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ORIGINAL RESEARCH

Exploring the Clinical Utility of Metagenomic Next-Generation Sequencing in the Diagnosis of Pulmonary Infection

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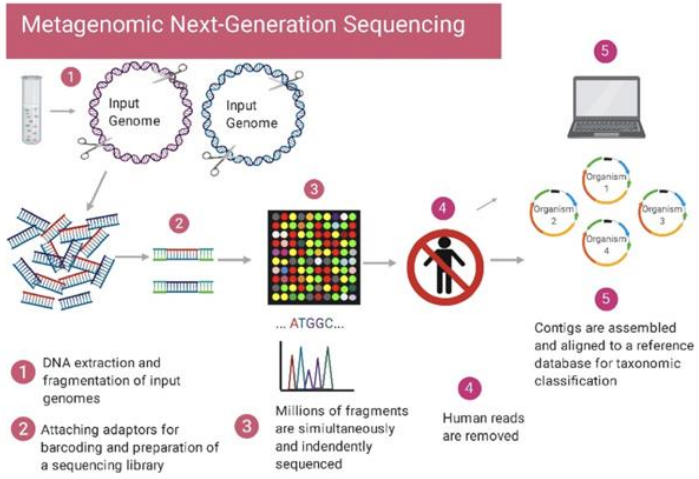

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Presented by
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- **Why carry out this study?**

Pulmonary infections remain important causes of morbidity and mortality in the world.

Improved diagnostic methods with better sensitivity, speed, and spectrum for pathogen detection are urgently needed.

We aimed to explore the real-world clinical application value and challenges of **metagenomic next-generation sequencing (mNGS)** for pulmonary infection diagnosis.

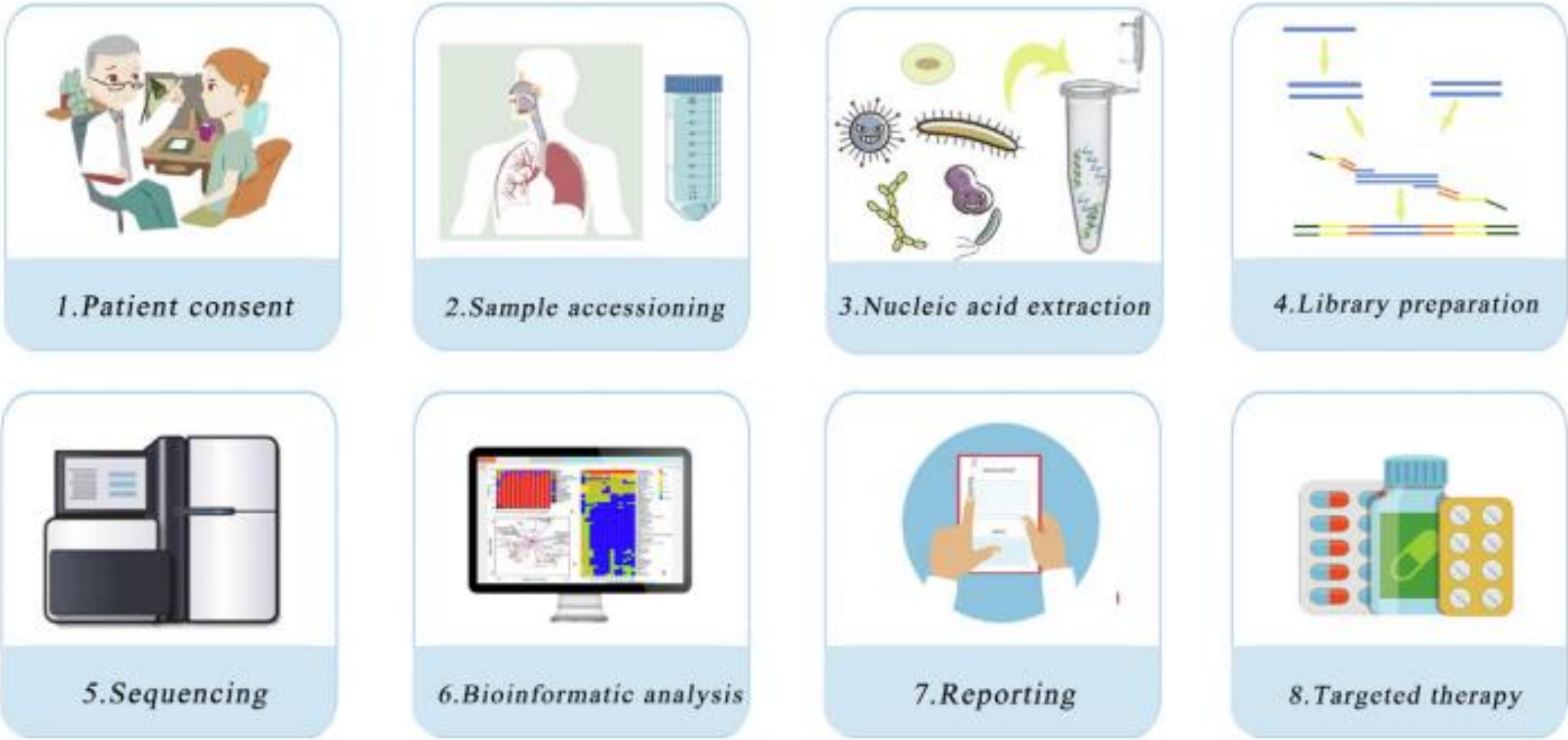
Although various assays exist, rapid and accurate diagnosis of causative pathogens is still difficult. Traditional culture methods, only used for fungal and bacterial detection, are time consuming and exhibit low rates of positive detection, and therefore cannot meet the clinical needs.

