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ORIGINAL RESEARCH

Clinical Utility and Therapeutic Strategy Value of Metagenomic Next-Generation Sequencing in Pulmonary Infection Among Cancer Patients

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Introduction: Cancer patients, particularly those with lung cancer, are highly susceptible to pulmonary infections due to both the disease itself and the immunosuppressive effects of treatments such as chemotherapy and immunotherapy. The objective of this study was to analyze pathogenic distribution characteristics of pulmonary infections in cancer patients and evaluated the guidance of metagenomic next-generation sequencing (mNGS) on clinical administration.

Methods: This retrospective study included 66 samples from cancer patients. Pathogens in patient specimens, encompassing peripheral blood, bronchoalveolar lavage fluid (BALF), sputum, and other samples, were identified using both mNGS and culture methods.

Results: Compared to culture methods, mNGS demonstrated a sensitivity of 95.5% across all samples. In terms of overall detections, Human gammaherpesvirus 4 was identified as the most frequently detected pathogen in cancer patients, while *Escherichia coli* and *Candida albicans* were ranked as the most common bacterial and fungal pathogens, respectively. During the perioperative period, non-surgical short-term treatment, non-surgical long-term treatment, and long-term treatment groups, *Escherichia coli* and *Achromobacter xylosoxidans* were all identified. Moreover, the treatment strategies for patients were timely adjusted based on the mNGS results, resulting in a significant improvement in clinical symptoms for 59.3% (16 out of 27) of the cancer patients.

Conclusion: mNGS is an advanced approach for pathogen detection in cancer patients, with commendable diagnostic performance demonstrated. The results of mNGS contribute to the rapid modification of clinical medication, which may improve the survival rate of cancer patients.

Keywords: cancer, metagenomic next-generation sequencing, pathogen, diagnostic performance

Background

Malignant tumors, posing a significant threat to human life and health, have emerged as the leading cause of mortality worldwide.[1] In 2022, it was estimated 19.9 million new cancer cases globally, with 4.8 million cases reported in China. Concurrently, cancer-related mortality in China constituted 26.4% of the global total.[2] Cancer patients are highly susceptible to severe infections, particularly pulmonary infections, primarily due to the nature of the disease, the effects of treatments, and overall immune dysfunction, which collectively increase their vulnerability to pathogen infections.[3] Emerging evidence highlights the direct link between various infectious pathogens and cancer development, identifying them as key carcinogenic factors.[4] For instance, *Helicobacter pylori* and human papillomavirus are recognized as primary causes of gastric and cervical cancers, respectively.[5] Additionally, inflammation driven by bacterial and viral

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infections has been shown to increase cancer risk, while infectious pathogens can interfere with the transmission of carcinogens through partial cross-immunity or immune assistance.[4] A more comprehensive understanding of the pathogenic spectrum of microorganisms in cancer patients is crucial for enhancing patient care across all stages of the cancer care cycle.

Currently, traditional pathogen diagnostic methods such as culture, smear microscopy, or PCR continue to be widely employed in clinical practice. However, these methods often face limitations such as challenges in pathogen culture, narrow scope, and reduced accuracy, all of which hinder rapid and precise diagnosis and treatment. Moreover, traditional microbial detection methods can only identify approximately 40% of pathogens,[6] resulting in the typical administration of empirical broad-spectrum antimicrobial therapy without a clear etiology. This not only fuels pathogen resistance but also leads to related toxic side effects. In fact, infection-related mortality was reported in 22.4% of lung cancer patients with severe radiation pneumonitis, with inappropriate empirical antimicrobial therapy and bacterial/fungal co-infection being identified as independent risk factors for death.[7]

Metagenomic Next-Generation Sequencing (mNGS) is a burgeoning detection technology boasting unique advantages such as broad coverage, high speed, and high sensitivity. It has shown immense potential in the diagnosis and medication guidance for various infectious pathogens.[8–10] However, there were comparatively few reports on the use of mNGS for pathogen detection in cancer patients. In this study, we examined the diagnostic performance and pathogenic profile distribution of mNGS in cancer patients. We also adjusted medication regimens based on mNGS results, aiming to contribute to improved prognosis in cancer patients.

Materials and Methods

Participants

A total of 66 samples were included in this retrospective analysis, obtained from 46 patients with confirmed diagnoses of cancer and pulmonary infection, which were collected at Jiangsu Cancer Hospital between November 2020 and October 2022. The final analysis incorporated patients who met the following inclusion criteria: 1) patients must be over 18 years old; 2) a confirmed diagnosis of cancer, including lung, esophageal, colorectal, bladder, nasopharyngeal cancers, among others; 3) patients with suspected pulmonary infections, such as fever, cough, expectoration, and other signs; 4) patients with treatment records. The exclusion criteria included: 1) patients were younger than 18 years old; 2) patients who did not undergo both concurrent culture and mNGS testing; 3) patients with incomplete clinical documentation. Demographic and clinical data of patients were extracted from the hospital's database. Individuals who were lost to follow-up records and failed sequencing were excluded from the cohort. The study was supported by Board and Ethics Committee of Jiangsu Cancer Hospital (IACUC-2208001). Informed consent was obtained prior to patient enrolment.

Culture Method

Standard culture methods were used to process clinical samples for the detection of pathogens and interpretation of results. In brief, the qualified samples were inoculated onto selective culture media, including blood agar and MacConkey agar. These were then incubated in a temperature-controlled environment for 24 to 48 hours, with the possibility of extending the incubation period for the detection of fastidious or slow-growing pathogens. Emergent colonies of suspected pathogenic origin were carefully selected and underwent further identification through a combination of biochemical tests, mass spectrometry analysis, and other relevant diagnostic assays.

