Clinical Application and Drug-Use-Guidance Value of Metagenomic Next-Generation Sequencing in Central Nervous System Infection

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Research Article

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Abstract

Background

Timely and precise etiology diagnosis is crucial for optimized medication regimens and better prognosis in central nervous system infections (CNS infections). We aimed to analyze the impact of mNGS tests on the management of patients with CNS infections.

Methods

We conducted a single-center retrospective cohort study to analyze the value of mNGS in clinical application. Three hundred sixty-nine patients with a specific CNS infection diagnosis were enrolled, and their clinical data were collected. CDI and DDI were defined in our study to describe the intensity of drug use in different groups. We also used LOH and mRS to evaluate whether the application of mNGS could benefit CNS infection patients.

Results

mNGS reported a 91.67% sensitivity in culture-positive patients and an 88.24% specificity compared with the final diagnoses. Patients performed with the mNGS test had less drug use, both total (58.77 vs. 81.18) and daily (22.6 vs. 28.12, p <0.1, McNemar) intensity of drug use, as well as the length of hospitalization (23.14 vs. 24.29). Patients with consciousness grading 1 and 3 had a decrease in CDI (Grade1, 86.49 vs. 173.37; Grade 3, 48.18 vs. 68.21), DDI (Grade1, 1.52 vs. 2.72; Grade 3, 2.3 vs. 2.45) and LOH (Grade1, 32 vs. 40; Grade 3, 21 vs. 23) with the application of mNGS. Patients infected with bacteria in CNS had a reduced CDI, DDI, and LOH in the mNGS Group, in contrast with the TraE Group. 49% of patients altered medication plans, and 24.7% of patients reduced drug intensity four days after mNGS reports, mostly due to the reduction of drug types.

Conclusion

mNGS showed its high sensitivity and specificity characteristics. mNGS may assist clinicians with more rational medication regimens and reduce the drug intensity of patients, of which the primary way was to reduce the variety of drugs, especially for severe patients and bacterial infections. mNGS has the potential value of improving the prognosis of CNS infectious patients.

Background

The central nervous system, or CNS, comprises the brain, the spinal cord, and associated membranes, and an infection of the CNS can be a life-threatening disease (1-5). The timely and accurate detection of pathogens is crucial to successfully diagnosing and managing center nervous system infections.

However, conventional infection diagnosis methods, such as microbial culture, targeted PCR et al., suffer from limited targets and long turn-around time, resulting in urgent recruitment of novel infection diagnosis techniques (6, 7).

Metagenomic next-generation sequencing (mNGS) has been recently used in infection diagnosis practice with satisfactory outcomes, significantly increasing pathogen detection sensitivity in various infections, including CNS infections(7). One study showed that mNGS increase the positive rate of pathogen detection by 13.1% from 55.6–68.7% in bacterial meningitis pathogens detection from cerebrospinal fluid (CSF)(8). In our previous study, mNGS could achieve a sensitivity and specificity in CNS infection of 90% and 98.57%, respectively [9], significantly reducing detection turn-around time (unpublished data). However, most studies have been focused on the diagnostic performance improvement value of mNGS. The lack of standard procedure, biased report criteria, and high economic cost made it challenging to evaluate the direct benefit acquired by the patients and public health system(8).

Molecular rapid diagnostic tests provide several opportunities to optimize antimicrobial selection to improve patient outcomes(9, 10). These microbiological results are used to guide the choice of antimicrobial drugs. Previous studies have reported the antimicrobial agent adjustments according to mNGS and Filmarray meningitis/encephalitis Panel. mNGS led to a change of treatment in 59 (37.1%) cases, including antibiotics de-escalation in 40 (25.2%) cases in respiratory infections (11). In pediatric CNS infections ,55.4% patients received antimicrobial de-escalation(12). Among immunocompromised patients, mNGS played a role in optimizing antibiotic use as well. Though several studies have made efforts to evaluate avoidance of antibiotic misuse and reduction of hospitalization period after the application of mNGS, there is still a lack of comprehensive evaluation of mNGS benefit in CNS infections. Additionally, a more precise evaluation of antimicrobial use needs to be performed.

In this study, we conducted a retrospective cohort study to evaluate the hospitalization period, antimicrobial drug types, and amount after mNGS employment, as well as the diagnostic yield of mNGS in CNS infection patients, aiming to comprehensively estimate the clinical benefits of mNGS and value of universal application in daily clinical practice.

Methods

Setting and Data Collection

This retrospective cohort study was conducted at Huashan Hospital of Fudan University. All the data for this study were collected from the Electronic Medical Records System of Huashan Hospital. The protocol for the conduct of this study was reviewed and approved by Huashan Hospital ethical committee. Patients or their surrogates signed informed consents for the lumbar puncture, and their information was collected from the Electronic Medical Records System.

Study Patients and samples

Patients older than 14 years with infection of the central nervous system (CNS) were eligible for inclusion if they were admitted to the Department of Infectious Diseases, Huashan Hospital of Fudan University, between March 2014 and December 2018. All the study participants were discharged with confirmed CNS infection as their primary diagnosis by physicians. We excluded patients with a previous diagnosis of CNS infection or results of a positive etiological test or effective treatments before admission, as we reasoned that these patients might be treated differently by clinicians based on known or suspected pathogen infection from previous treatment histories, which may lead to the bias of clinical decision making. Patients without lumbar punctures during the hospitalization were also excluded. Enrollment and exclusion criteria were listed in the supplementary information. CSF samples were obtained from all the patients and sent for routine and biochemical tests, CSF smear, as well as the traditional culture of bacteria, fungi, and tuberculosis.

Sample sequencing and data analysis

A volume of 1.5-3mL CSF samples from patient was collected according to standard procedures. A 1.5mL microcentrifuge tube with 0.6mL sample, enzyme, and 1g 0.5mm glass bead were attached to a horizontal platform on a vortex mixer and agitated vigorously at 2800-3200 rpm for 30 min. 0.3mL sample was separated into a new 1.5mL microcentrifuge tube, and DNA was extracted using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH) according to the manufacturer's recommendation.

Then, DNA libraries were constructed through DNA fragmentation, end-repair, adapter-ligation, and PCR amplification. Agilent 2100 was used for quality control of the DNA libraries. Quality qualified libraries were sequenced by the BGISEQ-50 /MGISEQ-2000 platform [2].

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 [3]. The remaining data by removal of low-complexity reads were classified by simultaneously aligning to four Microbial Genome Databases, consisting of bacteria, fungi, viruses, and parasites. The classification reference databases were downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4,945 whole-genome sequences of viral taxa, 6,350 bacterial genomes or scaffolds, 1064 fungi related to human infection, and 234 parasites associated with human diseases.

Diagnostic assessment of mNGS

We assessed the diagnostic performance of mNGS through the following steps. Firstly, we classified participants into two groups according to whether they have undergone the mNGS test: Patients who experienced traditional examinations only (TraE Group) and patients who underwent the mNGS test additionally (mNGS Group). Secondly, we calculated mNGS sensitivity compared to culture, mNGS sensitivity compared to culture=mNGS (positive) / culture (positive). Then the extra detection rate of mNGS compared to culture was statistically evaluated, mNGS extra detection rate= [mNGS (positive)-culture (positive)]/culture(positive). Moreover, we analyzed the accuracy rate of mNGS, and the accuracy

rate= mNGS (positive) & Case-consistent/mNGS (positive). Considering the composite criteria of recruiting, we will not discuss the specificity of mNGS here.

