Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Microbiological Diagnostic Performance of Metagenomic Next-generation Sequencing When Applied to Clinical Practice

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Background. Metagenomic next-generation sequencing (mNGS) was suggested to potentially replace traditional microbiological methodology because of its comprehensiveness. However, clinical experience with application of the test is relatively limited.

Methods. From April 2017 to December 2017, 511 specimens were collected, and their retrospective diagnoses were classified into infectious disease (347 [67.9%]), noninfectious disease (119 [23.3%]), and unknown cases (45 [8.8%]). The diagnostic performance of pathogens was compared between mNGS and culture. The effect of antibiotic exposure on detection rate was also assessed.

Results. The sensitivity and specificity of mNGS for diagnosing infectious disease were 50.7% and 85.7%, respectively, and these values outperformed those of culture, especially for *Mycobacterium tuberculosis* (odds ratio [OR], 4 [95% confidence interval {CI}, 1.7–10.8]; $P < .01$), viruses (mNGS only; $P < .01$), anaerobes (OR, ∞ [95% CI, 1.71–∞]; $P < .01$) and fungi (OR, 4.0 [95% CI, 1.6–10.3]; $P < .01$). Importantly, for mNGS-positive cases where the conventional method was inconclusive, 43 (61%) cases led to diagnosis modification, and 41 (58%) cases were not covered by empirical antibiotics. For cases where viruses were identified, broad-spectrum antibiotics were commonly administered (14/27), and 10 of 27 of these cases were suspected to be inappropriate. Interestingly, the sensitivity of mNGS was superior to that of culture (52.5% vs 34.2%; $P < .01$) in cases with, but not without, antibiotic exposure.

Conclusions. mNGS could yield a higher sensitivity for pathogen identification and is less affected by prior antibiotic exposure, thereby emerging as a promising technology for detecting infectious diseases.

Keywords. mNGS; interpretation; sensitivity; diagnostic; infection.

Infectious diseases remain leading causes of morbidity and mortality among all patient populations worldwide [1]. Pathogen identification of infectious disease is always difficult, which is a critical issue faced by infectious disease clinicians. The low detection rate of conventional culture methodology, especially that for fastidious organisms, makes precision diagnosis challenging in most patients. Culture-independent techniques such as serologic assay and nucleic acid amplification tests have proven useful for broadening the scope of detectable pathogens, but prior knowledge is necessary, which is sometimes impractical due to the complicated pathogen spectrum resulting from the popularity of international travel, etc. Previous literature suggested that up to 60% of cases were treated with no pathogen detected despite the comprehensive testing methods available [2–4]. The inability to obtain a targeted and timely diagnosis might delay precision antimicrobial treatment, leading to unnecessary broad-spectrum antibiotic usage, inducing antimicrobial resistance, and increasing healthcare costs.

Metagenomic next-generation sequencing (mNGS) is an unbiased approach that can theoretically detect all pathogens in a clinical sample and is especially suitable for rare, novel, and atypical etiologies of complicated infectious diseases [5]. In the near future, due to its sensitivity, speed, and cost-effectiveness considerations, mNGS might have the potential to become a routine diagnostic workup, partly replacing the traditional paradigm of serial tests [5]. However, literature relevant to clinical applications has mostly emerged as case reports or small-scale cohort studies, most of which have focused on virus detection from cerebrospinal fluid and plasma samples [6–12]. On the other hand, interpretation of mNGS results, especially those from respiratory specimens mixed with oral flora and colonizers [13–17], is challenging, which demands further investigation with larger cohorts. To these aims, the current study has been performed to expand mNGS testing to reach broader pathogens and samples while assessing its performance in real-life clinical practice.

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METHODS

Study Patients

We retrospectively reviewed 561 cases suspected of acute or chronic infection at Zhongshan Hospital in Shanghai, China, between April 2017 and December 2017. Using our inclusion/exclusion criteria (Figure 1), 511 samples were included for analysis and categorized into 3 groups defined as infectious disease (ID), noninfectious disease (NID), and unknown groups according to final diagnosis. Specimens were subjected to regular clinical microbiological assay as well as mNGS testing (BGI China) in a pairwise manner. The comparative study between mNGS and traditional methodology is described in Figure 1.

Metagenomic Next-generation Sequencing and Analysis

Sample Processing and Nucleic Acid Extraction

Blood samples were stored at room temperature, while all other specimens were stored in liquid nitrogen before testing. Volumes of 3–4 mL of blood were drawn from patients, placed in ethylenediaminetetraacetic acid tubes, stored at room temperature for 3–5 minutes before plasma separation and centrifuged at 1600*g* for 10 minutes at 4°C within 8 hours of collection. Plasma samples were transferred to new sterile tubes. Samples of 0.5–3 mL sputum or bronchoalveolar lavage fluid (BALF) were collected from patients according to standard procedures. Sputum was liquefied by 0.1% DTT (dithiothreitol) for 30 minutes at room temperature, and BALF could go directly to the next operation. Other types of samples (including tissue homogenates) were processed similarly to BALF. Then, 1.5-mL microcentrifuge tubes with 0.5 mL of sample and 1 g of 0.5-mm glass beads were attached to a horizontal platform on a vortex mixer and agitated vigorously at 2800–3200 rpm for 30 min. After agitation, 0.3 mL of the sample was separated into a new 1.5-mL microcentrifuge tube, and DNA was extracted using a TIANamp Micro DNA Kit (DP316, Tiangen Biotech) according to the manufacturer's recommendation.