Sample Collection

A total of 66 samples were procured, comprising 24 peripheral blood samples, 20 Bronchoalveolar Lavage Fluid (BALF) samples, 16 sputum samples, and six other body fluid samples. Specifically, blood samples were drawn under negative pressure using specialized anticoagulant tubes. A fibreoptic bronchoscope was employed to lavage the lesion multiple times before the BALF was fully retrieved and collected in the pharynx of the patient under local anaesthesia using a sterile tube. Sputum samples were obtained by having patients cough up their first morning sputum after a mouth rinse, ensuring that the sample was devoid of saliva, blood, or other contaminants. These samples were then transported to

Dinfectome Inc. (Nanjing, China) for mNGS assay processing. Concurrently, the same samples underwent culture testing in the hospital's clinical microbiology laboratory, following standard protocols.

Nucleic Acid Extraction

Samples were collected from patients according to standard procedures. Plasma was prepared from blood samples and circulating cell-free DNA (cfDNA) was isolated from plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's protocols. Sputum was liquefied by 0.1% DTT (dithiothreitol) for 20 minutes at 56°C before extraction. DNA was extracted from BALF, sputum and other samples using the TIANamp Magnetic DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. For each clinical sample, human cell lines and non-pathogenic bacteria were added as positive controls. Repeated nucleic acid extraction with tubes filled with UltraPure DNase/RNase-free distilled water was used as the no-template control (NTC).[11] In addition, each sample was spiked with both an Internal Control and a Unique Internal Control to assess the validity of the experimental procedures and to monitor for cross-contamination. The quantity and quality of DNA was assessed using the Qubit (Thermo Fisher Scientific) and NanoDrop (Thermo Fisher Scientific), respectively.

Library Preparation and Sequencing

All DNA libraries were prepared using the KAPA Hyper Prep kit (KAPA Biosystems, Boston, MA, USA). The quality of DNA libraries was assessed by the Agilent 2100 Bioanalyzer system (Agilent, Santa Clara, USA) and 75 bp single-end sequenced on Illumina NextSeq 550Dx (Illumina, San Diego, CA). Approximately 20 million reads were generated for each sample.

Bioinformatics Analysis

Pathogen identification was performed using an in-house bioinformatics pipeline as previously described.[12] The human host sequences were identified by mapping to the human reference genome (hs37d5) using bowtie2. Only reads that cannot be mapped to the human genome were retained, followed by alignment to the microorganism genome database (downloaded on April 2022, from ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/) for pathogen identification using Kraken2 (v2.0.7). Pathogenic microorganisms that meet the threshold were retained for the automated generation of clinical test reports.

Interpretation and Reporting

The mNGS pathogen detection pipeline had been previously described.[13–15] Positive mNGS results were based on the following criteria: 1) For Mycobacterium, Nocardia and Legionella pneumophila, the result was considered positive if a species detected by mNGS had a species-specific read number ≥1; 2) For bacteria (excluding Mycobacterium, Nocardia and Legionella pneumophila), fungi, virus and parasites, the result was considered positive if a species detected by mNGS had at least 3 non-overlapping reads; 3) pathogens were excluded if the ratio of microorganism reads per million of a given sample versus NTC was <10. The results were interpreted by a multidisciplinary team of microbiologists and infectious disease specialists.

Treatment Response Assessment

Patients were followed up for one month based on the standards outlined in the guidelines for the diagnosis and treatment of community-acquired pneumonia in Chinese adults, in order to evaluate the clinical responses to treatment.[16] This led to the classification of patients into four categories: i) improvement; ii) treatment failure; iii) referred for treatment; iv) lost to follow-up. Among the clinical criteria for improvement were as follows: 1) body temperature ≤37.8°C; 2) systolic blood pressure ≥90 mm Hg; 3) oxygen saturation ≥90%; 4) improvement in symptoms such as cough, chest tightness, and wheezing; and 5) imaging results indicating absorption and shrinkage of lung lesions. Treatment failure primarily comprises clinical deterioration, death, and potential mortality of the patients.

Statistical Analysis

McNemar's Chi-squared test was used to compare the differences in pathogen detections by culture and mNGS. Two-sample proportional z-test was used to compare the positive detection rates by two diagnostic methods. A two-sided P value of less than 0.05 was considered significant for all tests unless indicated otherwise (*P<0.05, **P<0.01, ***P<0.001)

Results

Clinical Characteristics of Patients

The study initially enrolled a total of 70 samples with suspected LRTIs, and after excluding patients without treatment records, 66 samples from 46 patients were included in the final analysis (Figure 1A). This included 19 patients who had two or more samples. The baseline characteristics of these patients are detailed in Table 1. The median age of patients was 69 years (range 43 to 82 years) and 17.4% (8/46) patients were female. Lung cancer (41.3%, n=19) was the most common types of cancer in this study. A total of 14 patients were treated with immunotherapy. Both mNGS and culture testing were performed on the collected samples. The mNGS report provided specific sequencing reads of all detectable microorganisms in the specimen that yielded valid data.