We evaluated the consistency between mNGS and clinical diagnosis: mNGS positive/Case consistent was determined as the mNGS detected potential pathogen is inconsistent with the final diagnosis, while mNGS positive/Case inconsistent represents inconsistent results between mNGS reports and final diagnosis.

Evaluation of clinical outcome

We employed Medical Research Council (MRC) Grade, Modified Ranking Scales (mRS), and length of hospitalization (LOH) to evaluate patient status. Participants were classified into 3 MRC grades according to their Glasgow Coma Scale(GCS)and their clinical manifestation (13): Grade1(GCS=15), Grade 2 (GCS of 11-14 or GCS of 15 associated with a focal neurological sign), and Grade3 (GCS $\leq$ 10). As reported, the patient's status can be divided into three categories according to mRS (14): Level 1 (good outcome, grade 0), Level 2(intermediate outcome, grade 1-2), and Level 3 (poor prognosis grade3-5). The LOH represented the length of the patient's hospital stay, and we used days as the unit of measurement.

In order to compare the intensity of antibiotic use between groups and avoid the incomparable defects caused by different types of drugs, we performed the calculation of Defined Daily Dose (DDD) (15). What is more, we introduced the concepts of cumulative drug intensity (CDI) and daily drug intensity (DDI). CDI was defined as the accumulated medication intensity of the patient during the hospital stay. CDI was equal to the sum of the drug intensity of all anti-infective treatments performed during hospitalization. DDI was the average daily medication intensity of the patient, DDI = CDI/ LOH.

The primary endpoint was the intensity of drug use and the length of hospital stays, while the secondary endpoint was the functional outcome at discharge according to the mRS.

Statistics analysis

For baseline characteristics, blood laboratory tests, and CSF laboratory tests, Kolmogorov–Smirnov test was used to determine the continuous variants described by medians when not. A Chi-square test was performed to evaluate independent binomial variables. Additionally, the Mann-Whitney test was used to compare the difference of baseline between TraE Group and mNGS Group. A P value <0.05 was considered significant. In the process of assessing diagnostic performance, sensitivity, specificity, and the additional detection rate was calculated according to the definitions above. Further, we analyzed the variance test and Mann–Whitney test to compare differences across mNGS subgroups. Statistical analyses and figures were conducted using the SPSS Version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.4.0 software (GraphPad Software, San Diego, CA, USA).

Results

General characteristics

In total, 369 patients were enrolled. Patients with confirmed etiological diagnosis before admission or those with a history of effective anti-infection treatment (n=153) were not included since medication history and etiological examinations would interfere with the clinicians' diagnosis and treatment. Besides, five patients without results of CSF laboratory tests were also excluded. Thus 211 participants were finally analyzed (Fig. 1). Fifty patients were diagnosed with encephalitis, 113 patients were affected with meningitis, and 38 patients were diagnosed with meningoencephalitis. As for the other ten patients, the exact location of the central nervous system infection could not be diagnosed. According to whether the patients have undergone mNGS tests, we divide patients into TraE Group and mNGS Group. The TraE Group we recruited included one hundred and thirteen patients who did not undergo the mNGS test as mNGS tests were not used at Huashan hospital until 2017. Therefore, for patients before 2017, their CSF samples were only performed with conventional tests, including CSF routine and biochemical tests, and culture of bacteria, fungi, and tuberculosis. Ninety-eight patients accepted the extra mNGS test according to clinical necessity, and they were classified into the mNGS Group. All the participants were categorized into bacterial (51 vs. 44), fungal (14 vs. 13), parasitic (1 vs. 3), viral (36 vs. 37), and unclassified infections (11 vs. 1), based on distinguishing whether or not mNGS was performed. Baseline characteristics of enrolled patients showed no significant difference among groups (Table.1).

Overall diagnostic performance of mNGS

We first performed etiology analysis, and it showed that, in the mNGS Group, culture reported 24 positive results, and mNGS detection revealed that *Mycobacterium tuberculosis* (n=7) was the most common potential pathogen. The top three causative pathogens identified were *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, and *Herpes Simplex Virus*-1 (HSV-1) (Fig. 3).

As shown in Fig. 2, mNGS reported a 91.67% (11/12) sensitivity compared to culture in the mNGS Group. The specificity compared to clinical diagnosis of mNGS was 88.24% (45/51). For traditional culture, the specificity compared to clinical diagnosis reached 92.31% (12/13). Therefore, mNGS owned a 39.8% extra detection rate to traditional culture, especially in virus detection. Compared with traditional culture methods, mNGS additionally detected Cytomegalovirus (CMV), Epstein-Barr virus (EBV), HSV-1, varicella-zoster virus (VZV), and Herpes simplex virus 6A (HSV6A) and adenovirus B1 in the mNGS Group.

Among 98 patients enrolled in the mNGS Group, 45 patients were categorized into mNGS (positive)/case consistent Group. In comparison, six patients were identified as mNGS (positive)/Case inconsistent including reports of *Candida parapsilosis* and *Rhodococcus* relatively in 2 patients who were finally diagnosed as viral infection, both *Prevotella intermedia* and *Streptococcus constellation* in patients diagnosed with *Aspergillus* infection, one report of *Rickettsia* in fungal meningitis, one detection of *Epstein-Barr virus* in bacterial meningitis and one report of *Oral Streptococcus* in tuberculosis meningoencephalitis patient. In culture-positive cases, mNGS reported one false-negative case of *Mycobacterium tuberculosis* (Fig. 3).

The comparison of drug use intensity between TraE Group and mNGS Group

Comparing the mNGS Group and the TraE Group, we found a significant difference in the CDI between the two groups during hospitalization (Fig. 4). The cumulative drug intensity during the hospitalization of the mNGS Group was lower than that of the TraE Group (81.18 vs. 58.77, respectively), with a decrease of 27.6%. In addition, we also found that the average DDI was lower in the mNGS Group as well (28.12 vs. 22.6 p=0.04), which meant the intensity of medication decreased by 19.6%.

Besides, we used the MRC grade (the definition of MRC grade as explained above) to classify patients according to their state of consciousness when they were admitted to the hospital. It could be observed that the patients in Grade1, which meant a worse state of consciousness and a more severe disease situation, the application of mNGS could decrease the median of DDI (4.57 vs. 2.24 p=0.0494) and CDI (73.25 vs. 48.06) during the hospital stay compared to TraE Group (Fig. 5A, D).