Other detection methods like polymerase chain reaction (PCR) and immunology techniques can only identify several specific pathogens.

Moreover, infections with multiple pathogens and the appearance of multidrug-resistant pathogens make the identification of the causative agent more difficult.

Metagenomic next-generation sequencing (mNGS) is a novel and promising approach which combines high-throughput sequencing with bioinformatics analysis.

- requires shorter analysis time,
- has a wide range of detectable pathogens (bacteria, fungi, viruses, and parasites can be simultaneously identified by a single assay)
- simple sample-processing
- is less affected by prior antibiotic use



Diao, Zhenli, et al. "Metagenomics next-generation sequencing tests take the stage in the diagnosis of lower respiratory tract infections." *Journal of Advanced Research* (2021).

We retrospectively reviewed the results of mNGS and conventional tests from 140 hospitalized patients with suspected pulmonary infections from January 2019 to December 2020.

The sample types included bronchoalveolar lavage fluid, lung tissue by transbronchial lung biopsy, pleural effusion, blood, and bronchial sputum.

Apart from the mNGS reports that our patients received, an extra comprehensive and thorough literature search was conducted.

Material and Methods

1-Patients and Sample Collection

We retrospectively reviewed **140 hospitalized patients** with **suspected pulmonary infections** in the Department of Respiratory and Critical Care, The Affiliated Wuxi Second People's Hospital of Nanjing Medical University from January 2019 to December 2020.

The inclusion criteria were as follows:

(1) patients who were suspected of having pulmonary infections;
(2) patients who agreed to undergo the mNGS examination;
(3) bronchoalveolar lavage fluid (BALF) samples and detection process passed quality control for mNGS; and
(4) patients whose medical data were recorded completely.

The exclusion criteria were as follows:

(1) patients who refused to undergo the mNGS examination;
(2) BALF samples or detection process failed to pass quality control for mNGS;
(3) patients with incomplete clinical and laboratory data;

Samples were sent to BGI Co., Ltd (Shenzhen, China) for sequencing as described.

The remaining specimens were sent to our microbiological laboratory.

The conventional tests were performed by culture of the bacteria or fungi in blood agar or in Sabouraud agar at 35°C for a maximum period of 5 days.

Bacteria or fungi culturing were performed by using the VITEK-II Compact automated microbiological system (bioMerieux, France).

An expanded database of over **330 species** of microorganisms can be identified using the new VITEK 2 Colorimetric Identification cards.

Other conventional diagnostic testing included

Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, cytomegalovirus (CMV), Epstein–Barr virus (EBV), adenovirus, and herpes simplex virus (HSV) **serological antibody detection**.

Xpert testing for Mycobacterium tuberculosis and so on.

The Xpert MTB/RIF test detects DNA from *Mycobacterium tuberculosis* complex and susceptibility to rifampin.

2- Metagenomic Next-Generation Sequencing and Analysis

2-1 Sample Processing and DNA Extraction

2-2 Construction of DNA Libraries

2-3 Sequencing and Bioinformatic Analysis

Construction of DNA Libraries

DNA libraries were constructed through DNA fragmentation (about 150 bp), end repair, adapter ligation, and PCR amplification.

An Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used for quality control of the DNA libraries (200–300 bp).

Then, libraries with confirmed quality were sequenced by the **BGISEQ-50 platform** (BGI Co., Ltd, Shenzhen, China)

Sequencing and Bioinformatic Analysis

High-quality sequencing data were generated by removing low-quality reads, followed by computational subtraction of human host sequences mapped to the human reference genome (hg19) using Burrows–Wheeler alignment.

The remaining data by removal of low-complexity reads were classified by simultaneously aligning to four microbial genome databases (bacteria, fungi, viruses, and parasites) which were downloaded from the National Center for Biotechnology Information (NCBI) (ftp://ftp.ncbi.nlm.nih.gov/genomes/).

RefSeq contains 4945 whole genome sequence of viral taxa, 6350 bacterial genomes or scaffolds, 1064 fungi related to human infection, and 234 parasites associated with human diseases.