Library Generation

DNA libraries were constructed through an end-repair method in which the adapters were added overnight, and polymerase chain reaction (PCR) amplification was used prior to analysis using an Ion Torrent Proton Sequencer (Life Technologies, Carlsbad, California). The quality of the DNA libraries was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California) combined with quantitative PCR to measure the adapters before sequencing. Qualified DNA libraries were prepared in a OneTouch system by emulsion PCR and then sequenced on the Ion Torrent Proton (Life Technologies, South San Francisco, California) sequencing platform.

Bioinformation Pipeline

High-quality sequencing data were generated by removing low-quality and short (length <35 bp) reads, followed by computational subtraction of human host sequences mapped to the human reference genome (hg19) using Burrows-Wheeler alignment. The

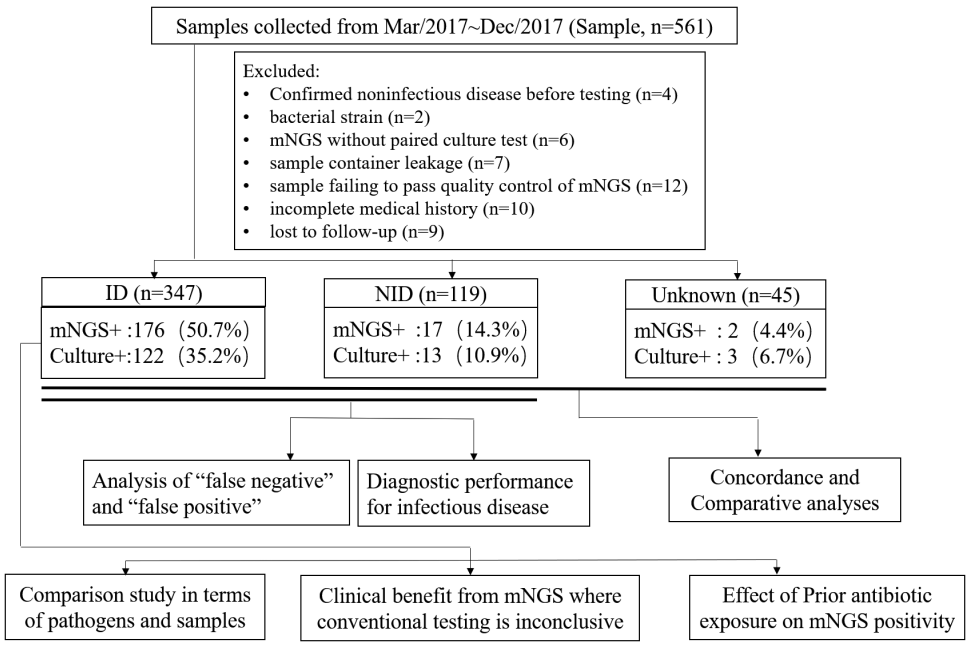

Figure 1. Flowchart of sample selection, classification, and comparison. From 561 samples, a total of 511 were selected for further analysis. Samples were divided into infectious disease (ID), noninfectious disease (NID), and unknown etiologies based on the retrospective diagnosis of the corresponding patients. All samples were examined for the concordance analysis of metagenomic next-generation sequencing (mNGS) and culture technique, while ID and NID patients were used to assess the diagnostic performance of the tests. Potential clinical benefits of mNGS were further analyzed in the ID group. Abbreviations: ID, infectious disease; mNGS, metagenomic next-generation sequencing; NID, noninfectious disease.

data remaining after removal of low-complexity reads were classified by simultaneous alignment to 4 microbial genome databases consisting of viruses, bacteria, fungi, and parasites. The classification reference databases were downloaded from National Center Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4189 whole-genome sequences of viral taxa, 2328 bacterial genomes or scaffolds, 199 fungi related to human infection, and 135 parasites associated with human diseases.

Criteria for a Positive mNGS Result

1. Bacteria (mycobacteria excluded), virus and parasites: mNGS identified a microbe (species level) whose coverage rate scored 10-fold greater than that of any other microbes according to Langelier's study [7].
2. Fungi: mNGS identified a microbe (species level) whose coverage rate scored 5-fold higher than that of any other fungus because of its low biomass in DNA extraction [2, 18].
3. Mycobacteria: *Mycobacterium tuberculosis* (MTB) was considered positive when at least 1 read was mapped to either the species or genus level due to the difficulty of DNA extraction and low possibility for contamination [19, 20]. Nontuberculous mycobacteria (NTM) were defined as positive when the mapping read number (genus or species level) was in the top 10 in the bacteria list due to the balance of hospital-to-laboratory environmental contamination [21] and low yield rate [22].

Examples of positive samples (proved by the culture method) are shown in Supplementary Tables 1–7. A variety of parameters were obtained from the sequencing platform, including mapping read number (species and genus level), abundance (species and genus level), and coverage rate. In consideration of confounding factors, such as nucleic acid contamination, total number of sequencing reads, and pathogen genome size [2], coverage rate was used as the measurement parameter in the present study (mycobacteria excluded).

Statistical Analysis

Comparative analysis was conducted by Pearson χ^2 test, Fisher exact test, or the McNemar test for discrete variables where appropriate. Data analyses were performed using SPSS 22.0 software. *P* values <.05 were considered significant, and all tests were 2-tailed.