Moreover, we divided patients into viral-, bacterial-, and fungal-infections subgroups based on the etiology diagnosis. We found that compared to the TraE Group, the median values of DDI (3.50 vs. 2.81) and CDI (70.33 vs. 53.00) of the mNGS Group in the subgroup of bacterial infections were lower. However, the application of mNGS didn't bring to the consistent decrease of DDI (viral-infection subgroup: 1.54 vs.1.50; fungal-infection subgroup: 1.78 vs. 2.37) and CDI (viral-infection subgroup: 21.19 vs. 26.83; fungal-infection subgroup: 36.60 vs. 47.00) in both viral-infection and fungal-infection subgroups (Fig. 6A, D).

Considering the confounding factor of co-infection and the differences between individuals, we believe that the DDI value of patients without co-infection could better reflect the advantage of mNGS in medication guidance since the length of hospitalization was excluded as an interference factor, too. In this regard, we regrouped patients into "co-infection" and "non-co-infection" groups, and the differences between the TraE Group and mNGS Group were analyzed (Fig. 7). We observed that the average of DDI value of the mNGS Group was lower than that of the TraE Group, which counted 2.25 and 2.72, respectively. Additionally, we excluded people with co-infection in different pathogenic subgroups and compared the DDI value with or without mNGS. Although the DDI value showed a decrease with the performance of the mNGS test in viral and bacterial infection subgroups, it didn't make a significant difference among fungal infections.

The positive impact of mNGS on patient prognosis

In addition to the intensity of medication, we observed that in the mNGS Group, the LOH of patients was shorter, which showed a decrease of 4.7% in the mNGS Group comparing with the TraE Group, as the average of LOH counted 23.14 (days) and 24.29 (days), respectively (Fig. 4). Among those patients with worse status on admission (MRC Grade 1), the application of mNGS brought to a significant shortening of LOH, and the median of LOH was 23.50 (days) and 40.00 (days) in the mNGS Group and TraE Group, respectively (Fig. 5B).

Furthermore, mRS was evaluated to reflect the patient’s nervous system function and self-care after discharge. According to the scores, patients were classified into three groups, of which Level 1 showed the best prognosis while Level 3 represented the worst. According to the analysis results, we could

conclude that the ratio of Level 3 was significantly less in the mNGS Group than that in the TraE Group (26.5% vs. 17.3%, respectively), and it might reflect that the use of mNGS could bring good to the improvement of patient's prognosis (Fig. 8).

Clinical decision making influenced by the application of mNGS

We further analyzed the impact of mNGS on doctors' clinical decision-making. We found that among 98 people in the mNGS Group, 48 (49%) patients' medication were altered, of which 15 (15.3%) patients' medication regimen was changed, and 24 (24.5%) patients experienced the drug de-escalation (the clinicians replaced high-escalation antibiotics with low-escalation antibiotics in 2 patients, while they still had an increase in DDI due to other reasons) as a result of mNGS application. The other nine people were discharged after the mNGS results feedback (Fig. 9A).

In addition, we compared the DDI at the mNGS examination day and four days after the mNGS results were reported among the mNGS Group. We came to the result that nine patients were discharged within four days after the mNGS report. Of the remaining 89 participants, 22 (24.7%) patients showed a decrease in DDI, and we further analyzed the detailed reasons for medication de-escalation: reduction of drug types (57.14%), reduction in medication dose (10.71%), drug replacement by lower-escalation ones (7.41%), and drug adjustments (25.00%) for the treatment of different pathogen types (Fig. 9B).

Discussion

Our retrospective study, which assessed the difference of the drug intensity and functional outcome of patients with CNS infection between groups performed with or without mNGS tests, identified a better prognosis and lower intensity of medication in patients who underwent mNGS tests. The improvement of medication intensity and prognosis due to the application of mNGS was significant in individuals with worse consciousness on admission or in people with CNS bacterial infection.

CNS infection is a life-threatening disease responsible for severe disability or death. CNS infections may depend on the therapeutic resources, including timely access to anti-infection therapy, appropriate antibiotic or antiviral drugs usage(5, 7, 16, 17). Several studies reported the superiority of mNGS for diagnosing pathogens in infectious diseases, comparing to conventional methods. Our data confirmed the high sensitivity and specificity of mNGS in the detection of pathogens, which were consistent with previous studies of mNGS (7, 18-20), as we found the sensitivity of the mNGS reached 91.67% compared to culture, and the specificity was 88.24% in contrast of final diagnosis. In addition, the extra-detection rate of mNGS was as high as 39.8% compared to culture. This study also revealed that mNGS was of excellent application value in the diagnosis of CNS infections.

Moreover, previous studies have confirmed the guidance role of mNGS in clinical treatment, as Hu et al. declared a considerable modification of infection diagnoses based on mNGS (21). Besides, another research showed that, among patients with suspected infection undergoing immunosuppressive

corticosteroid therapy, mNGS played a role in optimizing antibiotic regimes (22). However, these studies have failed to locate the study population on CNS infections accurately but have generally explored the role of mNGS in guiding clinicians to use drugs. Our study has uniquely evaluated the intensity of drug usage and its adjustment due to mNGS tests among patients with CNS infections. We revealed a difference in the intensity of anti-infection drugs used between participants performed with mNGS and those who did not, which confirmed that the application of mNGS could effectively reduce the medication intensity. We further evaluated the patients without co-infection and compared with the TraE Group; the CDI and DDI value of the mNGS Group still showed a noticeable reduction. As we excluded the confounding factors of co-infection, the benefit of mNGS on reducing drug intensity was further confirmed.

We also found that the application of mNGS might have a more significant impact on patients with bacterial CNS infection. As the data showed that in the bacterial and fungal infection subgroups, the CDI value of the mNGS Group was lower than that of the TraE Group, while the patients performed with mNGS tests in the viral and bacterial infection subgroups had lower DDI values. The reasons are as follows: Firstly, In the treatment of bacterial infections, the de-escalation of broad-spectrum antibiotics might bring more remarkable changes to the intensity of medication. Secondly, the clinical manifestations were typical; thus, the clinician's empirical judgments were more accurate; therefore, the intensity of medication showed less fluctuation. Thirdly, we generally did not diagnose patients as fungal infections when there was no etiological evidence. Most of them were performed with antibiotics empirically. The clinician might change the medication to fungal drugs after obtaining the mNGS reports; therefore, the intensity of the drug tended to increase.

Moreover, the people with the worst (Grade 3) and mildest (Grade1) neurological function seemed to be more likely to benefit from the mNGS examination, that was, reducing the length of hospitalization and drug use. It was possibly due to the reduction of medication and unnecessary hospitalizations based on the mNGS reports among patients with the mildest symptoms and patients in severe condition acquired optimized and targeted medications regimes, which speeded up the recovery of patients and reduced unnecessary medications. Therefore, we could draw to a conclusion that, for critical patients with CNS infection, the application of mNGS may be more valuable and instructive in diagnosis and treatment.