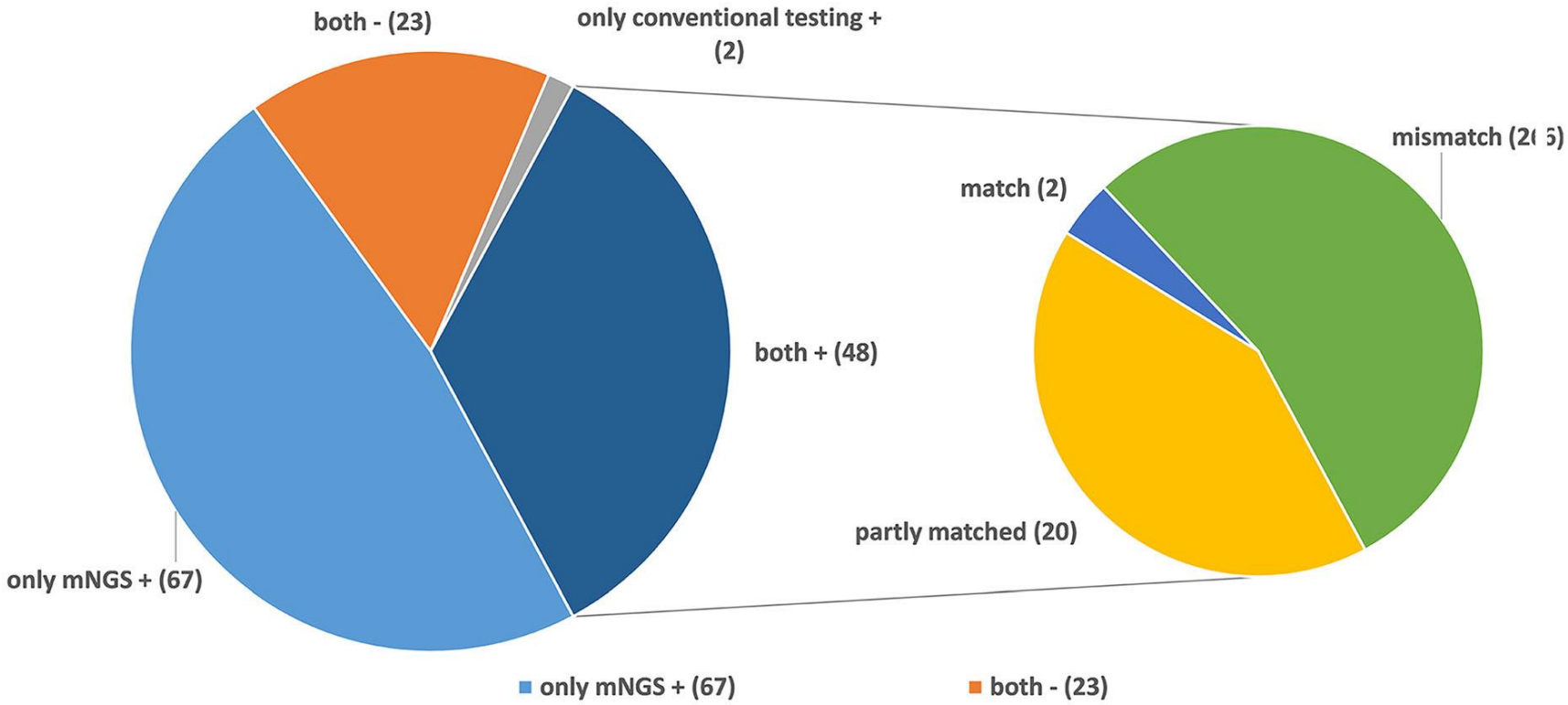
Results:

Table 1 Baseline characteristics of 140 patients

	Count	Percentage
Gender		
Male	62	44.28
Female	78	55.72
Age (years)		
≤ 40	18	12.86
> 40, ≤ 70	86	61.43
> 70	36	25.71
Basic illness		
Bronchiectasis	14	10.00
Chronic obstructive pulmonary disease	5	3.57
Previous history of tuberculosis	7	5.00
Bronchial asthma	1	0.71
Lung cancer	7	5.00
Diabetes	23	16.43
Connective tissue disease	16	11.43
Extrapulmonary malignancies	10	7.14
Sample type		
BALF	119	85.00
Lung tissue	9	6.43
Blood	5	3.57
Pleural effusion	6	4.29
Sputum	1	0.71
Chest CT scan		
Bilateral	89	63.57
Unilateral	51	36.43

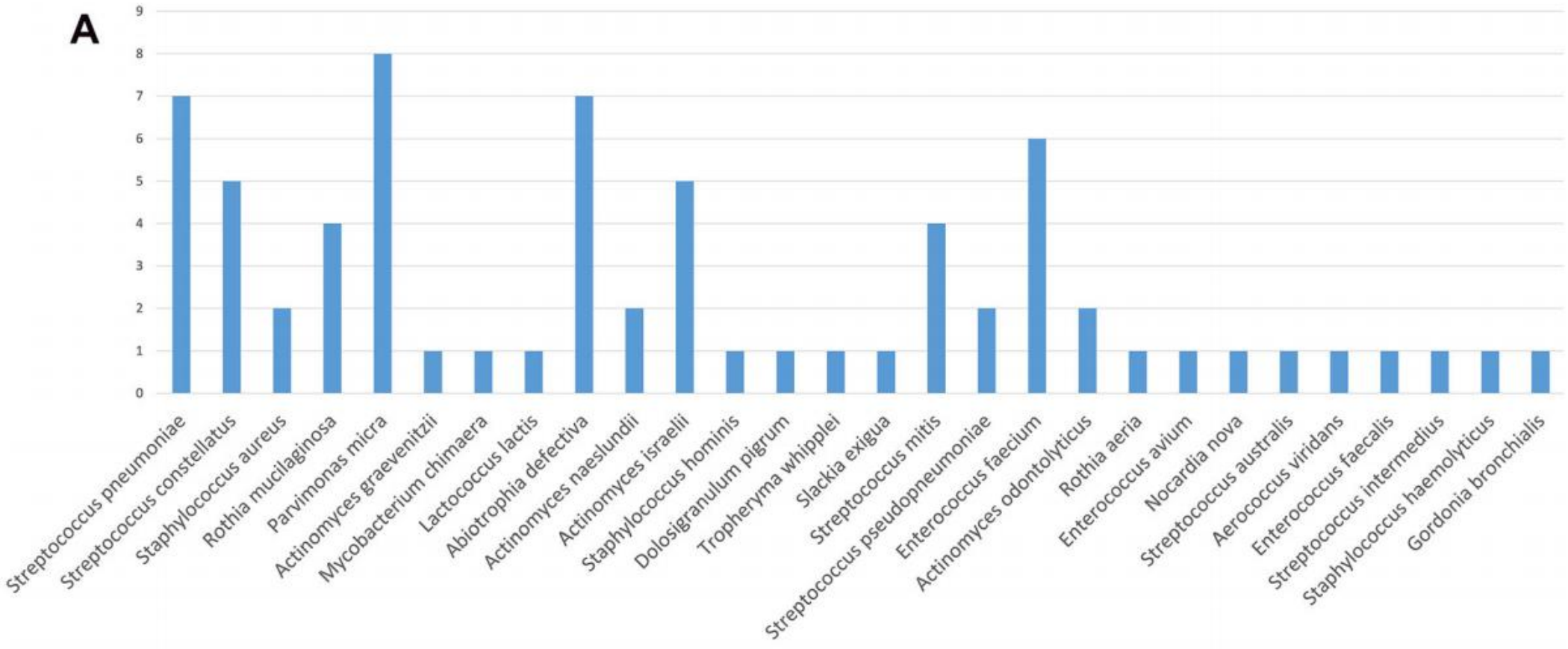
BALF bronchoalveolar lavage fluid, *CT* computerized tomography