Diagnosis Performance of mNGS in Detecting Pathogens in Cancer Patients

To evaluate the performance of mNGS in pathogen detection, we initiated a comparison of its positive detection rate against that of culture testing. A higher number of positive detections across different samples was yielded with mNGS compared to culture (Figure 1B). Furthermore, the positive detection rate of mNGS was significantly higher in blood and BALF samples as compared to culture (Figure 1B). Among the 66 samples, mNGS detected 32 fungal infections, while only 12 were detected by culture, thus

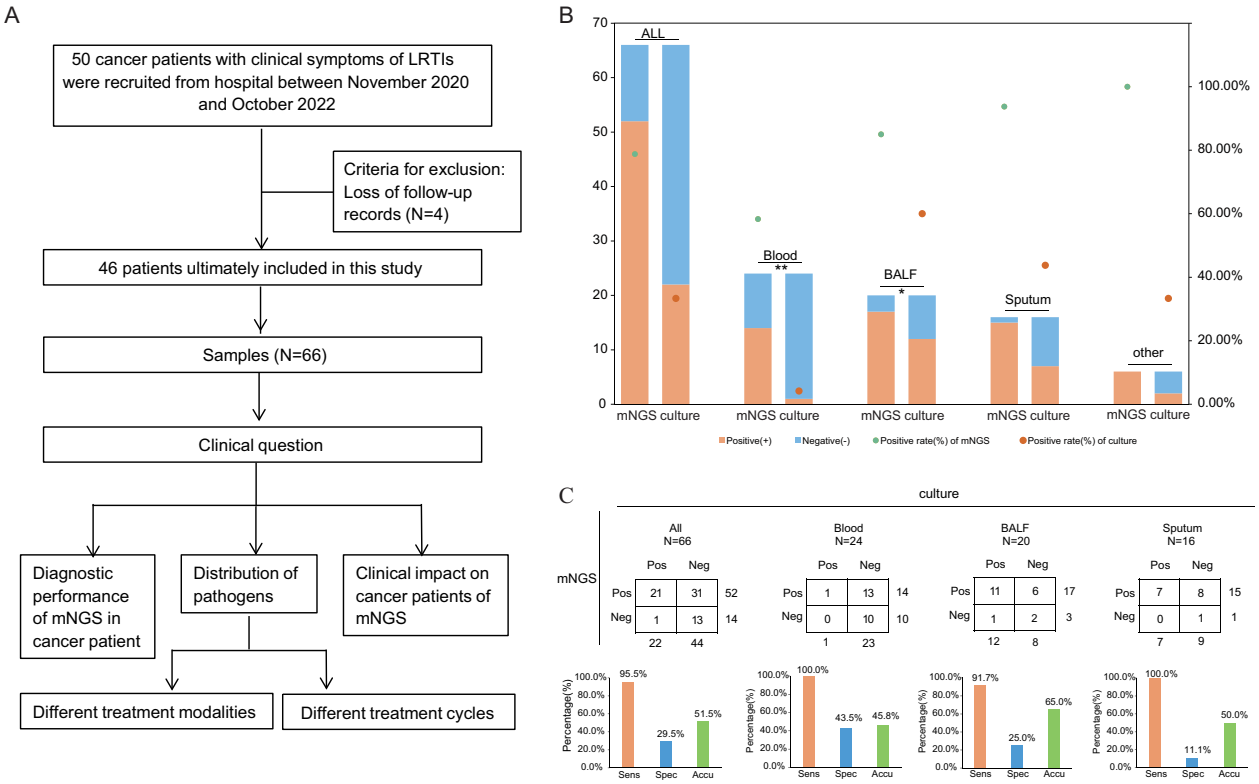

Figure 1 Evaluating the performance of mNGS in detecting pathogens of patients with cancer. (**A**) The enrolled patients and research questions. (**B**) Bar plots displaying positive and negative detections of mNGS and culture in different sample types **P<0.01, *P<0.05. (**C**) Bar plots illustrating the sensitivity, specificity and accuracy of mNGS in identifying pathogens in different sample types with the culture as the gold standard.

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Table 1 Patient Characteristics

Characteristics	Value
Patients	
Total	46
Male	38 (82.6%)
Female	8 (17.4%)
Age (years; median [IQR])	69 [64.75–74.0]
Cancer Type	
Lung cancer	19 (41.3%)
Esophagus cancer	12 (26.1%)
Nasopharynx cancer	3 (6.5%)
Bladder cancer	2 (4.3%)
Oral Cancer	2 (4.3%)
Malignant tumor of the tonsil	1 (2.2%)
Cholangiocarcinoma	1 (2.2%)
Bone cancer	1 (2.2%)
Laryngeal cancer	1 (2.2%)
Colon cancer	1 (2.2%)
Intracranial space	1 (2.2%)
Breast cancer	1 (2.2%)
Rectal cancer	1 (2.2%)
Therapy method	
Immunological therapy	14 (30.4%)
Non-immunotherapy	31 (67.4%)
Unknown	1 (2.2)

underscoring the superiority of mNGS in fungal detection. With culture as the gold standard, the sensitivity, specificity and accuracy of mNGS for pathogen identification in all samples were 95.5% and 29.5% and 51.5%, respectively (Figure 1C). The sensitivity of mNGS was relatively high (91.7–100%) in peripheral blood, BALF and sputum samples, although it exhibited lower specificity (11.1%) in sputum samples (Figure 1C). These findings clearly indicated that mNGS provides robust detection performance for diagnosing pathogens in cancer patients, though its performance may vary across different sample types.

