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Letter to the Editor

Targeted next generation sequencing is comparable with metagenomic next generation sequencing in adults with pneumonia for pathogenic microorganism detection

Dear editor,

We have read with great interest the study published in this journal by Peng et al.,[1] comparing the potential of metagenomic next-generation sequencing (mNGS) with that of comprehensive conventional microbiological tests (CMTs) as a front-line diagnostic in immunocompromised patients with suspected pneumonia. mNGS has markedly improved the efficiency of pneumonia's etiological diagnosis. Pathogens can be identified by mNGS include bacteria,[2] RNA and DNA viruses,[3] yeast and molds,[4] mycobacteria and parasites.[3] mNGS is of high value in detecting novel, rare and atypical pathogens, or the treated patients.[5] However, mNGS was expensive and greatly influenced by human genes, and it is not possible to conduct DNA and RNA dual process detection at the same time. Herein, we adopted a more rapid and economic technology, target next-generation sequencing (tNGS), to achieve an early diagnosis of respiratory infection, whose target detection were of only 153 pathogens (Supplementary Table 1) but covered for more than 95% of the respiratory infection.

From 1 February 2021 to 31 July 2021, adult patients with hospitalized pneumonia at the second affiliated hospital of Chongqing Medical University were recruited. Clinical characteristics including age, gender, underlying diseases, medication using history and personal history, were collected. Accessory examination results including blood routine, C reacting protein (CRP), procalcitonin (PCT) and imaging findings were extracted during hospitalization. Blood samples and qualified lower respiratory tract specimens (LRS), including sputum, bronchoalveolar lavage fluid (BALF), pleural effusion and lymph node tissue were obtained from patients after hospitalization and preferred to be collected before the antimicrobial therapy began. mNGS and tNGS detection was performed parallelly on qualified LPS in all enrolled patients by the Chongqing KingMed Diagnostics. And for mNGS detection, only the DNA process was performed.

A total of 102 patients were included. The median age was 63.7 years old. Sixteen (15.7%) patients were over 80 years old, 63 (61.8%) were male, and 65 (63.7%) suffered from different underlying diseases. Patients' symptoms and comorbidities reported at the time of hospitalization were described in Supplementary Tables 2. Their vital signs and main accessory examination results at admission were described in Supplementary Tables 3.

The overall microbial detection rate of tNGS and mNGS were 82.17% (106/129) and 86.51% (109/126), respectively. And there was no significant difference between them ($p = 0.41$) (Table 1). The top 12 microorganisms detected by tNGS and mNGS were shown in Fig. 1A. *EB virus, Streptococcus pneumoniae* and *Haemophilus influenzae* were the top three microorganisms detected both by tNGS and mNGS detection. The detected numbers of *Candida albicans, Human herpesvirus 5, Acinetobacter baumannii, MTBC, Pneumocystis yersini, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Mycoplasma pneumoniae* were located at the 5th / 5th, 6th / 6th, 7th / 10th, 8th / 11th, 9th / 8th, 10th / 7th, 11th / 9th and 12th / 12th positions in tNGS and mNGS test, respectively. 20 cases of *Rhinovirus A/B/C* were detected by tNGS and none by mNGS. *Human herpesvirus 7* was detected in 33 cases by mNGS, but not by tNGS (Fig. 1B).

Bacteria, viruses, fungi and others accounted for 51.76% (103/199), 34.67% (69/199), 12.56% (25/199) and 1.01% (2/199) among the microorganisms detected in tNGS, respectively (Fig. 1C). Meanwhile, bacteria, viruses, fungi and others detected by mNGS accounted for 47.09% (105/223), 38.12% (85/223), 13.45% (30/223) and 1.35% (3/223), respectively (Fig. 1D). In addition, we also detected 20 species of microorganisms with the number of 57 by mNGS detection (Fig. 1E). However, they were not included in the panel of our designed 153 respiratory pathogenic microorganisms, sorting to 8 bacteria, 5 viruses, 5 fungi and 2 others (Fig. 1F).