In total, we reported 49% of altered medication and 24.7% of decreased drug usage, reflecting the potential value of mNGS in guiding the clinical medication plan and benefiting the precise use of antibiotics. We further analyzed the reasons for decreased drug intensity, and the reduction of drugs counted for the most, counting 57.14%. It can be easily explained that the patients might be treated with multiple drugs simultaneously on admission due to the unclear etiology diagnosis. However, later, the clinician could identify the target pathogen with mNGS tests and exclude previously suspected pathogen, therefore reduced the types of drugs. Moreover, there were other reasons for the decrease of drug intensity such as drug adjustment, drug downgrade, and reduction of drug dose, since, with the report of mNGS, clinicians might find the pathogenic type targeted by the empirical medication was different from the actual type of infection, then the type of medication was changed. Furthermore, narrow-spectrum anti-

infection drugs might be used precisely according to the target pathogen reported by mNGS. The previous study of Filmarray meningitis/encephalitis Panel has shown that it could, to some extent, improve the antibiotic regimens on CNS infection patients (10, 23, 24); however, the research focused on the bacterial meningitis patients, without an overall population of CNS infected patients restricted by the limited scope of its pathogen examination and the study design itself.

We used the mRS to assess the patient's self-care ability and neurological impairment at the time of discharge. We found that the patients' probability of being included in the poor prognosis (Level 3) would decrease from 26.5–17.3% result of mNGS application, showing the ability of mNGS in improving the results of patients with CNS infections. The conclusion could be reached that the application of mNGS in CNS-infected populations improved patients' prognosis and optimized the drug intensity of patients during hospitalization. The previous study reported a better improvement rate among patients who adjusted medication according to mNGS than those performed with medication empirically (22). We speculate that the mNGS' rapid, accurate, and broad pathogen detection characteristics precisely identified pathogens types, thus accelerating the workup and treatment. Besides, CNS infection is a severe and dangerous infectious disease, demanding timely therapy, while CSF culture has difficulty giving timely feedback due to its low positive rate and time-consuming characteristics. Therefore, the application of the mNGS test would promote the implementation of the optimal treatment, thus leading to rapid remission of the disease and a better prognosis.

Our study has several strengths, as our retrospective research analyzed a group of patients with high homogeneity of the CNS infection, ensuring that the same group of doctors made their medication plan, thus reflecting the changes in medication usage and prognosis accurately under the influence of mNGS. Our study also has limitations inherent to its retrospective design. The relatively small sample size of parasite CNS infections and unclassified ones in our study, and therefore we could not yet conclude the value of clinical medication guidance of mNGS in these groups. In addition, the results of mNGS may be interfered with by many factors, thus in the future, a larger sample size, multi-center, and prospective study are needed to verify the application value of mNGS in CNS infections.

Conclusion

This retrospective cohort study demonstrated that mNGS exhibited superiority over traditional culture and could better detect CNS infections. Additionally, mNGS could shorten hospital stays and improve patient outcomes, especially for severe patients and bacterial infection. Besides, the application of mNGS significantly reduced the daily drug intensity used on CNS infection patients with better consciousness on admission. Therefore, for clinical decision-making, mNGS might be applied to assist in rational and precise drug use, reducing abuse of antibiotics and thus, preventing antibiotic resistance.

Abbreviations

CDI: cumulative drug intensity

CMV: Cytomegalovirus

CNS: central nervous system

CSF: cerebrospinal fluid

DDD: Defined Daily Dose

DDI: daily drug intensity

EBV: Epstein-Barr virus

GCS: Glasgow Coma Scale

HSV-1: Herpes simplex virus 1

HSV 6A: Herpes simplex virus 6A

LOH: length of hospitalization

mNGS: metagenomic next-generation sequencing

MRC: Medical Research Council

mRS: modified Ranking Scales

VZV: varicella-zoster virus

Declarations

Ethics approval and consent to participate

The study was approved by the Huashan Hospital ethical committee the methods were carried out in accordance with the approved guidelines of the institution.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

K.L and H.C.Z conceived this research and wrote the paper, K.L, HCZ and Yi Zhang collected and analyzed the clinical data; Yang Zhou provided technical support of mNGS; Z.F.F, H.Y.W, Y.H.Z, M.X.F and J.Y.S were sub-investigators in this research, performed data validation and cleanup for this study. J.W.A, Q.C and W.H.Z supervised data collection and analysis, and paper draft. All authors read and approved the final manuscript.

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Tables

Table 1: Demographical characteristics of enrolled patients.

Characteristic	TraE Group	mNGS Group	p value
Age			
Median (IQR) – yr	46 (31-57.5)	44 (29-57)	0.505
Distribution – no. (%)			0.911
13-18 yr	5 (4.4)	6 (6.1)	
19-25 yr	13 (11.5)	11 (11.2)	
26-40 yr	30 (26.6)	23 (23.5)	
41-60 yr	41 (36.3)	40 (40.8)	
>60 yr	24 (21.2)	18 (18.4)	
Male sex – no. (%)	68 (60.2)	66 (67.3)	0.281
Syndrome – no. (%)			0.132
Meningitis only	67 (59.3)	46 (46.9)	
Encephalitis only	22 (19.5)	28 (25.6)	
Meningitis with encephalitis	17 (15.0)	21 (21.4)	
Unclassified	7 (6.2)	3 (3.1)	
CNS infection – no. (%)			0.089
Bacterial infection	51 (45.1)	44 (44.9)	
Viral infection	36 (31.9)	37 (37.7)	
Fungal infection	14 (12.4)	13 (13.3)	
Parasitic infection	1 (0.9)	3¶ (3.1)	
Unclassified	11 (9.7)	1 (1.0)	
Immunosuppression – no. (%)	21 (18.6)	10 (10.2)	0.086
Glasgow coma score+ – no. (%)			0.103
Grade 1	11 (9.7)	18 (18.4)	
Grade 2	16 (14.2)	8 (8.2)	
Grade 3	86 (76.1)	72 (73.4)	
Body temperature – median (IQR), °C	37.0 (36.7-37.6)	37.0 (36.5-38.0)	0.819
Blood laboratory examination – median (IQR)			

WBC, *10^9/L	7.62 (5.72-10.22)	7.33 (5.79-9.47)	0.743
Neutrophil, %	74.1 (62.8-80.8)	72.2 (64.3-79.3)	0.937
C-reaction protein, mg/L	6.6 (3.0-20.1)	5.4 (3.0-25.2)	0.851
Procalcitonin, ng/mL	0.06 (0.04-0.12)	0.06 (0.04-0.11)	0.721
CSF laboratory examination – median (IQR)			
WBC, *10^6/L	75.0 (9.0-160.5)	100.0 (23.0-250.0	0.104
Protein, mg/L	1287.0 (743.5-2310.5)	1359.0 (730.0-2425.0)	0.617
Multinuclear cell, %	20.0 (10.0-32.8)	16.0 (10.0-35.0)	0.638
Chlorides in CSF, mmol/L	116 (110-119)	116 (107-120)	0.988
Glucose in CSF, mmol/L	2.4 (1.9-2.8)	2.30 (1.80-2.83)	0.796

IQR, interquartile range. **TraE Group**, traditional examination group. **mNGS Group**, metagenomic next-generation sequencing group. **WBC**, white blood cell. **CSF**, cerebrospinal fluid.