Pathogen Identification Based on mNGS

To learn about the pathogenic spectrum of cancer patients, statistical analysis of mNGS results was performed. A total of 33 microorganisms were detected in the enrolled 66 samples. Multiple species were detected in 33 of the samples with positive mNGS results, including seven samples that detected both bacteria, fungi, and viruses (Figure 2A). Single-pathogen bacteria were most commonly detected (Figure 2B). Notably, Human gammaherpesvirus 4 was the most frequently identified pathogen in cancer patients in terms of overall detections, as it was found in 19 samples. We also identified co-detection of more than one pathogen in this study (n=45), with Human gammaherpesvirus 4 being the most common type detected alongside other species. Moreover, among all the identified pathogens, *Escherichia coli* and *Candida albicans* were the most commonly detected bacterial and fungal pathogens, found in eleven and ten samples respectively. Furthermore, etiological analyses were conducted on 33 samples of bronchoalveolar lavage fluid and sputum, which were categorized based on varying cancer types (Figure 2C). Fungal pathogens, such as *Candida albicans* and *Aspergillus fumifatus*, were more prevalent in patients with lung cancer. Conversely, viral pathogens, such as Human alphaherpesvirus 1, were more frequently detected in patients with digestive tract cancers.

Effect of Treatment Modalities on Pathogen Composition

Cancer patients were particularly prone to infections due to the immunosuppressive effects of their disease and associated treatments. These treatments could potentially influence the distribution of pathogens. As depicted in Figure 3, patients

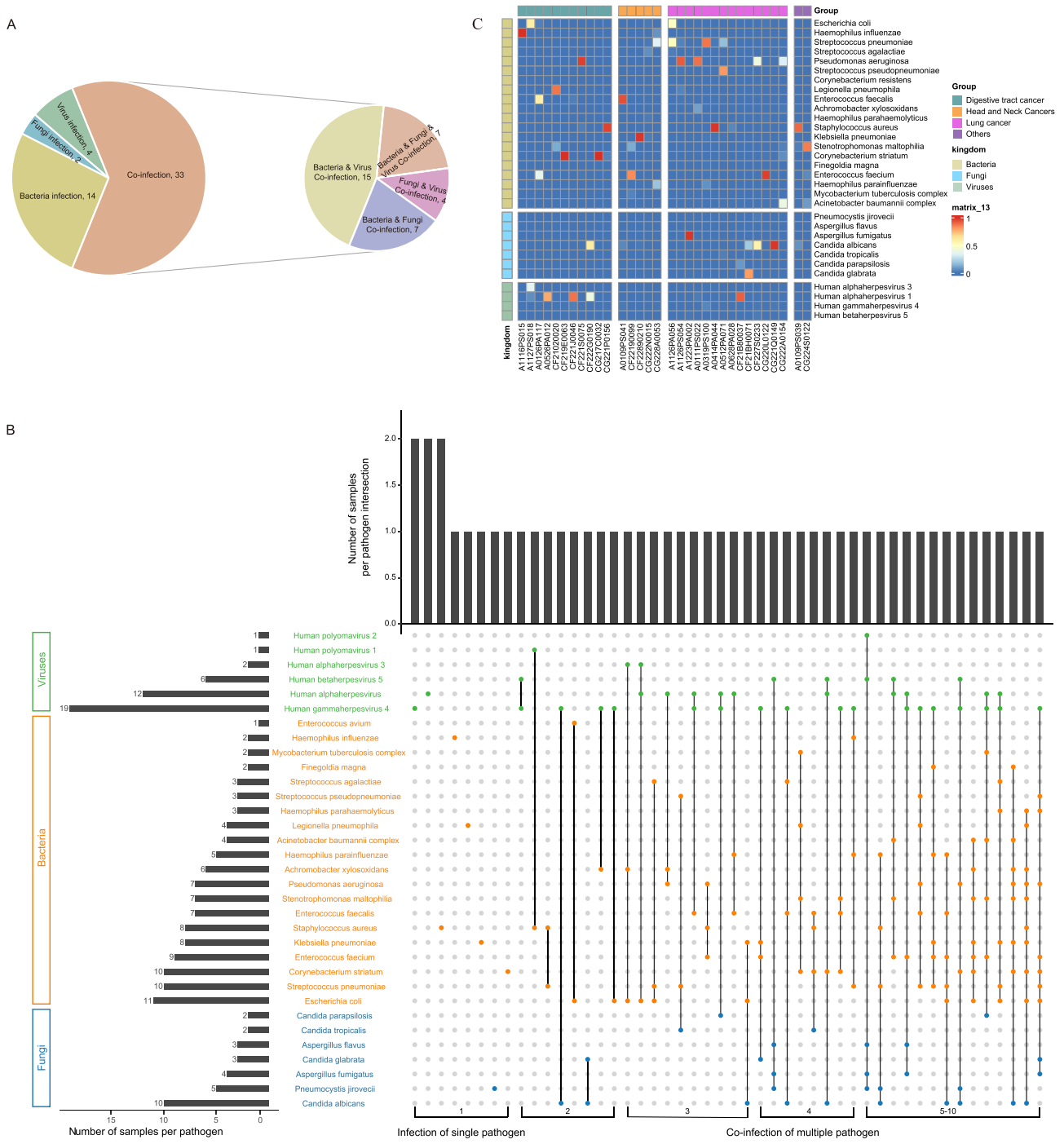

Figure 2 Pathogen identification based on mNGS. (**A**) Diagnoses of the enrolled patients were established by mNGS. (**B**) Number of samples of each pathogen and detection or co-detection type identified by mNGS testing. The causative pathogens are shown in colored dots and connected with a vertical line in each coinfection. This figure was plotted using UpSetR. (**C**) Heatmap of pathogen composition of different cancer species.

under no immunotherapy exhibited a broader spectrum of pathogens. Certain pathogens, including *Helicobacter pylori, Staphylococcus hominis* and Human polyomavirus 2, were exclusively detected in patients undergoing immunotherapy. Conversely, *Enterococcus faecalis, Acinetobacter baumannii complex, Enterobacter cloacae complex, Mycobacterium tuberculosis complex*, and *Streptococcus pseudopneumoniae* were solely identified in the non-immunotherapy group. However, the prevalence of *Stenotrophomonas maltophilia, Enterococcus faecium*, and *Human alphaherpesvirus* exhibited significant differences between the two groups.