RESULTS

Sample and Patient Characteristics

Demographic features of the patients in the current study are provided in Supplementary Tables 8 and 12. In the ID group (347 [67.9%]), the majority of patients were diagnosed with lower respiratory system infections (255/347 [73.5%]), followed by skin and soft tissue infections (32/347 [9.2%]) and intra-abdominal infections (17/347 [4.9%]) as shown in Figure 2*A*. In the ID group, 158 (45.5%) patients were diagnosed with confirmed pathogens by the conventional technique (Supplementary Figure 1). The remaining specimens were subdivided into the NID (119/511 [23.3%]) and unknown (45/511 [8.8%]) groups, with the respective etiology described in Supplementary Table 9 and Supplementary Figure 2. Most of our sample types belonged to the respiratory system, with 149 of 511 (29.2%) from BALF, 143 of 511 (28.6%) from sputum, and 55 of 511 (10.8%) from pleural fluid, followed by tissue (44 [8.6%]), pus (43 [8.4%]), and plasma (38 [7.4%]) (Figure 2*B*).

Diagnostic Performance Comparison of mNGS and Culture

Comparison of Diagnostic Performance for Differentiating ID From NID

The positivity rates of mNGS and culture tests for the ID, NID, and unknown groups are illustrated in Figure 3*A*. To compare the diagnostic efficiency for differentiating ID from NID, 466 samples were included for further study. The negative predictive values and positive predictive values of diagnosing infectious disease by mNGS were 37.4% and 91.2%, respectively, with the negative likelihood ratio and positive likelihood ratio being 0.57 and 3.55. As expected, mNGS increased the sensitivity rate by approximately 15% in comparison with that of culture (50.7% vs 35.2%; $P < .01$), while the specificity difference was not significant (85.7% vs 89.1%; $P = .39$) (Figure 3*B*). Furthermore, for cases where specific pathogens were clinically suspected, the sensitivities for detecting MTB, NTM, *Aspergillus*, and *Cryptococcus* by mNGS were 45.7%, 29.8%, 61.5%, and 43.8%, respectively. In addition, mNGS was inferior to serum antigen testing for detecting *Cryptococcus* (43.8% vs 81.3%; $P = .01$; Supplementary Table 10).

Concordance Between mNGS and Culture for Pathogen Detection

In our results, mNGS and culture were both positive in 96 of 511 (18.8%) cases and were both negative in 274 of 511 (53.6%) cases. Ninety-nine samples were positive by mNGS only (19.4%) and 42 were positive by culture only (8.2%). For double-positive samples, the 2 results were completely matched in 65 of 96 cases and totally mismatched in 9 of 96 cases (Figure 3*C*). The remaining 22 cases were found to be "partly matched," indicative of at least 1 overlap of pathogens when polymicrobial results were observed.

"False Positives" and "False Negatives" of mNGS

In the ID group, up to 54 culturable pathogens were missed by mNGS. Among these "mNGS false-negative" cases, 11 culture results were paradoxical with clinical diagnosis, 9 were detected by mNGS without meeting our positive criteria, and the remaining 30 were completely unidentifiable by mNGS. For the "mNGS false-positive" cases in the NID group, possible reasons included colonization (4/15), potential concomitant infection with NIDs (4/15), and likelihood of latent infection (3/15) (Table 1).

Comparison of mNGS and Culture Testing by Pathogens and Samples

Comparison Analysis at the Pathogen-type Level

Among the 257 microbes isolated, MTB (53/257) was the most commonly detected pathogen, followed by NTM (30/257),