Figures

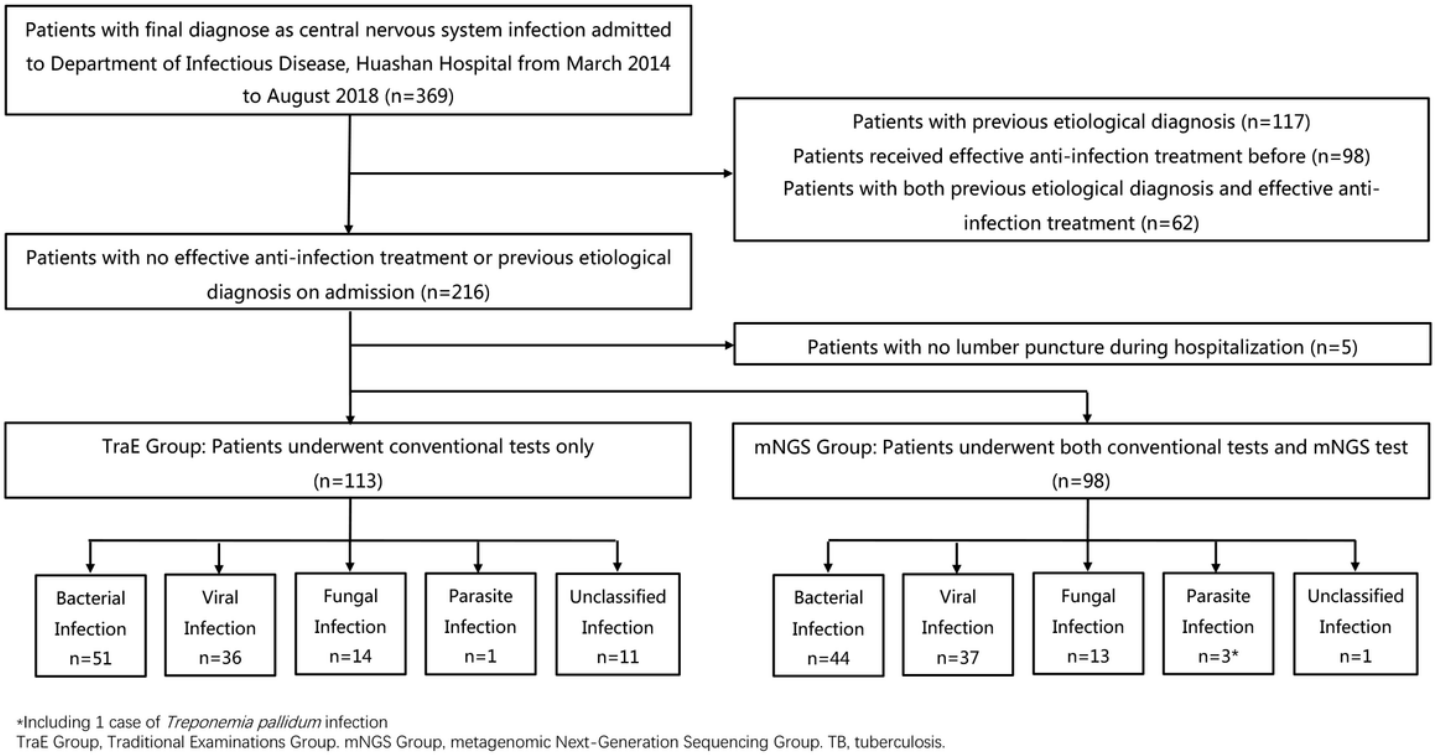

Figure 1

Flowchart of enrollment and classification

Methods	Sensitivity compared to culture	Concordance rate compared with final diagnosis	Additional detection rate
mNGS	91.67% (11/12)	88.24% (45/51)	39.80% (39/98)
Culture	—	92.31% (12/13)	—

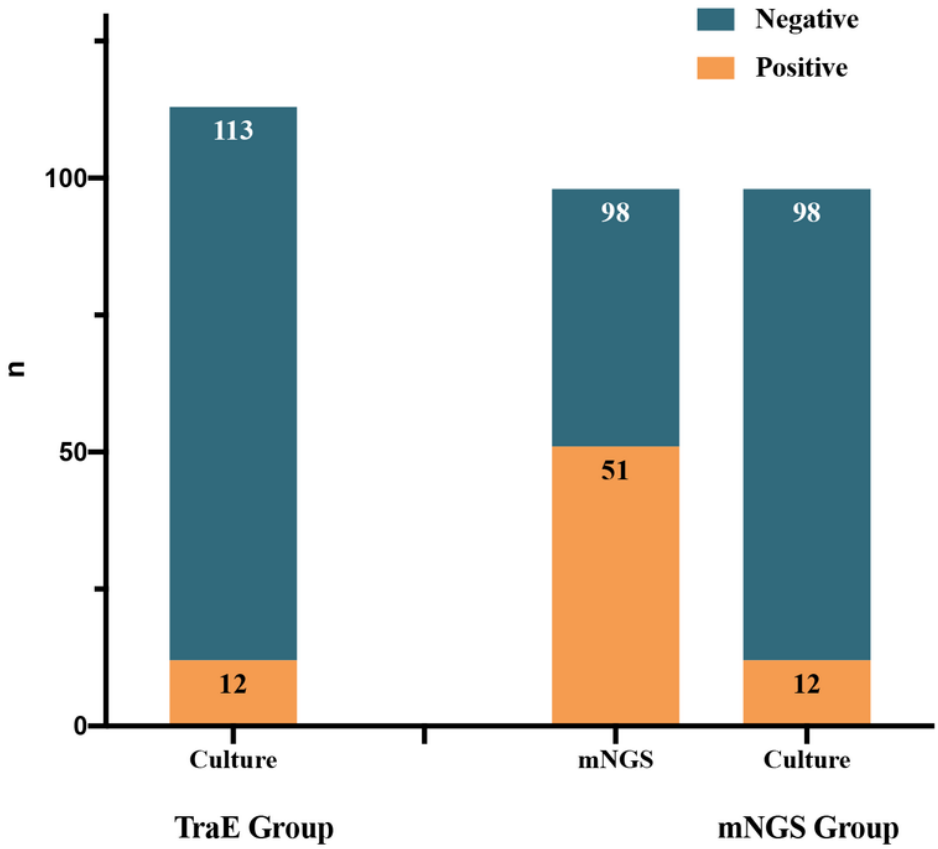

Figure 2

Diagnostic performance of mNGS. The sensitivity and additional detection rate of mNGS compared to culture and specificity compared to clinical diagnosis. TraE Group, traditional examination group. mNGS Group, metagenomic nextgeneration sequencing group.