Concordance Between mNGS and Conventional Diagnostic Testing

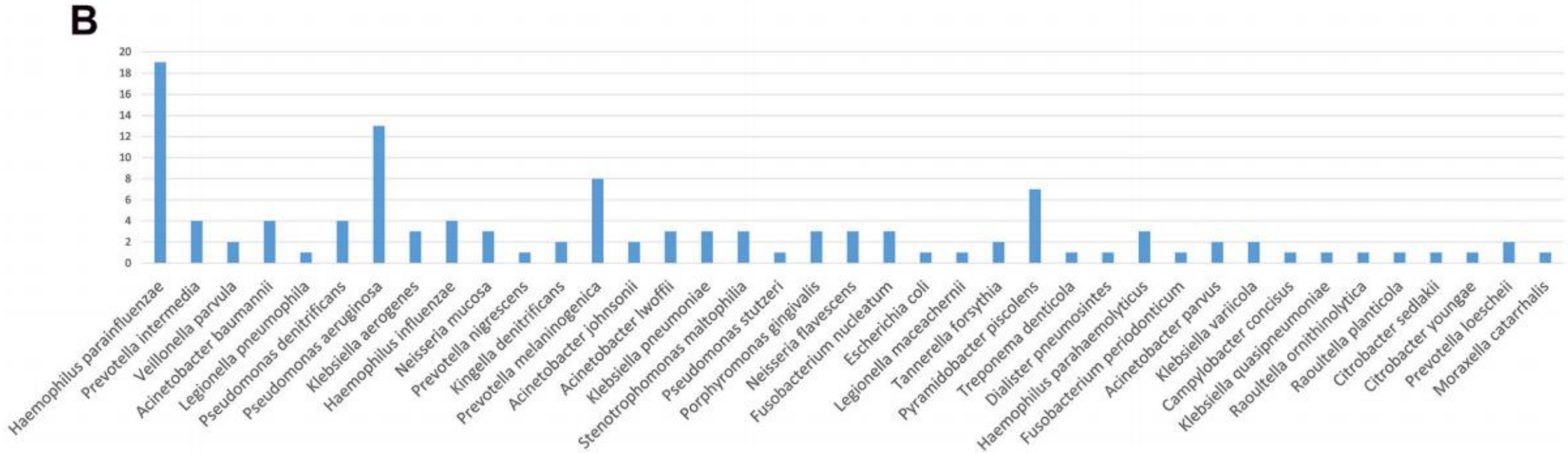


Comparison of Pathogenic Detection Between mNGS and Conventional Diagnostic Testing