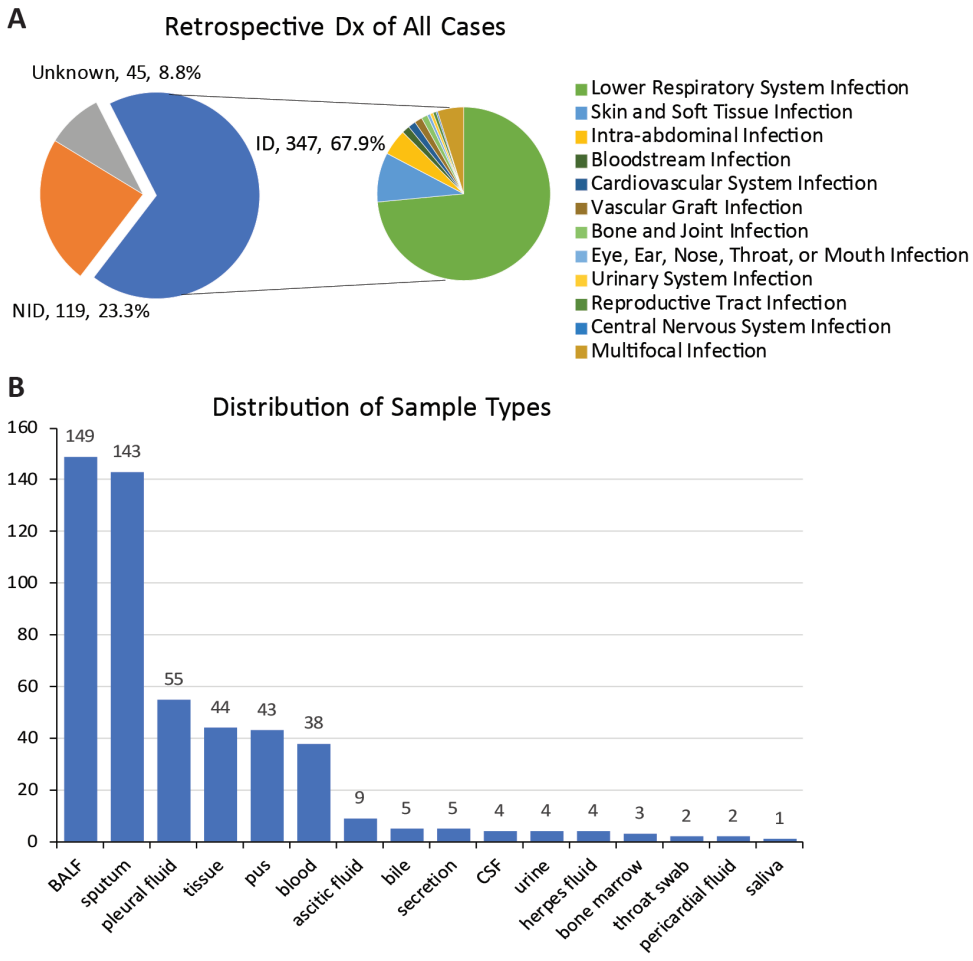

Figure 2. The distribution of the infectious diseases and clinical specimens in the present study. *A*, Pie charts demonstrated the etiology of disease based on retrospective diagnosis. In the infectious disease group, the vast majority of patients were diagnosed with lower respiratory tract infection (255/511 [49.9%]). *B*, Correspondingly, the respiratory tract specimens accounted for the greatest proportion of our samples. Abbreviations: BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; Dx, diagnosis; ID, infectious disease; NID, noninfectious disease.

virus (25/258), and *Aspergillus* (26/258). The percentage of mNGS-positive samples was significantly higher than that of culture-positive samples in terms of MTB (odds ratio [OR], 4 [95% confidence interval {CI}, 1.7–10.8]; $P < .01$), virus (mNGS only; $P < .01$), anaerobe (OR, ∞ [95% CI, 1.71–∞]; $P < .01$), and fungus (OR, 4.0 [95% CI, 1.6–10.3]; $P < .01$) (Figure 4*A*). *Nocardia* was also observed to have a higher yield rate by mNGS than that by culture, although the difference was not significant ($P = .25$) due to the small sample size. Interestingly, some strains of anaerobe (2/3), *Nocardia* (2/2), and *Cryptococcus* (4/4) microbes were initially culture negative and were later isolated with adjusted culturing conditions based on mNGS results.

Comparison Analysis at the Sample-type Level

In sputum and tissue sample types, we found a significantly higher sensitivity in detection by mNGS vs culture (Supplementary Figure 3), whereas the overall sensitivity of mNGS did not differ among sample types (Figure 4*B*). However, we observed a higher positivity in BALF than that in sputum ($P < .01$; Figure 4*C*) for NTM but not MTB, *Aspergillus*, or *Cryptococcus* (Supplementary Figure 4).

Potential Implications of Clinical mNGS Testing

Effect of Antibiotic Exposure on Pathogen Detection

In our analysis, 181 of 347 (52.2%) patients were exposed to antibiotics prior to mNGS and culture testing, while the remaining patients were not exposed. There was no significant difference in the positivity rate between mNGS and culture in nonexposed patients (43.3% vs 36.7%; $P = .10$). However, given prior antibiotic usage, the positivity rate of mNGS was drastically higher than that of culture (52.7% vs 34.4%; $P < .01$; Figure 5*A*).

Diagnosis Assisted by mNGS for Patients Without Identifiable Etiology by Conventional Testing

Among 347 samples, 70 (20.2%) were mNGS positive, while the comprehensive conventional method was inconclusive, including *Pneumocystis pneumonia* in plasma, *Mycoplasma hominis* in the articular cavity, *Legionella pneumophila* in hand pus,