Effect of Treatment Cycles on Pathogen Composition

These cancer patients were categorized into four groups based on different treatment durations: perioperative period, non-surgical short-term treatment, non-surgical long-term treatment, and long-term treatment. The pathogen compositions of each group were then analyzed. Two species, *Escherichia coli* and *Achromobacter xylosoxidans*, were detected in all four groups of patients (Figure 4A and B). Based on the species detected, *Candida glabrata, Candida parapsilosis, Aspergillus fumigatus*, and *Aspergillus flavus* were only detected in the long-term treatment group, possibly indicating that patients receiving surgical treatment were less likely to have fungal infections than other patients (Figure 4B). *Legionella pneumophila, Streptococcus agalactiae*, and *Enterobacter cloacae complex* showed higher detection in perioperative patients. Which indicated that perioperative patients were more susceptible to bacterial infections, while long-term surgical treatment patients were more susceptible to fungal and viral infections.

The Influence of mNGS Detection on Clinical Diagnosis and Treatment

We further analyzed the influence of mNGS on clinical decision-making by physicians (Figure 5). In a cohort of 41 patients with positive results from mNGS, the mNGS facilitated the identification of the causative pathogen in 27 cases, while corroborating the clinically anticipated pathogen in eleven cases. Nevertheless, for two patients, their mNGS results were interpreted as contamination. Furthermore, a single case had a detected result that was found to be incongruous with clinical manifestations. In the group of patients with negative mNGS results, mNGS assisted in excluding infection for all five cases.

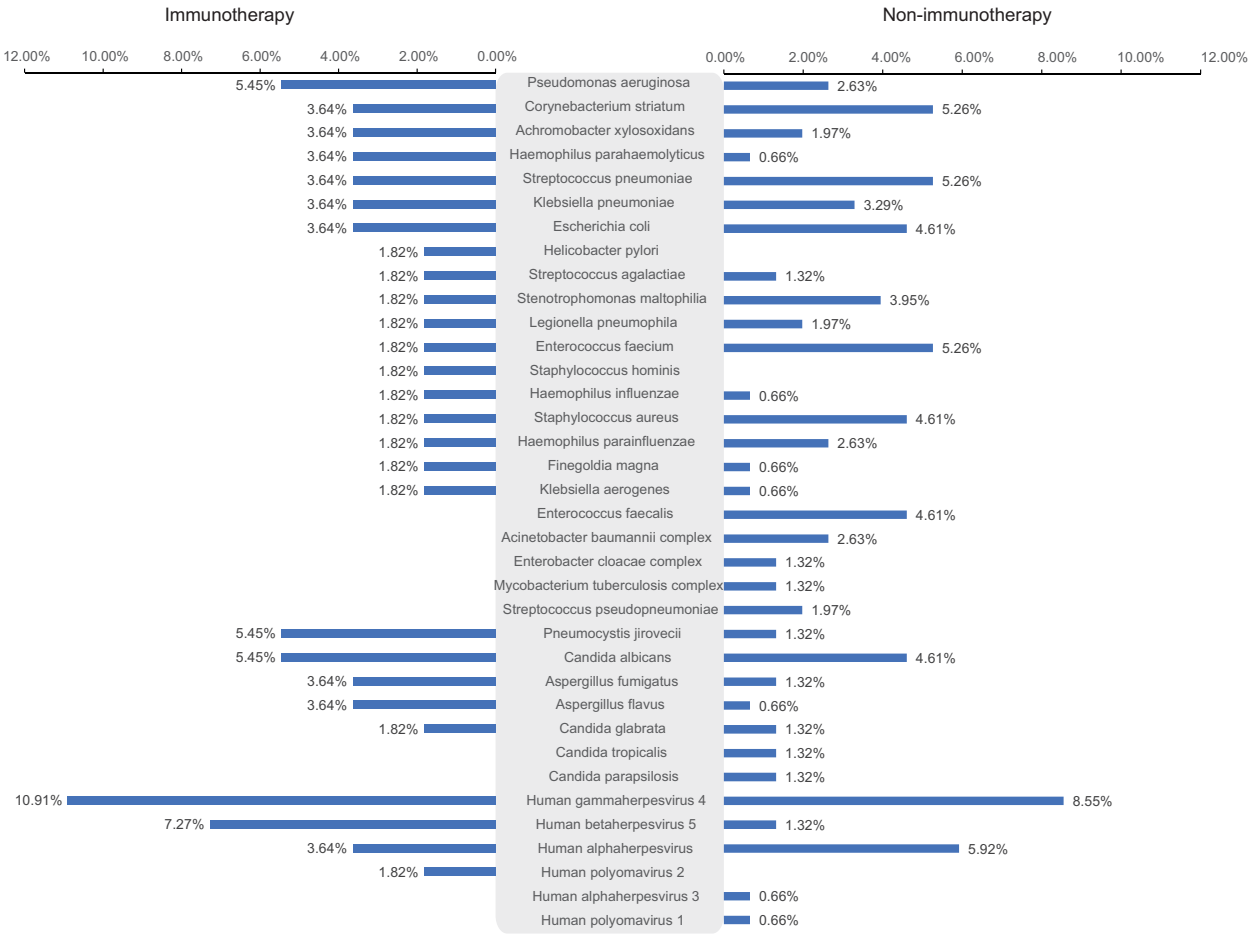

Figure 3 Distribution of pathogens in the immunotherapy and non-immunotherapy groups. Y-axis showed the species detected by mNGS, while the X-axis showed the number of samples in which the species was detected as a percentage of all pathogens.