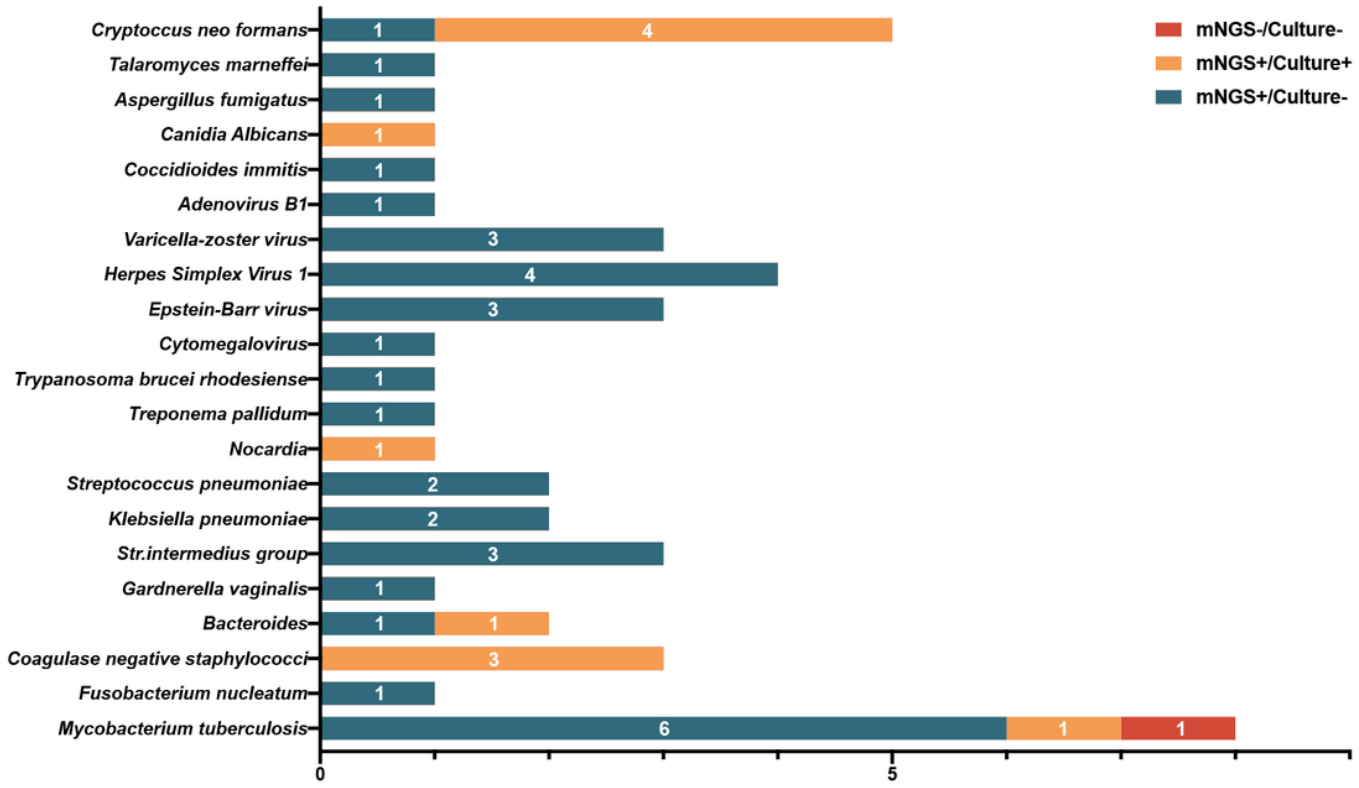

Figure 3

Distribution of detected pathogen by mNGS and culture.

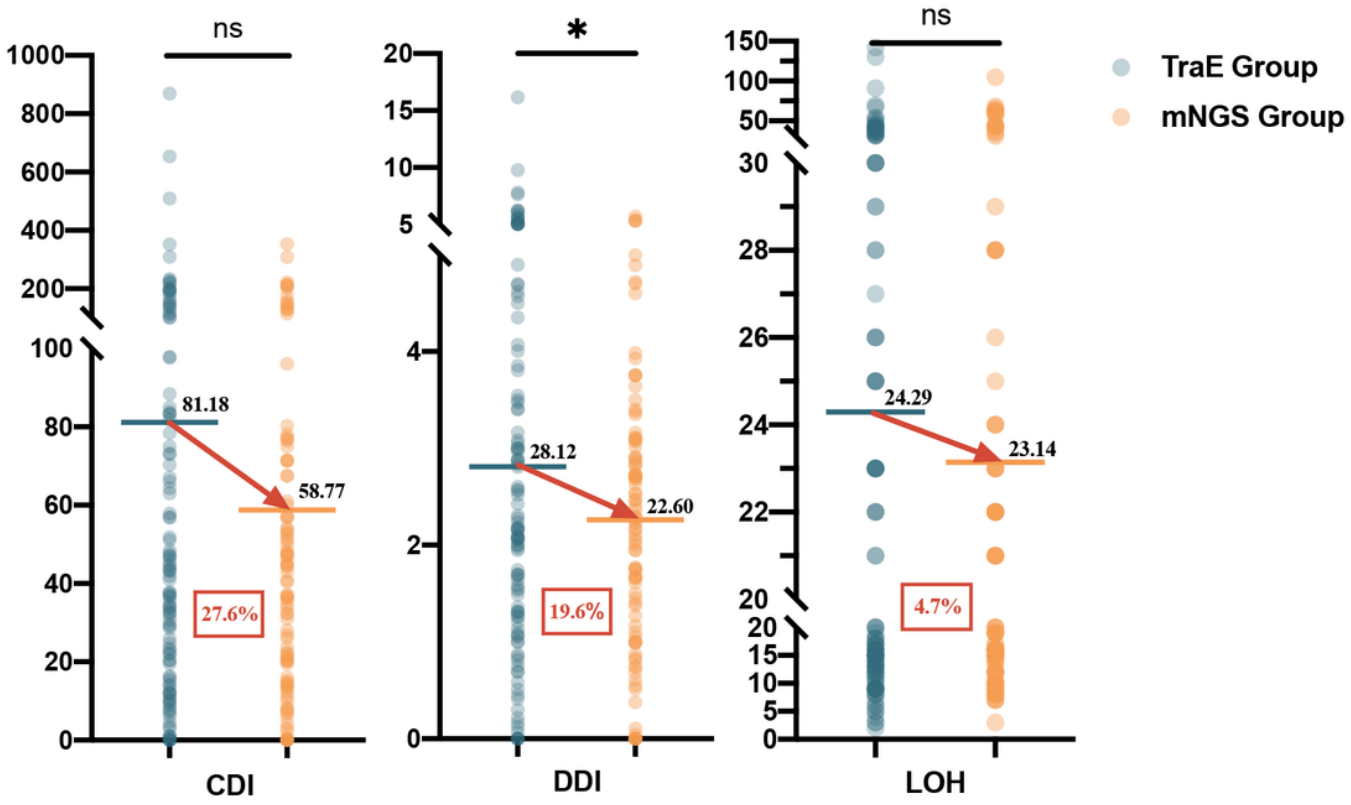

Figure 4

Comparison between TraE Group and mNGS Group in average of CDI, DDI and LOH. The number in the red box showed the decline percentage between the two groups. TraE Group, traditional examination group. mNGS Group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