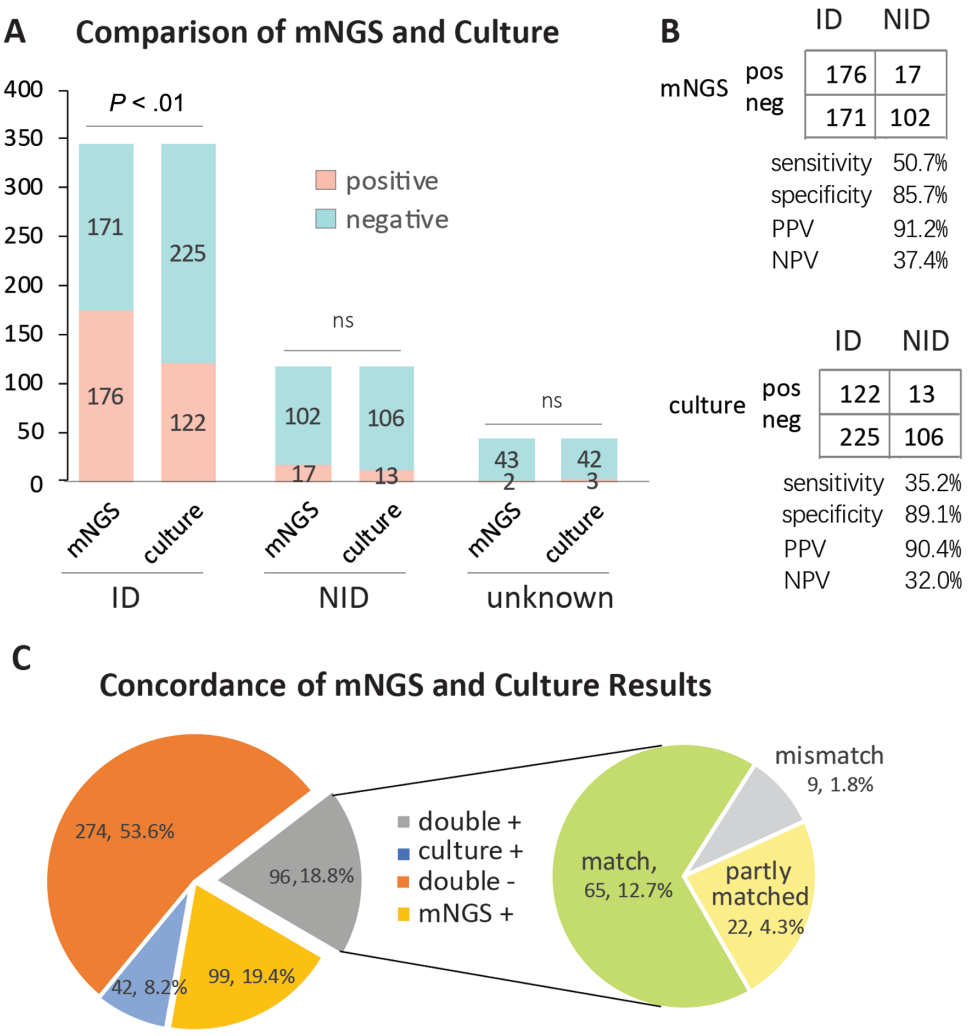

Figure 3. Positivity rate comparison and concordance analysis between metagenomic next-generation sequencing (mNGS) and culture for infectious disease (ID; n = 347), noninfectious disease (NID; n = 119), and unknown (n = 45) samples. *A*, The number of positive samples (y-axis) for pairwise mNGS and culture testing is plotted against the ID, NID, and unknown groups (x-axis). *B*, Contingency tables formatted in a 2 × 2 manner showing the respective diagnostic performance of mNGS and culture testing for differentiating ID from NID. Sensitivity was increased by approximately 15% in mNGS compared with culture (50.7% vs 35.2%; *P* < .01), while specificity remained similar. *C*, Pie chart demonstrating the positivity distribution of mNGS and culture for all samples from 3 groups. For the double-positive subset, a high proportion of complete matching (65/96) and partial matching (at least 1 pathogen identified in the test was confirmed by the other) (22/96) was seen, with only 9 conflicts between mNGS and culture results. Abbreviations: ID, infectious disease; mNGS, metagenomic next-generation sequencing; NID, noninfectious disease; NPV, negative predictive value; ns, no significant difference; pos, positive; neg, negative; PPV, positive predictive value.

Bartonella henselae in the lymph node, *Nocardia* in brain and lung tissue, *Cryptococcus* in skin, and *Rhizopus microsporus* in BALF (Supplementary Figure 5). Based on mNGS diagnosis, 9 (13%) identified microbes confirmed the clinical diagnosis, while up to 43 (61%) modified the initial diagnosis. Fourteen (20%) pathogens were uncertainly associated with clinical diseases, whereas 4 (6%) were discrepant with clinical diagnosis (Figure 5*B*). The pathogens were more frequently uncovered (58.6%) by an empirical antibiotic regimen (Figure 5*C*).

Potential Inappropriate Antibiotic Usage for Patients With Virus Isolates

Among the 27 viruses from 24 patients, the most commonly identified viruses were Epstein-Barr virus (n = 8), followed by torque teno virus (n = 4), herpes simplex virus 1 (n = 4) and cytomegalovirus (n = 4). Almost one-half of patients were considered immunocompromised hosts (15/27) and diagnosed with a hospital-acquired infection (13/27). There was a considerable percentage of patients (14/27) prescribed broad-spectrum antibiotics, while 10 of 27 patients were suspected of inappropriate antibiotic usage (Table 2 and Supplementary Table 11).

DISCUSSION

In our study, we systematically compared detection by mNGS and culture in a pairwise manner and found mNGS to be advantageous in several aspects. First, mNGS is noted for its superior feasibility in detecting fungi (OR, 4.0 [95% CI, 1.6–10.3];

Table 1. Analysis of "False Positive"[a] (n = 15) and "False Negative"[b] (n = 54) Results