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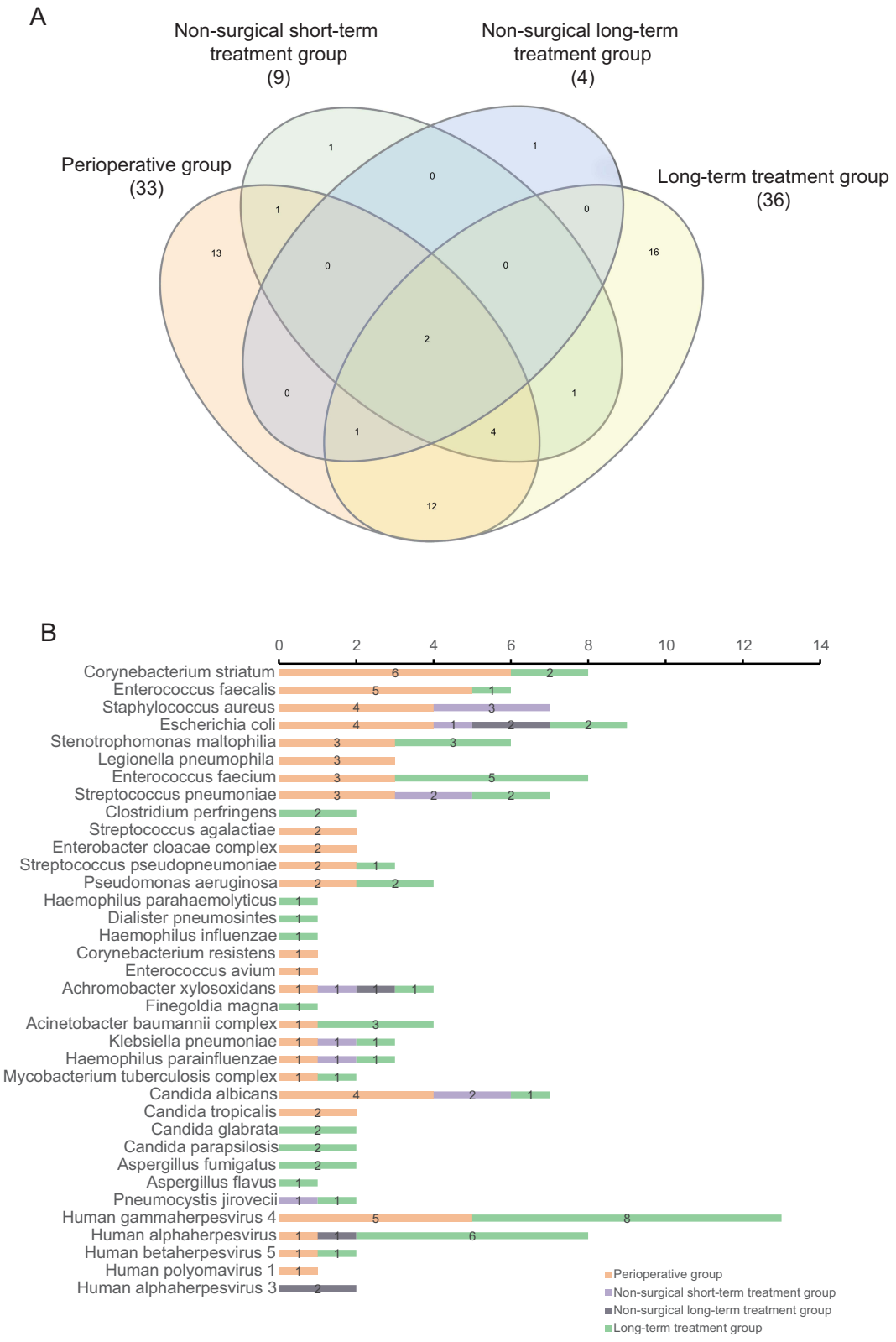

Figure 4 Distribution of pathogens under different treatment cycles. (**A**) Venn diagrams for four different treatment cycles. (**B**) Pathogen distribution in four different treatment cycles groups. Y-axis indicated the species detected by mNGS, while the X-axis illustrated the number of samples in which the species was detected.

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In assessing the influence of mNGS results on the clinical medication approach for patients, we found that among the 27 patients in whom the pathogen was discovered, twelve patients had their treatment medication switched based on the mNGS results. Additionally, eight patients underwent escalation therapy, and one patient began treatment with a novel therapeutic agent, while the remaining six patients continued with their previous medication. Among these patients, 59.3% (16/27) exhibited a marked amelioration in clinical symptoms; however, 22.2% (6/27) experienced a deterioration in their condition, with some cases resulting in death. Among the five patients in whom mNGS contributed to ruling out infection, one patient continued their original treatment for personal reasons, while antibiotic use was discontinued in the other four patients. Eventually, three of these patients achieved substantial clinical improvement, whereas one patient succumbed, largely attributable to the primary cancer.