MRC Grade	Groups	CDI (IQR)	DDI (IQR)	LOH (Day, IQR)
Grade 1	TraE Group	73.25 (20.50-227.88)	4.57 (1.74-5.13)	40.00 (14.00-42.00)
	mNGS Group	48.06 (35.67-160.48)	2.24 (1.49-3.51)	23.50 (19.25-43.25)
Grade 2	TraE Group	47.25 (30.98-138.32)	3.45 (2.10-5.95)	17.50 (9.00-28.75)
	mNGS Group	47.21 (9.50-134.05)	2.09 (1.00-4.86)	21.50 (14.75-39.75)
Grade 3	TraE Group	37.50 (13.70-83.27)	2.08 (1.04-3.08)	18.50 (12.75-28.25)
	mNGS Group	36.70 (14.81-57.00)	2.28 (1.14-2.88)	16.00 (12.00-22.00)

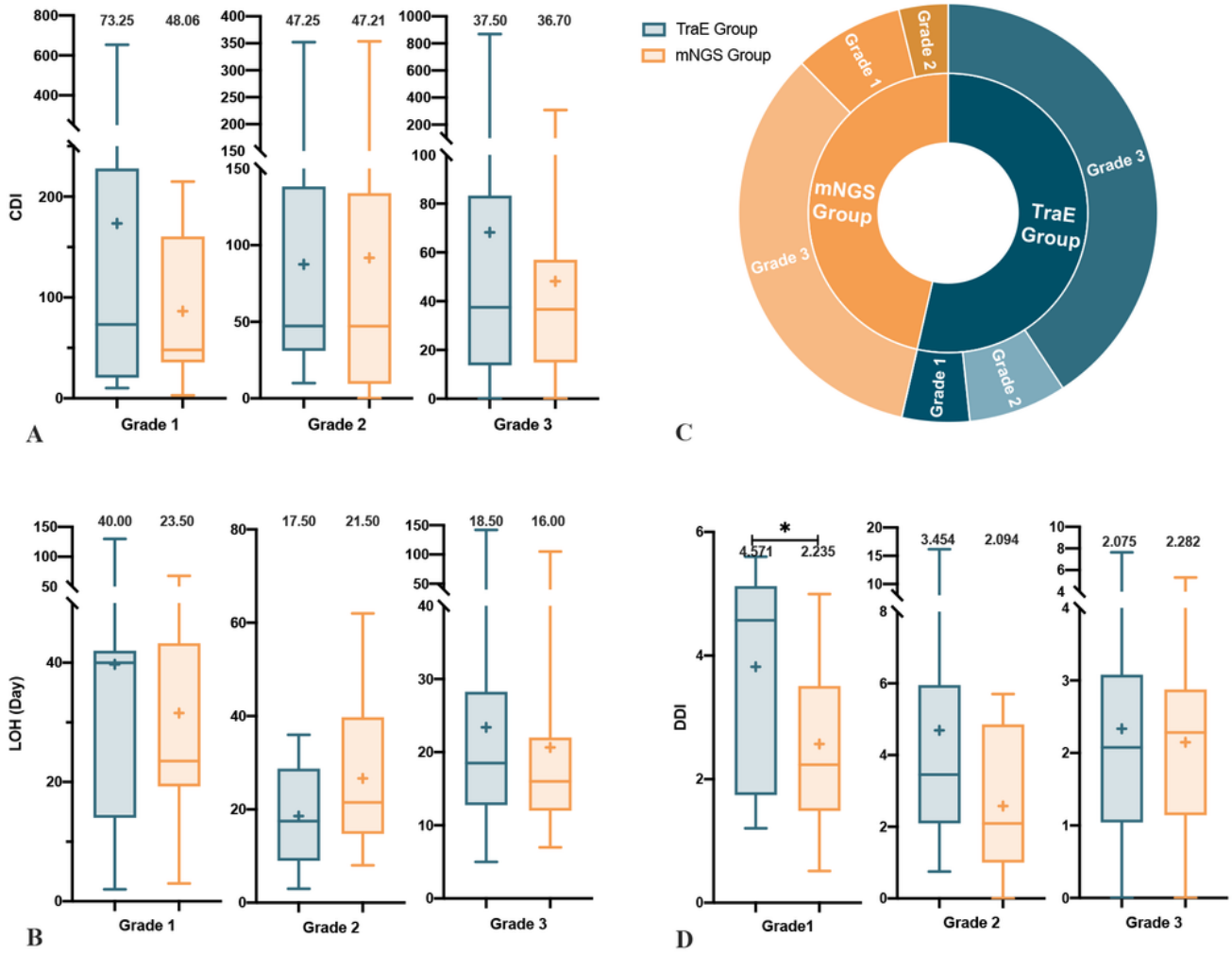

Figure 5

Intensity of drug use and length of hospital stay in patients with different GCS at admission. (A) Comparison of the median CDI between TraE Group and the mNGS Group in Grade1, 2, and 3. (B) Comparison of the median LOH between TraE Group and the mNGS Group in Grade1, 2, and 3. (C) The composition of Grade 1, 2, and 3 in the TraE Group and the mNGS Group respectively. (D) Comparison of the median DDI between TraE Group and the mNGS Group in Grade1, 2, and 3. Medical Research Council (MRC) grade 1 indicates a Glasgow coma score of 15 (on a scale of 3 to 15, with lower scores indicating reduced levels of consciousness) with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less. TraE Group, traditional examination group. mNGS Group, metagenomic nextgeneration sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

CNS Infection	Groups	CDI (IQR)	DDI (IQR)	LOH (Day, IQR)
Viral Infection	TraE Group	21.19 (7.15-40.13)	1.54 (0.61-2.46)	13.00 (9.00-16.00)
	mNGS Group	26.83 (8.13-47.90)	1.50 (0.78-2.50)	16.00 (12.00-22.00)
Bacterial Infection	TraE Group	70.33 (32.67-155.17)	3.50 (2.00-5.08)	23.00 (16.00-36.00)
	mNGS Group	53.00 (24.83-105.10)	2.81 (2.02-3.58)	21.00 (12.50-32.50)
Fungal Infection	TraE Group	36.60 (23.13-97.68)	1.78 (1.26-2.58)	30.50 (16.50-47.00)
	mNGS Group	47.00 (28.70-104.83)	2.37 (1.08-2.80)	21.00 (13.00-58.00)