Pathogens Detected Only by mNGS in the NID Group				
Sample No.	Patient No.	Diagnosis	mNGS Result	Possible Explanation
Sputum-95	PT318	Dermatomyositis	*Enterococcus faecium*	Likely colonization
Sputum-3	PT074	ABPA	*Mucor circinelloides*	Likely contamination
Blood-9	PT137	Postchemotherapy pneumonitis	Virus (TTV)	Potential cause of inflammation
Tissue-13	PT137	Postchemotherapy pneumonitis	Virus (TTV)	Potential cause of inflammation
Tissue-7	PT111	UCTD	Virus (EBV)	Potential cause of inflammation
Tissue-17	PT185	Lymphoma	Virus (EBV)	Possible cause of lymphoma
Sputum-51	PT185	Lymphoma	Virus (HAdV4)	Unknown
BALF-45	PT194	Pneumonolipoidosis	Virus (CMV)	Unknown
Sputum-22	PT108	COP	MTB	Overinterpretation
Tissue-30	PT294	Lung cancer	MTB	Overinterpretation
Ascitic fluid-5	PT327	Intra-abdominal cancer	MTB	Overinterpretation
Sputum-61	PT218	Old lesion	*Acinetobacter baumannii*	Likely colonization
Sputum-96	PT318	Dermatomyositis	*A. baumannii*	Likely colonization
Sputum-141	PT016	ABPA	*Pseudomonas aeruginosa*	Likely colonization
Pleural fluid-11	PT141	Castleman disease	Anaerobe	Unknown

Culturable Pathogens Missed by mNGS in the ID Group				
		Possible Explanation		
Microbe	Count	Clinically Unsupported Microbes	"Weak" Positive	Not Detected
NTM	11	0	0	11
MTB	7	0	0	7
Streptococcus	5	1	1	3
Escherichia coli	4	1	1	2
Klebsiella pneumoniae	4	2	0	2
Enterococcus	3	0	0	3
Aspergillus	3	0	0	3
A. baumannii	3	0	3	0
P aeruginosa	3	0	1	2
Haemophilus influenzae	2	2	0	0
Candida	2	0	0	2
Staphylococcus epidermidis	2	2	0	0
Staphylococcus aureus	2	0	2	0
Citrobacter citrate	1	1	0	0
Chryseobacterium meningosepticum	1	1	0	0
Acinetobacter junii	1	1	0	0
Total	54	11	8	35

Abbreviations: ABPA, allergic bronchopulmonary *Aspergillosis*; BALF, bronchoalveolar lavage fluid; CMV, cytomegalovirus; COP, cryptogenic organizing pneumonia; EBV, Epstein-Barr virus; HAdV, human adenovirus 4; ID, infectious disease; mNGS, metagenomic next-generation sequencing; MTB, *Mycobacterium tuberculosis*; NID, noninfectious disease; NTM, nontuberculous mycobacteria; PT, patient; TTV, torque teno virus; UCTD, undifferentiated connective tissue disease.

[a]In the NID group, "false positive" occurred if mNGS identified culture-negative microbes.

[b]In the ID group, "false negative" was considered when culturable pathogens were missed by mNGS.

$P < .01$) and viruses (mNGS only) in general and for several bacterial pathogens including anaerobes (OR, ∞ [95% CI, 1.71 to ∞]; $P < .01$), MTB (OR, 4 [95% CI, 1.7–10.8]; $P < .01$), and potentially *Nocardia* (a trend without a significant difference [$P = .25$]). In addition, the rapid feedback of mNGS might hasten clinical decision making and guide clinical laboratories to improve culture conditions for fastidious organisms. Moreover, we have identified, for the first time, that a substantial percentage of infection diagnoses were modified based on mNGS (61.4%), whereas most empirical antibiotic regimens are inappropriate for the detected pathogens (58.6%). Finally, according to our data, the mNGS yield rate is less likely to be affected by prior antibiotic usage, which is consistent with previous reports showing that, in contrast with cultures [23], pathogenic DNA may survive longer in plasma [24], facilitating the clinical practice of complex infectious diseases.

Although approximately 15% higher than that of traditional pathogen culture, our positivity rate (50.7%) of mNGS appeared