Discussion

With 4.57 million new cancer cases and 3 million deaths, China ranked first in the world both new cases and deaths.[17] Surgical procedures, chemotherapy, radiotherapy and immunotherapy are the current major methods of cancer treatment. These treatments may cause significant immunosuppression, leaving cancer patients vulnerable to infection.[8] However, there is a number of pathogens ranging from bacteria to viruses and fungi that can be challenging to diagnose. CMT methods include microbial culture, serology, antigen/antibody assays and polymerase chain reaction (PCR), which are limited by their time consuming and low pathogen positivity rates.[18] The detection rate and utility of routine blood cultures for pneumonia had been reported to be extremely low at 0–14%, regardless of severity and risk.[19]

In contrast, mNGS as a culture-independent detection technique allows unbiased detection of all pathogens in a sample within a short turnaround time. In the present study, mNGS showed a higher positive detection rate compared to culture both in blood, BALF and in the overall sample. Specifically, for blood samples, the higher detection rate by mNGS is primarily attributed to its enhanced ability to identify pathogenic microorganisms compared to blood culture diagnostics.[20] However, it is also possible that mNGS detected pathogens originating from non-blood sources, such as skin flora. Furthermore, the sensitivity of mNGS ranged from 91.7% to 100% in different samples, indicating that mNGS could detect a wide range of pathogens. The most common site of infection in patients with advanced cancer was the

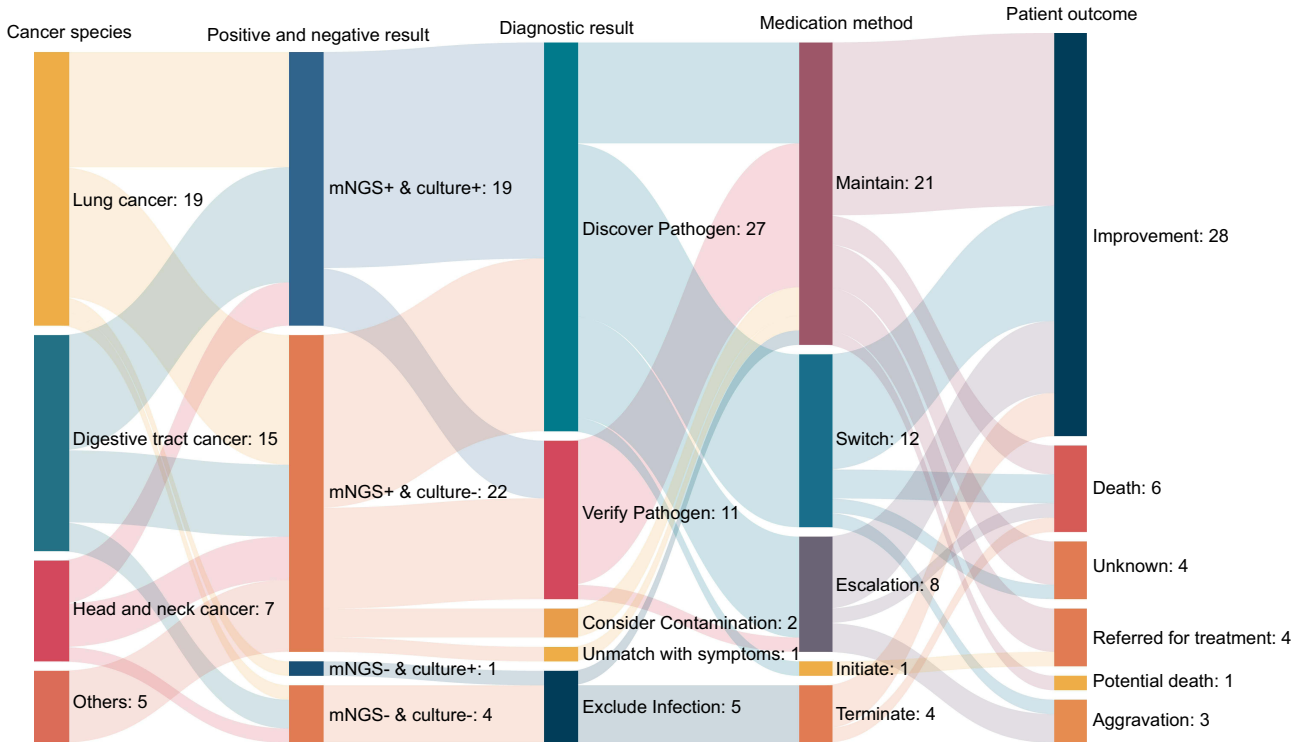

Figure 5 mNGS-directed treatment modalities in cancer patients.

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respiratory tract (48.6%), followed by the urinary tract (12.3%) and wounds (4.1%).[21] mNGS has good performance in the diagnosis of respiratory infections, mainly in terms of high sensitivity, positive detection rate and short turnaround time.[13,22,23] In summary, these demonstrated the exceptional diagnostic performance of mNGS in diagnosing pathogens in cancer patients.

Some microorganisms have been found to be closely associated with the development of cancer. For example, HPV is associated with the development of cervical and oral cancers,[24] *Helicobacter pylori* is an important risk factor for gastric cancer[25] and *Campylobacter jejuni* indirectly promotes the development of colorectal tumors.[26] Besides, coinfections in cancer patients tend to result in worse clinical outcomes.[27] Therefore, understanding the pathogenic profile of cancer patients has positive clinical implications. The pathogens of hospital-acquired infections isolated from lung cancer surgery patients included *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and viruses related to the mechanism of lung carcinogenesis include HCMV, influenza virus, measles virus, etc.[28]