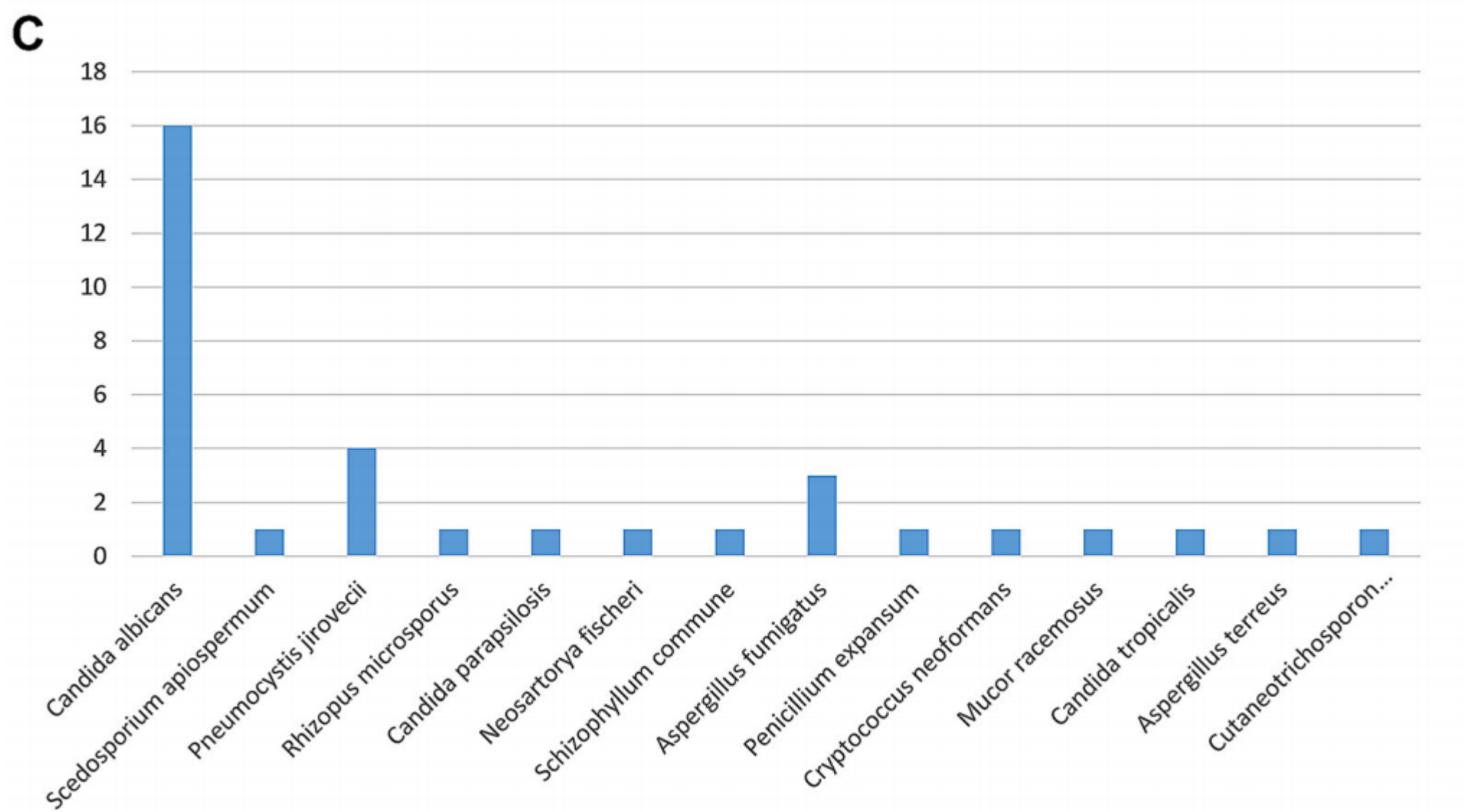
Significant differences were noticed in the positive detection rates of pathogens between mNGS and conventional diagnostic testing (115/140, 82.14% vs 50/140, 35.71%, $P < 0.05$).



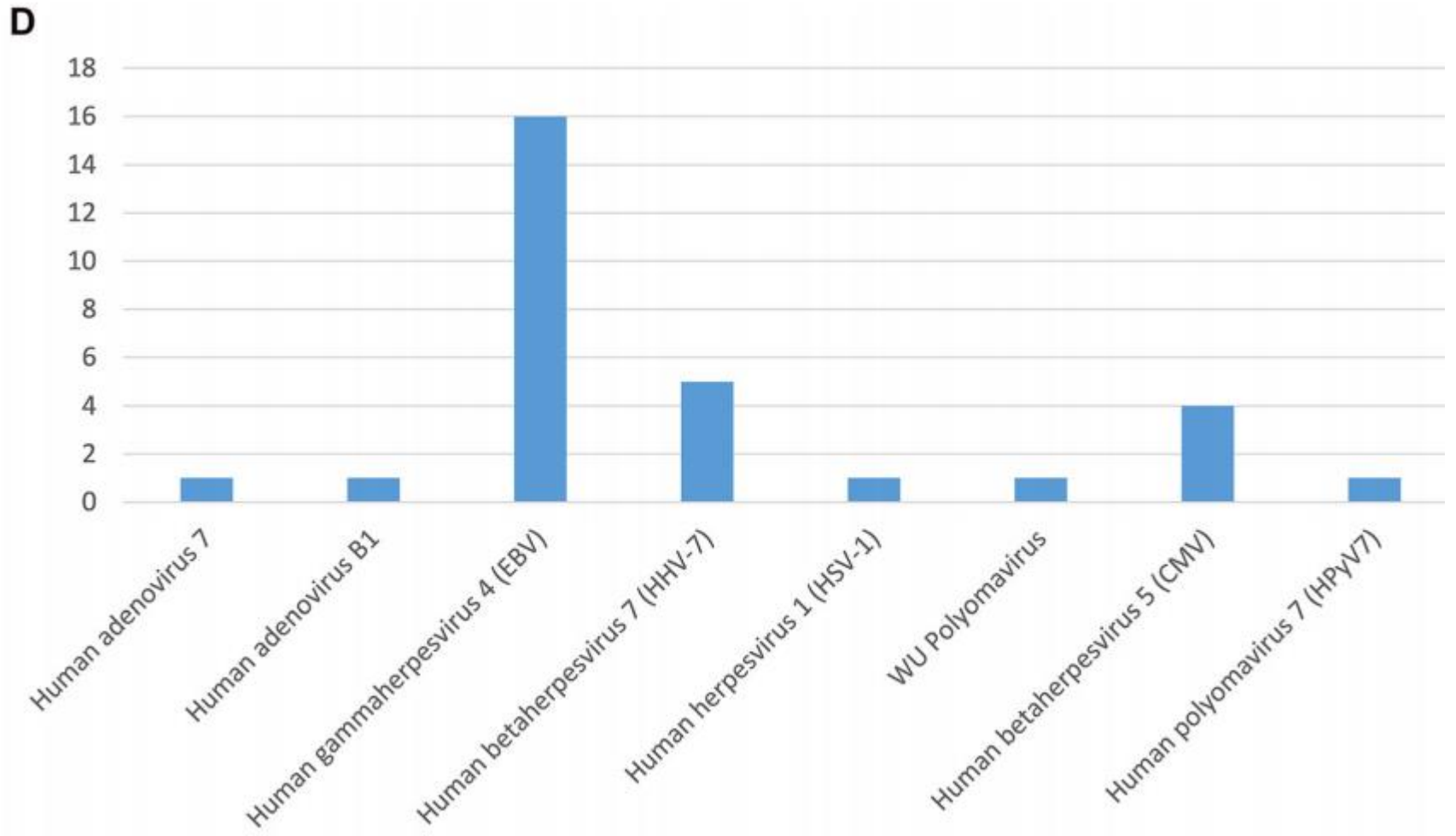
Species distribution of a **Gram-positive bacteria** detected by mNGS.