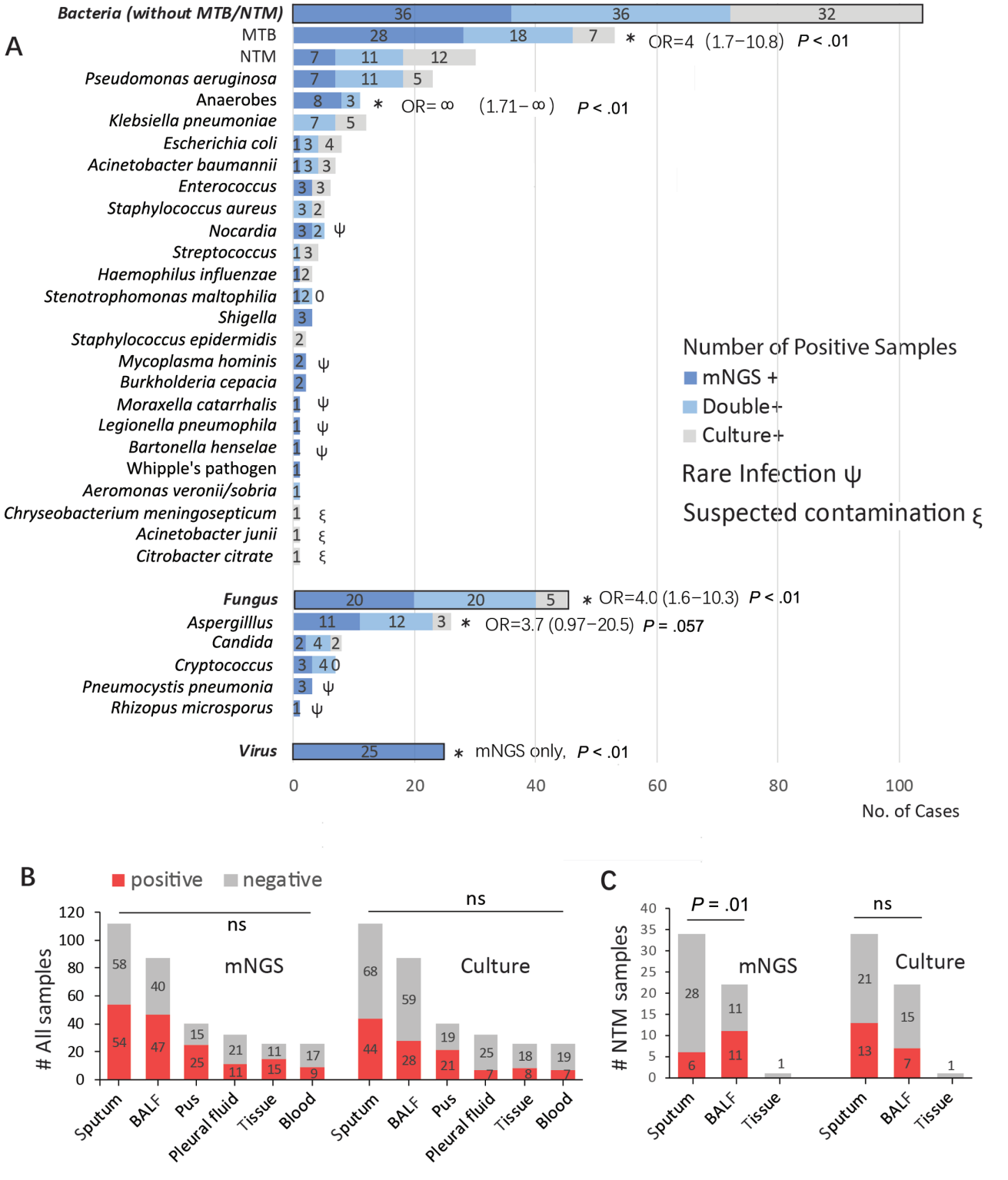

Figure 4. The overlap of positivity between metagenomic next-generation sequencing (mNGS) and culture for different infectious diseases and sample types. A total of 31 different pathogens were detected in the infectious disease group with their corresponding frequencies plotted in histograms (*A*). *Mycobacterium tuberculosis* (MTB) and anaerobes demonstrated a superior positivity rate in mNGS than that in culture ($P < .01$), while in general, the fungi and viruses, but not bacteria (excluding MTB/nontuberculous mycobacteria [NTM]), were found to be significantly more detectable in mNGS than in culture. Interestingly, the overall positivities of mNGS and culture were unaffected by sample types (*B*), except for NTM, where mNGS tends to be more likely to yield a positive finding in bronchoalveolar lavage fluid compared with sputum (*C*). Values in parentheses indicate the 95% confidence interval. Abbreviations: BALF, bronchoalveolar lavage fluid; mNGS, metagenomic next-generation sequencing; MTB, *Mycobacterium tuberculosis*; ns, no significant difference; NTM, nontuberculous mycobacteria; OR, odds ratio.