Human herpesvirus 7 was the most abundant microorganism (33/57, 57.89%) detected in mNGS besides tNGS panel. Another major difference in the detection of tNGS and mNGS was *Rhinovirus A/B/C. MTBC* was detected in 12 cases of tNGS and 6 cases of mNGS ($p = 0.16$). *Klebsiella pneumoniae* was detected in 7 cases of tNGS and 10 cases of mNGS ($p = 0.42$). The detection rates of *Mycoplasma pneumoniae* in tNGS and mNGS were all low, 1.6% (2/129) and 2.4% (3/126), respectively ($P = 0.63$) (Fig. 1G).

Patients with pneumonia at outpatient department can be effectively controlled under the treatment of empirical antibiotics. However, the detection of pathogenic microorganisms is particularly important for patients with serious illness, or the ones with poor empirical treatment outcomes. Early distinguish of causative pathogens can significantly improve the prognosis of them.[6] In recent years, mNGS is currently widely used in the clinical practice of various infectious diseases, including respiratory infections. However, mNGS was not perfect.

Here, the microorganisms detected by tNGS or mNGS in our data were consistent with the generally recognized pathogenic bacteria of pneumonia.[7] The top two bacteria detected were consistently *Streptococcus pneumoniae* and *Haemophilus influenzae*t. *MTBC* was detected in 12 cases in tNGS and 6 cases in mNGS. There was no significant difference in the detection efficiency between them. However, from the perspective of technique, in the process of removing human gene background of mNGS, it may affect the detection rates of G negative bacteria, viruses and intracellular bacteria, which resulted in a higher false negative.

In terms of virus, *EB virus* was the No. 1 to be detected in both methods. Most scholars tend to believe that *EB virus* was one of the normal microorganisms colonized in human respiratory tract.[8] However, such consistent results also confirmed the accuracy and wide coverage of the metagenome detection technology. More, 20

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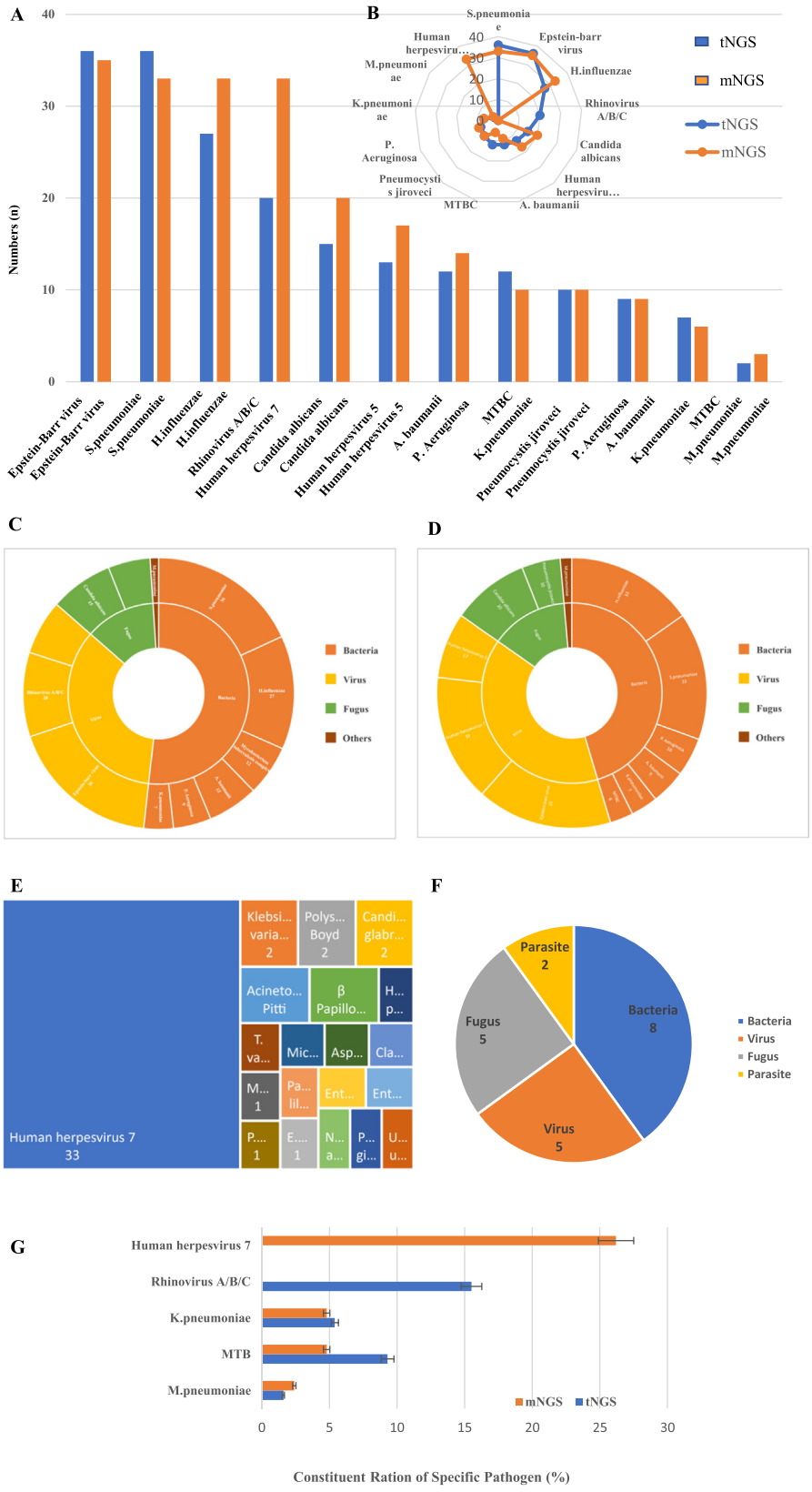

