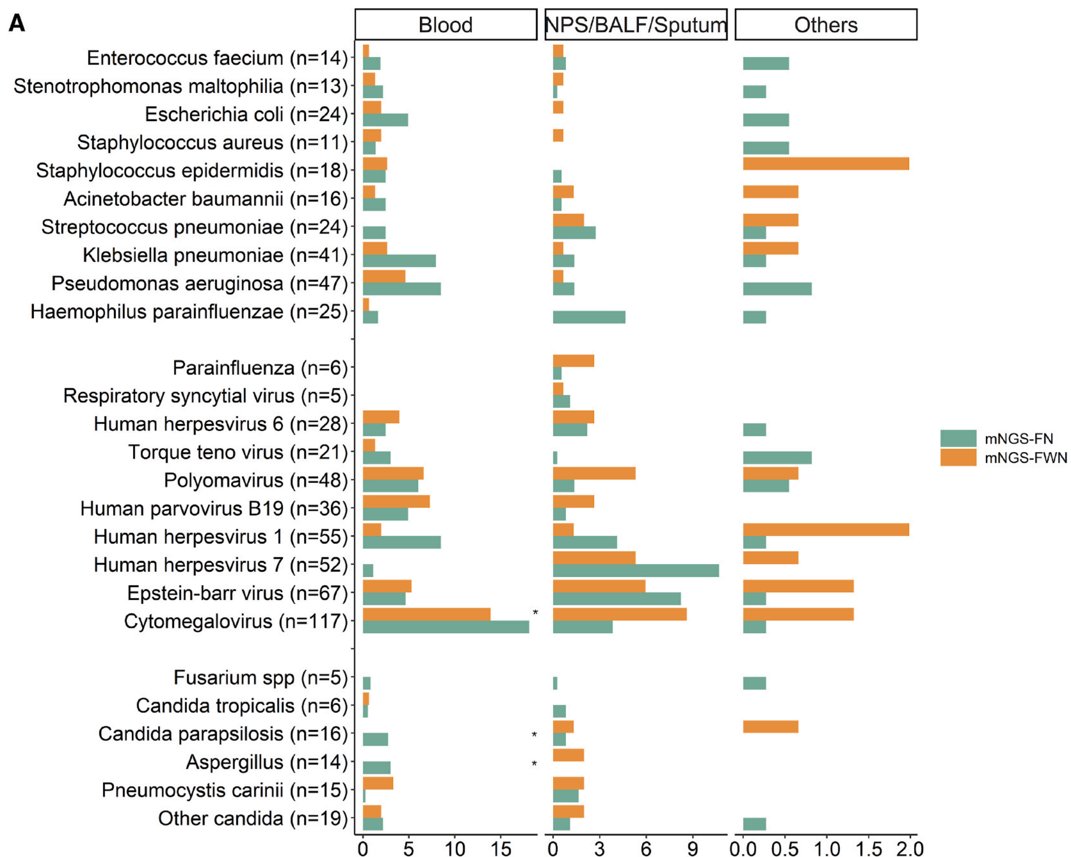

Fig. 4. The distribution of organisms in different samples between the FN and FWN groups after standardizing the samples based on the total number of patients. Figure demonstrates the differences in microbial community distribution between FN and FWN patients in blood, respiratory (NPS/BALF/sputum) and other samples. *P < 0.05. BALF: bronchoalveolar lavage fluid, NPS: nasopharyngeal swab. Other fluids include cerebrospinal fluid, urine, and pus.

3.5.2. Concordance of mNGS-Reported pathogens with clinical diagnosis in FN and FWN patients

The clinical concordance of bacteria, viruses and fungi reported by mNGS were compared between patients with FN and FWN separately. As shown in Table 2, the rates of probable of virus and fungus were much lower in patients with FN than those with FWN (Table 2).

3.6. Clinical concordance of mNGS results based on the type of specimens in patients with FN and FWN

Of 358 blood specimens, the “probable” rates in the neutropenic patients were lower than that in the non-neutropenic patients (44.8 % (38.2–51.4 %) *vs.* 60.3 % (49.0–71.5 %), $p = 0.022$), Cohen’s h = 0.311. Of the 81 NPS specimens, the “probable” rates in the neutropenic patients were lower than that in the non-neutropenic patients as well (42.6 % (29.4–55.8 %) *vs.* 70.6 % (48.9–92.2 %), $p = 0.044$), Cohen’s h = 0.573, while the “unlike” rate in neutropenic patients was higher compared to that in non-neutropenic patients (40.7 % (27.6–53.8 %) vs. 11.8 % (0–27.1 %), $p = 0.028$), Cohen’s h = 0.684. Among the 45 BALF specimens, there were no statistically significant differences in the "probable," "possible," and "unlikely" rates between patients with neutropenia and those without neutropenia (Table 3).