The above pathogens were also identified in our study, with Human gammaherpesvirus 4 being the most detected virus regardless of the grouping conditions. Human gammaherpesvirus 4 as a human oncolytic virus is associated with malignant lymphoma, nasopharyngeal cancer and gastric cancer, and Human gammaherpesvirus 4 genes promote tumorigenesis by interacting with oncogenes to drive the cell cycle process.[29] Our research found that *Candida albicans* was the most frequent fungus. *Candida albicans* is an opportunistic pathogen that is typically considered a commensal organism in the oral cavity. However, in patients with compromised immune function or underlying pulmonary diseases, the detection of Candida in the respiratory tract holds clinical significance.[30] Due to the immunosuppressed state of cancer patients, produces osteogenic by-products, triggers inflammation and induces molecular patterns thereby promoting the development of cancers, including oral cancer.[31,32] Some cancer patients will develop antibiotic resistance due to complications in the treatment process. For instance, cancer patients with *Streptococcus pneumoniae* toxemia showed a high level of resistance to ceftazidime.[33] It was a reminder that learning about the pathogenic spectrum of cancer patients might also serve as a reference when choosing antibiotic therapy. As in our study, the clinical dosing regimen was adjusted according to the pathogens detected by mNGS, and the results suggested that the adjusted patients had a lower mortality rate. The analysis of pathogen distribution characteristics could more accurately guide the rational selection and application of clinical antibiotics. Preliminary results of this have been obtained in another study.[34]

Immunotherapy for cancer had been making remarkable progress in recent years, generally with good tolerability and fewer side effects, achieving pleasant clinical responses in solid tumors such as melanoma, non-small cell lung carcinoma and bladder cancer.[35,36] In our study, we found that immunotherapy patients with cancer had fewer pathogenic species detected than non-immunotherapy patients in terms of pathogen detection, but there was no regular variation in specific species. Probably, we could tentatively speculate that immunotherapy could reduce the risk of infection associated with the treatment process compared to other treatment modalities such as surgery. Our research has revealed that *Staphylococcus hominis* is only detected in patients undergoing immunotherapy. *Staphylococcus hominis* is a commensal coagulase-negative staphylococcus that typically does not cause disease in humans; however, it is increasingly recognized as a potential opportunistic and nosocomial pathogen capable of infecting immunocompromised patients,[37] leading to conditions such as sepsis, infectious endocarditis, and endophthalmitis.[38,39] Whereas *Helicobacter pylori* is primarily known as a major cause of chronic gastritis and gastric ulcers. However, studies have also shown the presence of *H. pylori* in the oropharynx[40] and suggested its potential impact on the respiratory system.[41] Furthermore, the possibility of contamination during the sampling process should not be underestimated. Improper handling during the collection of respiratory samples may inadvertently introduce gastrointestinal flora, including *H. pylori*, into the samples.

Meanwhile patients undergoing surgery, particularly those in a perioperative setting, are vulnerable to bacterial infections, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[42,43] This susceptibility is due to a number of factors, including exposure to anesthesiologists, surgical procedures, and the hospital environment itself.[42] In addition, the prolonged treatment cycles typical of cancer patients, coupled with their extended exposure to hospital pathogens, often result in compromised immune systems, making these patients more prone to fungal and viral infections.[44,45] The findings of this study corroborate these observations, further underlining the utility of mNGS in aiding cancer patients to promptly identify pathogenic organisms. Thus, a comprehensive understanding of the infection susceptibilities of

different patient groups to specific pathogens can guide clinical decision-making processes, foster the creation of targeted prevention and intervention measures, and ultimately enhance postoperative care and outcomes in cancer patients.

A noteworthy finding from our study was the evaluation of mNGS-directed medication adjustment outcomes in cancer patients. Our results indicated that the majority of patients who received treatments targeting pathogens identified by mNGS exhibited an improvement in their clinical symptoms, while only a minority experienced disease progression. This finding aligns with previous research,[46–48] lending further support to the notion that clinical medication guidance based on mNGS results has a positive impact on patients' clinical outcomes. While the specific treatment strategy for each cancer patient is determined by the unique combination of infections and comorbidities, mNGS exhibits considerable potential in pathogen detection and holds significant value in guiding clinical treatments. Nonetheless, it is worth noting that despite the ability of mNGS to significantly reduce detection time and its high sensitivity, the detection cost remains a significant factor limiting its widespread application compared to culture methods.

However, there were other limitations in this study, like the lack of sample size and the final number of samples enrolled was only 66. Additionally, without the support of other clinical data, our findings only present pathogen detection under different subgroups to provide a reference for subsequent studies. Furthermore, we did not conduct a direct comparison between mNGS-guided treatment and traditional empirical treatment, which limits the clinical applicability of our results. Future research could potentially focus on a prospective exploration of infection sources in cancer patients and the adverse outcomes of co-infections, integrating both mNGS results and laboratory data.

In conclusion, mNGS has shown promising diagnostic accuracy in identifying pathogens among cancer patients. A comprehensive understanding of the pathogenic spectrum in these patients can significantly influence the appropriate selection of clinical antibiotics, potentially improving patient outcomes. Hence, clinicians should remain vigilant regarding infections in cancer patients and consider integrating mNGS into their diagnostic workflows where appropriate.

Data Sharing Statement

The pathogen reads of our study were deposited in the Genome Warehouse in the National Genomics Data Center (https://ngdc.cncb.ac.cn/) under project PRJCA028738.

Ethics Approval and Consent to Participate

This study was approved by the ethics committee of Jiangsu Cancer Hospital (IACUC-2208001) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was collected from each patient upon sample collection according to the protocols approved.

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Author Contributions

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

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Disclosure

Weiwei Wu, Jia Liu, and Miao Zhu are employees of Dinfectome Inc., Nanjing, Jiangsu, China. The other authors declare no competing financial interests.

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