Fig. 1. Microorganism identified in adult patients with pneumonia by tNGS and mNGS detection. (A) showed the numbers of top 12 microorganism identified by tNGS and mNGS detection. (B) Radar map showed the distribution of top 13 microorganism identified by tNGS and mNGS detection. Categories of microorganism identified in adult patients with pneumonia by tNGS (C) and mNGS (D) detection. (E) showed the detail species which were identified by mNGS but not by tNGS detection; (F) showed the categories of microorganism identified by mNGS but not by tNGS detection. (G) Specific microorganism identified in adult patients with pneumonia by tNGS and mNGS detection.

Table 1
Comparison the Detection Rates of tNGS and mNGS in Respiratory Samples.

	tNGS			mNGS				
Sample type	Cases (n)	Positive cases (n)	Positive rate (%)	Cases (n)	Positive cases (n)	Positive rate (%)	x^2	*P*
Sputum	35	34	97.14	33	30	90.91	1.19	0.28
BALF	84	68	80.95	84	76	90.48	3.11	0.08
Hydrothorax	9	3	33.33	9	3	33.33	0.00	1.00
Tissue	1	1	100					
Total	129	106	82.17	126	109	86.51	0.67	0.41

Rhinoviruses A/B/C were detected in tNGS, but none in mNGS. This was because *Rhinoviruses A/B/C* are RNA virus, and the mNGS test in this study only carried out the DNA process. Other common viruses, such as *influenza* and *parainfluenza*, were detected in a small amount in both methods (only 1), which may be related to the fact that the time of our research is not the season of influenza.

Collectively, we presented the first data on comparing the test efficiency of tNGS and mNGS detection in respiratory samples. And we observed tNGS is effective in detecting respiratory pathogens, whose cost was a quarter.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics approval

Written informed consent was obtained from the patient before any clinic choices were made and for the publication of any potentially identifiable images or data included in this article.

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Declaration of Competing Interest

The authors have declared no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.08.022.

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