4. Discussion

Immunocompromised pediatric patients face an elevated risk of infection, a situation that is frequently compounded by coexisting conditions and the adverse effects of chemotherapy in hematologic and oncologic care. Prompt and precise detection of the offending pathogens is vital for diminishing morbidity and mortality, thereby enhancing patient prognosis and outcomes [25]. Recent strides in mNGS technology have markedly improved our capacity to identify and profile an extensive spectrum of microorganisms. Importantly, mNGS has emerged as a highly sensitive and impartial tool for pathogen detection, offering particular benefits in vulnerable populations such as immunocompromised patients, including those experiencing febrile neutropenia.

A pioneering facet of our study is the scrutiny of how neutrophil counts affect mNGS results in pediatric cohorts. We’ve discovered

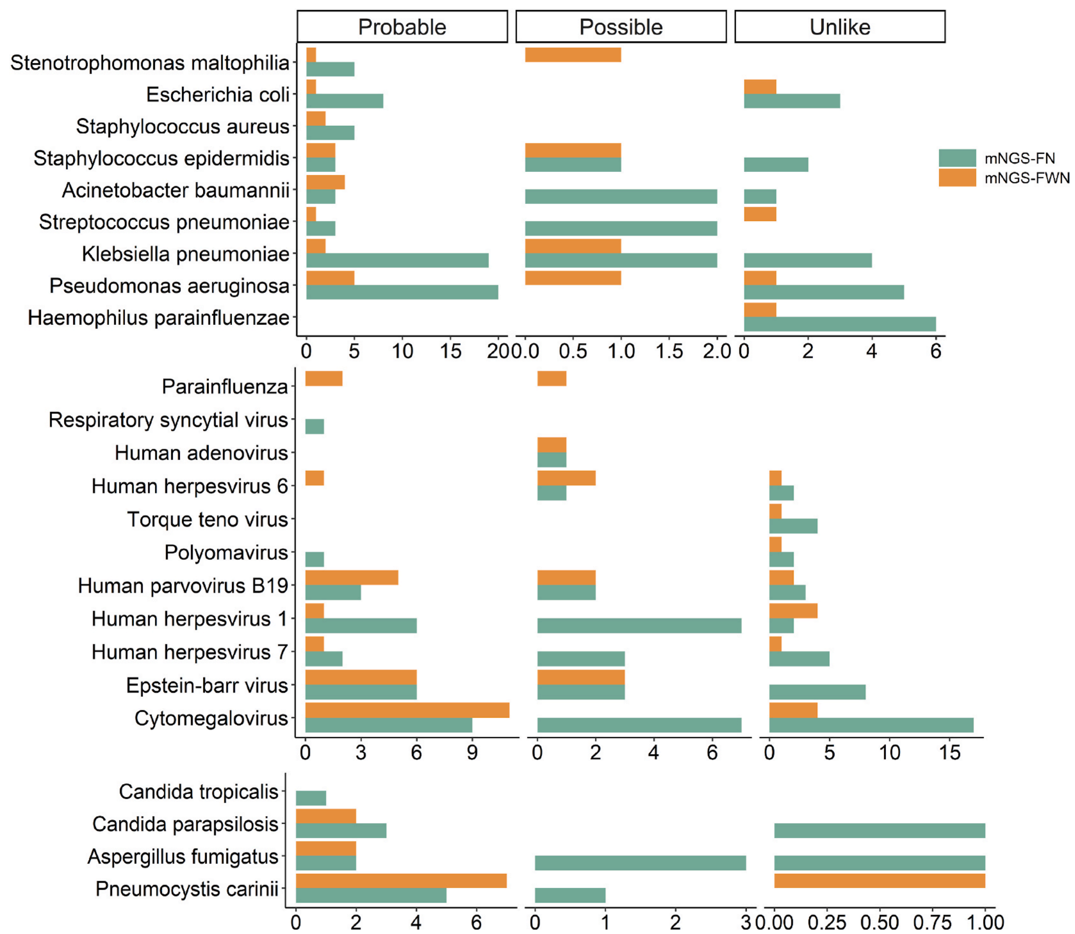

Fig. 5. Clinical concordance of mNGS results based on the type of specimens in patients with FN and FWN. It depicts the count and distribution of "Probable", "Possible", and "Unlikely" groups within the microbial communities, comparing these categories between FN and FWN patients.

Table 2
Comparison of the clinical consistency between FN and FWN groups in different pathogens.