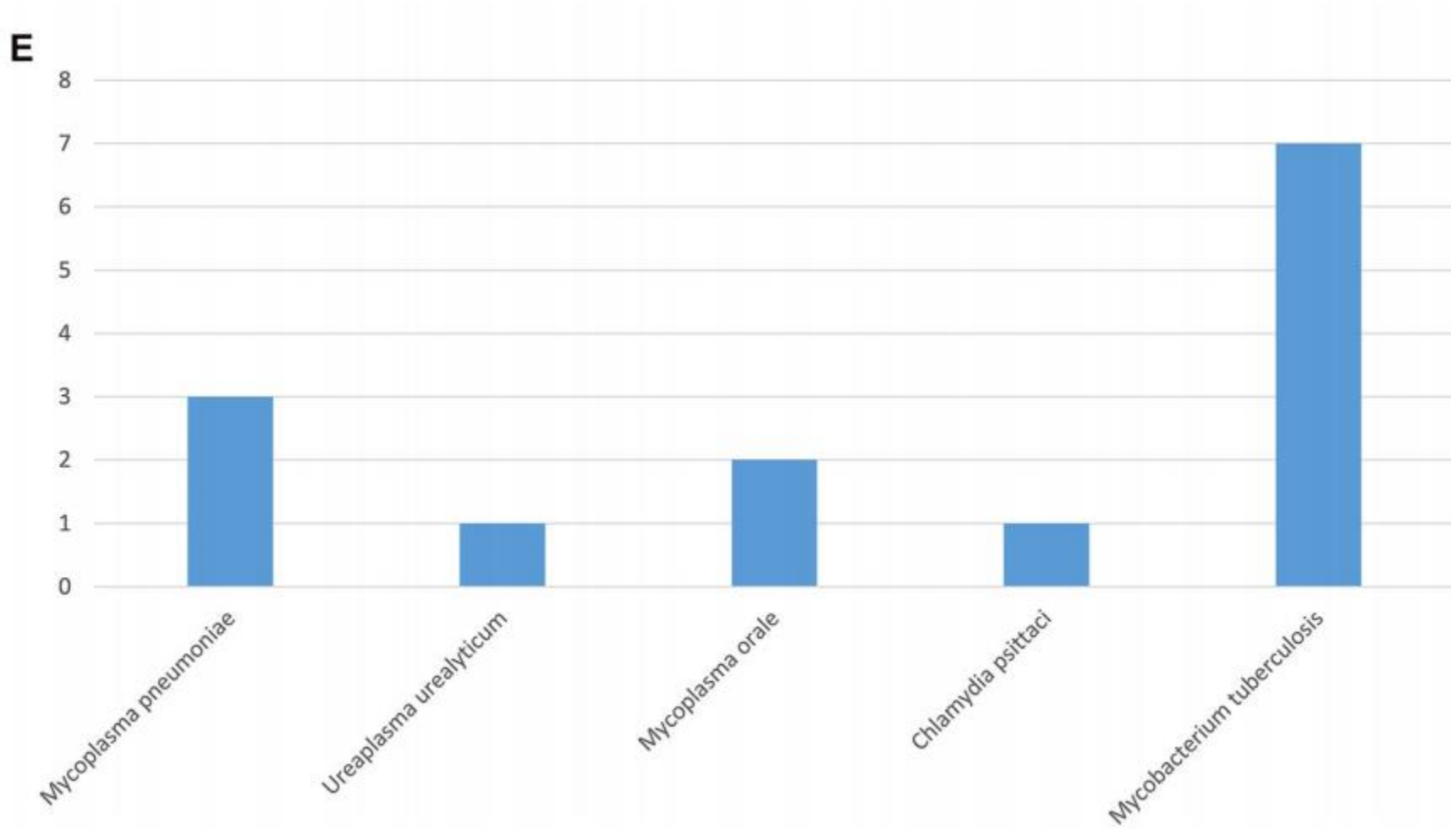
Species distribution of a **Gram-negative bacteria** detected by mNGS