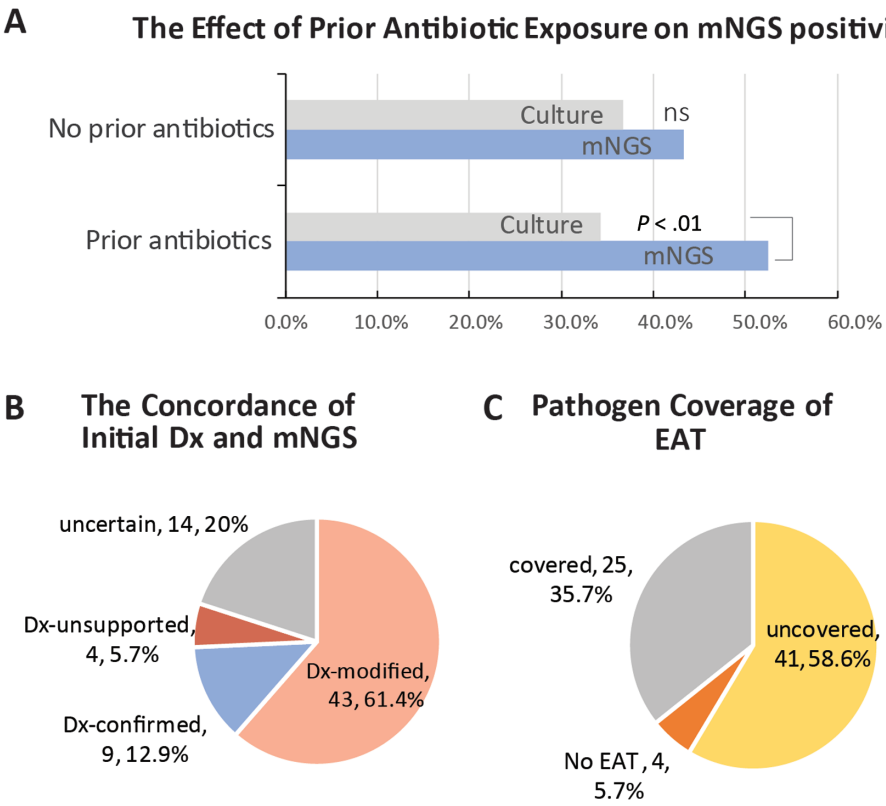

Figure 5. The clinical implications of metagenomic next-generation sequencing (mNGS) testing in real-world settings. *A*, A significantly higher yield of positivity was observed for mNGS compared with that of culture in samples with, but not without, prior antibiotic exposure ($P < .01$), suggesting that mNGS was less affected by prior antibiotic exposure. *B*, For microbes identified by mNGS where conventional testing was negative, the primary empirical diagnosis was frequently confirmed by (12.9%) or modified (61.4%) according to mNGS, whereas only 5.7% was considered unreliable (diagnosis unsupported) and 20% uncertain. *C*, Pathogens were more frequently uncovered (58.6%) by an empirical antibiotic regimen, while a smaller proportion (35.7%) was covered by the initial antimicrobial treatment. Abbreviations: Dx, diagnosis; EAT, empirical antibiotic therapy; mNGS, metagenomic next-generation sequencing; ns, not significant.

to be lower than expected. Previous studies reported a wide variety of sensitivity ranging from 36% [7] to 100% [8]. One possible reason is our inclusion of all patients suspected of infectious disease on a larger scale rather than pathogen-confirmed patients only as our comparator, which has been discussed in previous study [25]. Another reason may be associated with the sample types included. Most of our samples were derived from the respiratory tract, which is typically contaminated with oral

Table 2. Clinical Characteristics of Patients With Virus Isolates (n = 27)

		HAI		Immunosuppressed Patients		Broad-spectrum Antibiotics[a]		Suspected Inappropriate Antibiotic Usage		Treatment Responsive	
Type of Virus	% Coverage	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
EBV (n = 8)	30–98	3	5	4	4	4	4	2	6	2	6
TTV (n = 4)	8–69	2	2	2	2	2	2	1	3	1	3
CMV (n = 4)	7.4–29	4	0	4	0	3	1	3	1	1	3
HSV-1 (n = 4)	27–98	2	2	2	2	4	0	3	1	1	3
VZV (n = 2)	99–100	1	1	0	2	0	2	0	2	2	0
JC virus (n = 2)	96–97	0	2	1	1	0	2	0	2	2	0
HboV (n = 1)	87	0	1	0	1	0	1	0	1	1	0
HSV-1/EBV (n = 1)	96/97	1	0	1	0	1	0	1	0	1	0
HSV-1/TTV (n = 1)	97/9.6	0	1	1	0	0	1	0	1	1	0
Total (N = 27)	…	13	14	15	12	14	13	10	17	12	15

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAI, hospital-acquired infection; HboV, human bocavirus; HSV-1, herpes simplex virus 1; TTV, torque teno virus; VZV, varicella zoster virus.

[a]Carbapenem, tigecycline, oxazolidine, and glycopeptides were included. Other antibiotics are described in the Supplementary Materials.

normal flora, commensal organisms, and colonizers [13], leading to a relatively lower purity than other sample types [7]. On the other hand, the positivity criteria in our study might also result in lower sensitivity, as 8 of 54 "false-negative" microbes were identified by mNGS but discarded due to failure to meet our criteria. However, by using such criteria, a high specificity of mNGS comparable with culture (85.7% vs 89.1%; $P = .39$; Figure 3*B*) could be attained. Notably, our result suggested that the sensitivity of mNGS is not superior to that of culture for recognizing common bacteria (excluding MTB and anaerobes), which is consistent with a previous report that, compared with sequencing, culture is able to identify the vast majority (74%) of bacterium-associated pneumonia [26]. Therefore, we concluded that the mNGS technique for detecting common bacterial infections might not be as advantageous as it is for other microbes.

The turnaround time in our cohort was 32–36 hours for DNA-Seq and might be further reduced to approximately 24 hours in the near future due to the localization of the sequencing platform on site at our hospital. In contrast, the average feedback time of pathogen culture is ≥3 days for bacteria, 7 days for fungi, and 45 days for mycobacteria. Although the current per sample cost is higher (3000 Ren Min Bi [RMB]) than that of any single regular methodology (culture test for 600–700 RMB, *Cryptococcus* antigen test for 320 RMB, *Aspergillus* serological test for 600 RMB, and T.SPOT for 600 RMB), approximately 40% of the infectious diseases in our department could be inconclusively diagnosed, making pathogen screening with the "testing bundle" of traditional techniques less cost-effective.

Our study is not without limitations. In our results, the most frequently identified pathogen was mycobacteria, while the majority of samples were derived from the lower respiratory tract, which may lead to biased conclusions if generalizing to a broader scale of clinical infectious disease. Moreover, our untargeted mNGS is still not truly comprehensive. RNA-Seq data, for example, were not concomitantly tested with DNA sequencing, which might provide valuable complementary information such as RNA virus and microbial transcriptome alterations. Finally, our mNGS tests were delivered to the centralized laboratory rather than an in-house microbiology laboratory, which may sacrifice sensitivity rate because of reduced viability due to increased turnaround time from bedside to bench. However, we suggest that mNGS could yield higher sensitivity for early identification of fastidious and time-consuming microbes (eg, MTB, virus, anaerobe, and fungus) and assist clinical decision making regarding the antibiotic regimen; in addition, mNGS appears to be less affected by prior antibiotic exposure. With appropriate patient selection, sample handling, and data interpretation, mNGS could emerge as a promising technology for precision diagnosis and tailored therapy for clinical infectious diseases.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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