	FN	FWN	*P*-value
Total			
Probable	46.8 %	65.6 %	0.002
Possible	22.3 %	14.1 %	
Unlike	31.0 %	20.3 %	
Bacteria			
Probable	59.0 %	67.1 %	0.438
Possible	17.1 %	14.3 %	
Unlike	24.4 %	18.6 %	
Virus			
Probable	42.9 %	60.0 %	0.042
Possible	22.9 %	14.1 %	
Unlike	34.3 %	25.9 %	
Fungus			
Probable	65.8 %	92.9 %	<0.001
Possible	19.7 %	0.0 %	
Unlike	14.5 %	7.1 %	

P values were derived from comparing the composition ratios of all subgroups within this category using the chi-square test or Fisher's exact test (two-sided).

that the efficacy of mNGS is modulated by neutrophil levels, exhibiting a heightened detection rate and a more diverse microbial array relative to CMT. Not only does our research validate prior studies attesting to mNGS's enhanced performance over CMT, but it also introduces an additional layer of insight by illustrating the specific impact of neutrophil counts on mNGS outcomes in the pediatric

Table 3
Comparison of the clinical consistency between FN and FWN groups in different specimens.

	BLOOD			NPS			BALF		
	FN	FWN	*P*	FN	FWN	*P*	FN	FWN	*P*
Probable	44.8 %	60.3 %	0.022	42.6 %	70.6 %	0.044	57.9 %	75 %	0.235
Possible	24.4 %	15.1 %	0.095	16.7 %	17.6 %	0.925	21.1 %	16.7 %	1.000
Unlike	30.8 %	24.7 %	0.320	40.7 %	11.8 %	0.028	21.1 %	8.3 %	0.380

P values obtained from comparing the composition ratios of groups using the chi-square or Fisher's exact test (two-sided).

demographic. Furthermore, our results underscore the distinct characteristics of NGS detection across various types of pathogens and specimen types, offering valuable insights for the field. This study has profound implications for the development of tailored detection strategies in pediatric care, emphasizing the need for individualized approaches based on our findings.

Our analysis revealed variances in pathogen detection rates between FN and FWN patients, with mNGS often uncovering polymicrobial infections in both cohorts [18,26–28]. This observation is consistent with prior studies that identified viruses as the predominant pathogens in pediatric infections, succeeded by bacteria and fungi [29–33]. Such insights are vital for informing empiric antibiotic therapies, particularly in high-risk FN children, where local antibiogram data and pathogen epidemiology are crucial [34,35]. Additionally, the identification of viral pathogens through mNGS could enable the targeted use of antiviral medications, possibly elucidating the observed insufficient response to antibiotic treatments in FN patients [36].

In our analysis, sequencing read counts were comparable between FN and FWN patient groups, challenging the notion that diminished white blood cell counts correlate with increased microbial DNA presence in blood samples [19]. Our findings align with a study that neutropenia was less influential on mNGS's yield [37].The divergence in outcomes across different studies may be attributed to variations in sample types—from peripheral blood to BALF and nasopharyngeal swabs—timing of sample collection [19] (such as during clinical shock or after antibiotic treatment), and patient demographics, with our research being focused exclusively on pediatric patients, unlike other studies that include adults. Moreover, disparate immunosuppressive regimens across patient cohorts might influence host defense mechanisms and infection susceptibility, further accounting for the inconsistent findings.

Our research additionally uncovers that organisms detected by mNGS are less likely to be true pathogens in patients with FN compared to those with FWN, as evidenced by a greater incidence of "unlikely" categorizations within the FN cohort. This variation hints at the necessity of factoring in false positives, such as commensal bacteria and DNA viruses, when interpreting mNGS data. Our results are consistent with those presented by Niles DT and colleagues, who observed that 49.7 % of the pathogens identified by mNGS in a pediatric patient population were deemed credible agents of infection [14]. Yet, despite these complexities, mNGS has proven to be highly valuable in immunocompromised patients, with a substantial share of the detected organisms bearing clinical significance [11].

In our study, the consistency of mNGS detection varied between the FN and FWN groups. Notably, FWN patients exhibited a higher "probable" detection rate in blood and nasopharyngeal swab specimens compared to FN patients. Conversely, the rate of "unlikely" findings was elevated in samples from patients without neutropenia. While mNGS demonstrated enhanced sensitivity over CMT in CSF, BALF, and blood samples [27], it did not show a significant advantage in sputum, tissue, and pus specimens [31]. The notably high positive detection rate of mNGS (88.3 %) in pulmonary infection cases, particularly among those with hematologic malignancies, underscores its diagnostic value [38,39]. Furthermore, the wide variety of viruses identified in the respiratory specimens of children may more accurately indicate their immune system status rather than act as direct agents of respiratory infections [40].