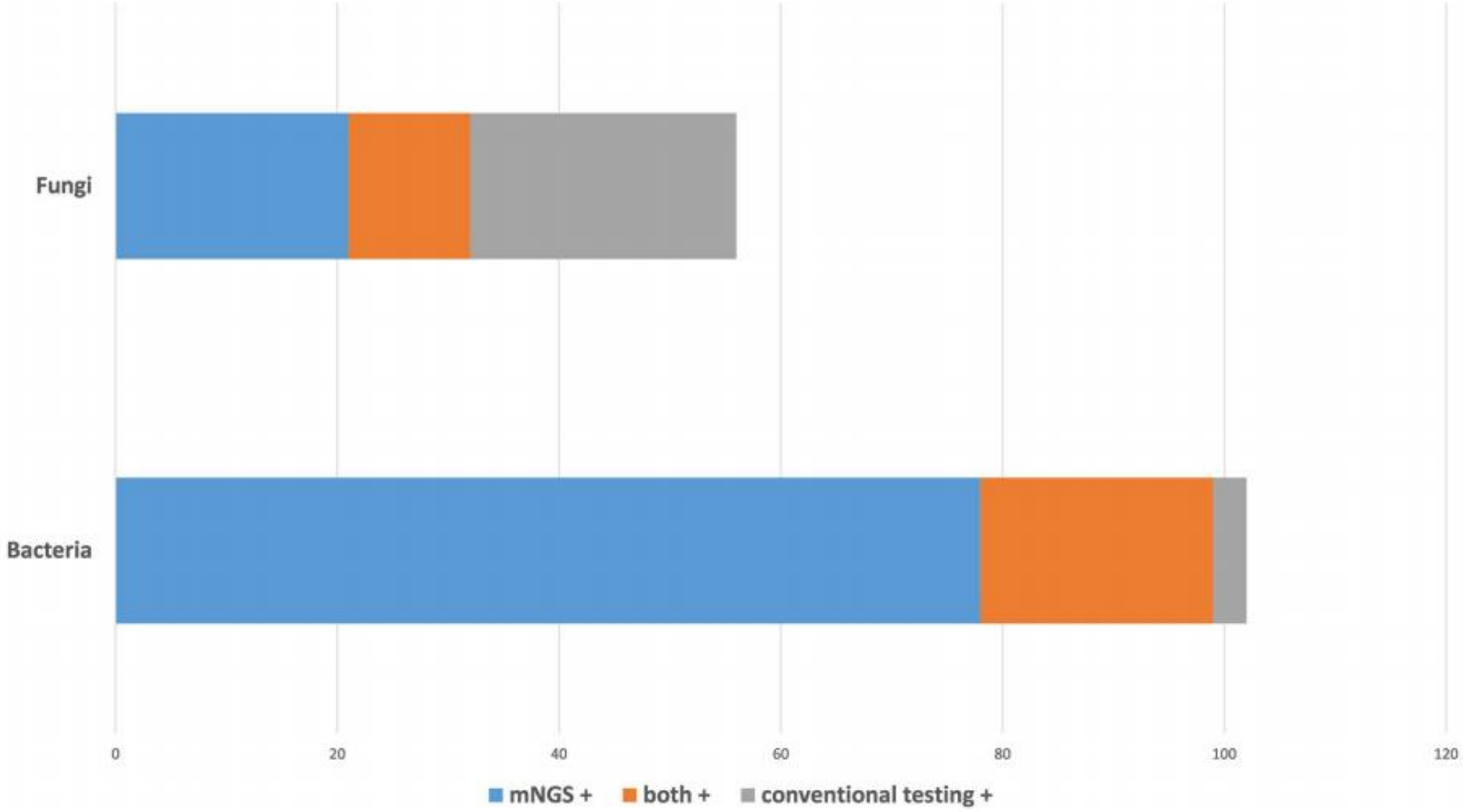
Species distribution of fungi detected by mNGS



Species distribution of viruses detected by mNGS



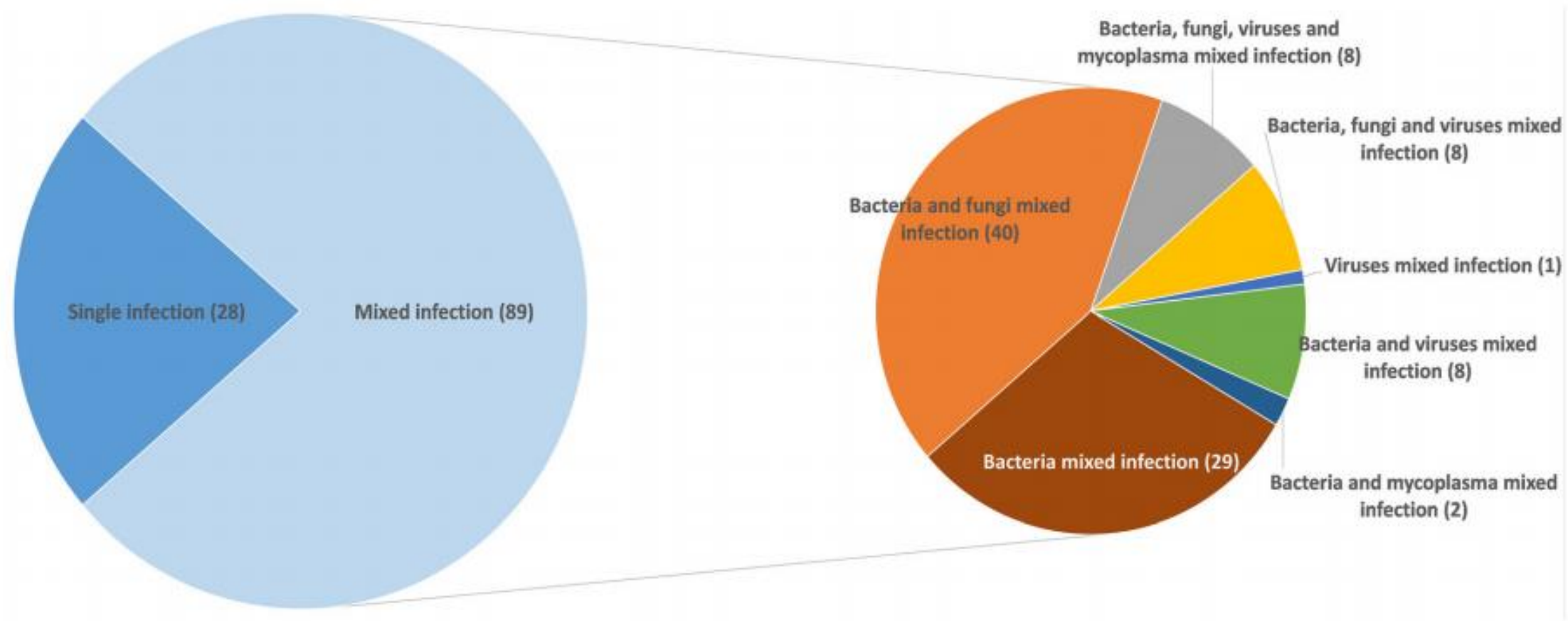
Species distribution of other pathogens (Mycoplasma, Chlamydia psittaci, M. tuberculosis) detected by mNGS.