Our study has several limitations. Firstly, its execution in a single-center setting limits how broadly our findings can be applied. The unique demographic characteristics and clinical practices at different centers could influence the transferability of our results to other populations. Secondly, the retrospective design of our study could introduce selection bias, potentially affecting the observed outcomes. Factors such as the timing of mNGS sample collection and any antibiotic treatment received before mNGS sampling could impact the detection rates and types of pathogens identified. Additionally, inconsistencies in the protocol for sample collection and processing may have contributed to variable results. Also, the high cost of mNGS resulted in selection bias, as not all febrile patients underwent this testing, potentially affecting the representation of infections detected.

Despite these limitations, our findings provide valuable insights into the influence of neutrophil counts on mNGS outcomes in pediatric patients. However, our conclusions are drawn from observations in pediatric patients under specific conditions, and while they offer significant insights, they may require adaptation for applicability to other patient populations or in different investigative settings. We plan to address these issues in future prospective studies to enhance the value of our findings. Additionally, future research should focus on the prospective validation of our findings and investigate how the nuanced understanding of neutrophil counts' impact on mNGS outcomes can inform and enhance clinical management strategies for pediatric patients.

In conclusion, the findings from our study reveal that mNGS yields a comparable rate of pathogen detection in patients with FWN to that in patients with FN. Significantly, bacterial pathogens were more frequently identified in the FN group compared to the FWN group, suggesting an increased vulnerability to bacterial infections among neutropenic pediatric patients. Moreover, the detection rate of "probable" pathogens, indicating greater clinical relevance, was lower in the FN group than in the FWN group. Additionally, we found higher clinical concordance in pathogen detection within blood and NPS samples in the FWN group compared to the FN group. Interestingly, there was no significant difference in the sequencing reads between FN and FWN patients, highlighting the robustness of mNGS in detecting pathogens across different neutrophil count levels. These findings emphasize the importance of considering the neutrophil count in pediatric patients when interpreting mNGS results for effective infection management.

Data availability statement

The datasets produced and analyzed during this study are not publicly accessible due to ethical constraints; however, they can be obtained from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Di Wang: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Investigation, Data curation. **Haipin Chen:** Validation, Software, Methodology, Formal analysis, Data curation. **Cheng Zhao:** Software, Methodology, Investigation, Formal analysis, Data curation. **Hua Song:** Investigation, Data curation. **Jingying Zhang:** Data curation. **Fenying Zhao:** Formal analysis, Data curation. **Juan Liang:** Data curation. **Weiqun Xu:** Data curation. **Yongmin Tang:** Writing – review & editing, Supervision, Data curation. **Xiaojun Xu:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix

Sample Collection and Testing Methodologies

To ensure the accuracy of microbial detection in various samples from patients, meticulous collection and handling techniques were employed for blood, nasopharyngeal swabs, sputum, BALF, as follows:

Blood samples were collected from each patient using aseptic techniques to minimize the risk of contamination, with approximately 5 mL of blood drawn. The collected blood was then placed into culture bottles prepared for testing under both aerobic and anaerobic conditions. These bottles were incubated at 37 °C, and bacterial growth was continuously monitored for up to 7 days.

Nasopharyngeal swabs were collected using sterile, flexible swabs. These were inserted into the nostril until resistance was encountered, indicating contact with the nasopharynx, and then rotated to ensure secretions were absorbed. Immediately after collection, the swab was placed into viral transport media and dispatched to the laboratory for further analysis.

For sputum samples, patients who could produce sputum were asked to do so directly into a sterile container, preferably in the morning before eating or drinking anything. To ensure the samples accurately represented lower respiratory tract secretions and minimized saliva contamination, patients were instructed to perform a deep cough.

BALF from patients with suspected pulmonary infections was collected through bronchoscopy, following standard procedures to ensure the sample's quality, such as a recovery rate greater than 40 %, over 95 % of surviving cells, less than 10 % red blood cells (excluding trauma/bleeding factors), and 3–5 % epithelial cells, with intact smear cells showing no deformation. A portion of this fluid was then cultured: after centrifugation, the precipitate was inoculated onto blood agar plates for bacterial growth and other agar plates for fungal cultures.

Upon arrival at the laboratory, all specimens were promptly processed under conditions tailored to the specific type of test being performed. For culture tests, the samples were prepared in a sterile environment to avoid any contamination. When the tests involved mNGS analysis, the preparation of samples focused on preserving nucleic acids to ensure accurate sequencing results.

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