The percentage of mNGS-positive patients was significantly higher than that of conventional testing positive patients **with regard to bacterial detection** (P <0.01), but no significant differences were found with regard to fungal detection (P = 0.67)

Streptococcus infantis, Oribacterium parvum, Prevotella pallens, Kingella oralis, Mogibacterium timidum, Treponema maltophilum, Dialister invisus, Streptococcus parasanguinis, Neisseria subflflava, Rothia dentocariosa, Campylobacter showae, Actinomyces johnsonii, Johnsonella ignava, Cardiobacterium hominis, Scardovia wiggsiae, Cardiobacterium valvarum, Desulfomicrobium orale, Fretibacterium fastidiosum, Actinomyces gerencseriae, etc.

were not interpreted as pathogens, because they were known as normal flora of the oral cavity, respiratory tract, or the skin and were not considered to cause pulmonary infection so far (via literature searches).



Mixed infections for various pathogens detected by mNGS and conventional test

Comparison of mNGS and Conventional Test in the Diagnosis of Pulmonary Infection

Table 3 Performance of mNGS and conventional testing in diagnosis of pulmonary infection

Diagnostic testing	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %
mNGS	89.17 (83.61–94.73)	75.00 (63.48–86.52)	95.54	53.57
Conventional laboratory-based diagnostic testing	50.00 (40.98–59.02)	81.82 (65.70–97.94)	93.65	23.38

95% CI 95% confidence intervals, *PPV* positive predictive value, *NPV* negative predictive value

The sensitivity of mNGS in pulmonary infection diagnosis was much higher than that of conventional test (89.17% vs 50.00%; P <0.01), but the specificity was the opposite, with no statistically significant difference (75.00% vs 81.82%; P > 0.05).

Discussion

- In our study, mNGS exhibited better performance than conventional test for detecting bacteria ($P < 0.01$), whereas it was not superior to conventional test with regard to fungal detection ($P = 0.67$). This was inconsistent with previous findings.

Possible explanations for this divergence are due to different diseases, different sample types, and different test conditions of mNGS and conventional test.

In terms of **mixed infection diagnosis**, the proportion of mixed pulmonary infections in our patients was much higher than we originally thought.

We found that a total of 69 (69/ 140 = 49.29%) cases were positive for mixed infection by mNGS only. However, when combined with conventional test results, the positive ratio of the mixed infection increased to 63.57% (89/140).

These results are in agreement with Fang et al. when combining mNGS and conventional test .

These results indicate that the combination of mNGS and conventional test contributes to the diagnostic ratio of the mixed pulmonary infection.

This study found that the most common combinations were bacterial–fungal coinfection and bacterial–bacterial coinfection.

The inconsistency between our study and other studies might result from the low proportion of immunocompromised patients in this study. Another possible explanation is that our hospital is located in the lower reaches of the Yangtze River, a high humidity area in which fungal infections are common. C. albicans was the major fungal pathogen in this region

mNGS

is a bias-free assay that can detect all pathogens in the environment; however, this test method itself cannot distinguish pathogenic microorganisms from colonizing microorganisms, background microorganisms, and contaminated microorganisms. For now, no authoritative guide is available to the interpretation of the mNGS report and therefore this remains a great challenge for clinicians.

In order to interpret the results of mNGS more accurately and objectively, in our study, we conducted an extra comprehensive and thorough literature search in PubMed in an attempt to identify published cases of pulmonary infection due to pathogens that we detected, without time limits.

We found out in mNGS reports that our patients received that some microorganisms which do **not cause human pulmonary infections** (e.g., Cardiobacterium hominis),

some microorganisms which **are not pathogenic in humans**, or some microorganisms whose pathogenicity is as yet unclear (e.g., Bacteroides vulgatus) were all included in the pathogenic microorganism list.

This problem is relatively easy to solve by reading related literature.

The most troublesome issue is **opportunistic pathogens**. Some microorganisms, for instance, S. constellatus, are part of the normal flora of the oral or respiratory tracts of humans, but pulmonary infections due to them have been widely reported; on the contrary, although reports have shown that some microorganisms, such as A. israelii, can cause respiratory infection, the number of articles was quite limited (sometimes only one to two).


Thank you!