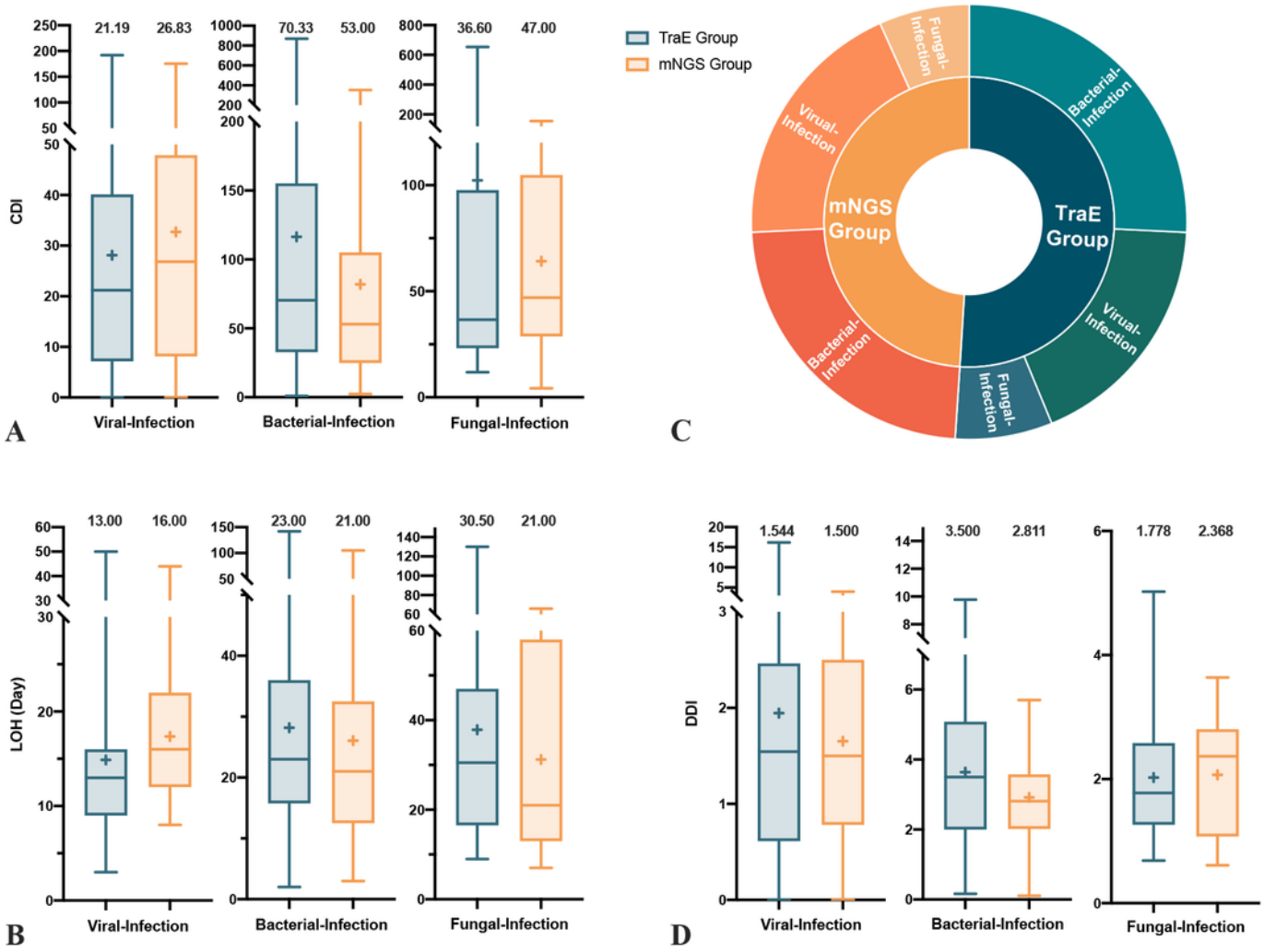

Figure 6

Intensity of drug use and length of hospital stay in patients with different pathogens infections. (A) Comparison of the median CDI between TraE Group and the mNGS Group in viral-, bacterial- and fungal-infection. (B) Comparison of the median LOH between TraE Group and the mNGS Group in in viral-, bacterial- and fungal-infection. (C) The composition of different kind of infections in the TraE Group and the mNGS Group respectively. (D) Comparison of the median DDI between TraE Group and the mNGS

Group in viral-, bacterial- and fungal-infection. TraE Group, traditional examination group. mNGS Group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

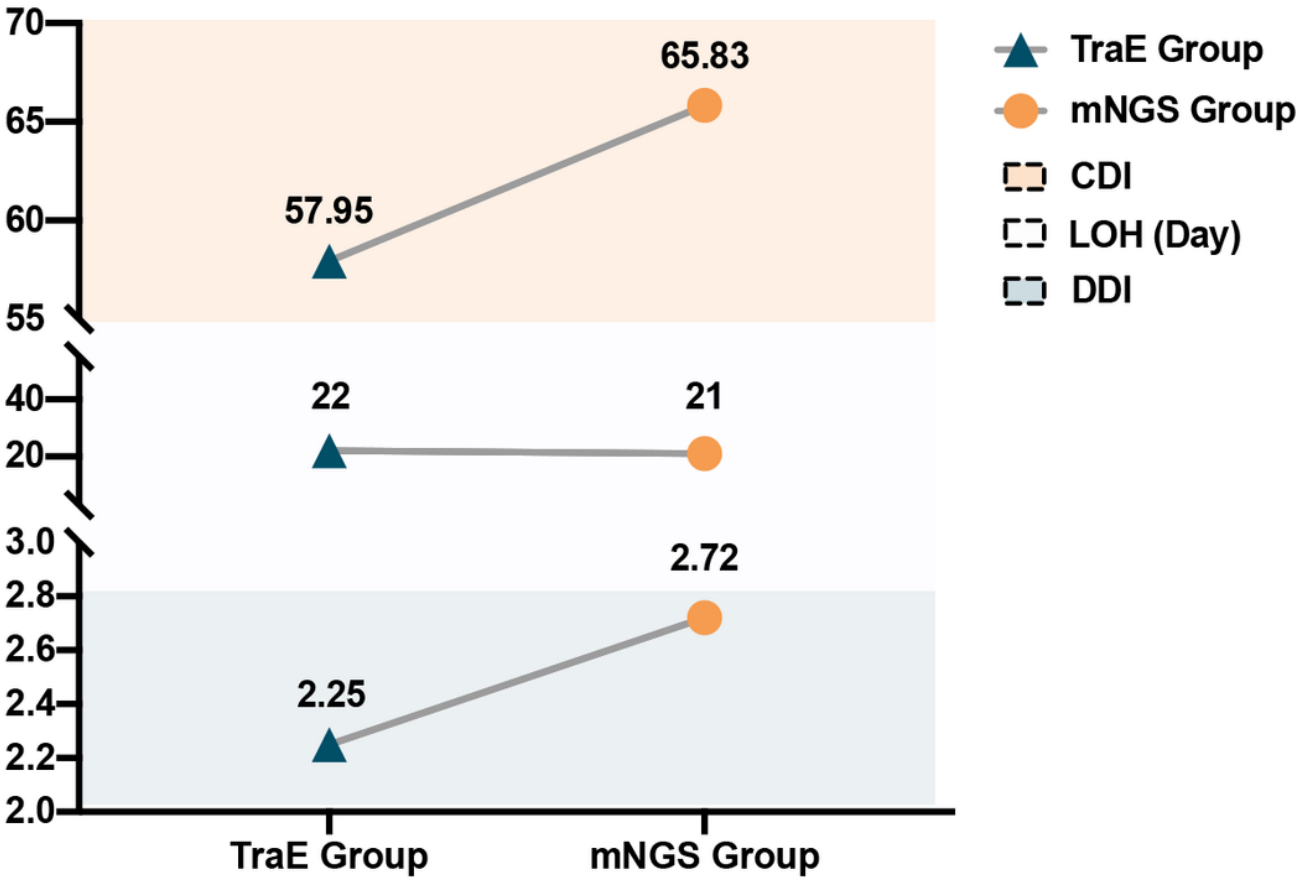

Figure 7

Comparison between TraE Group and mNGS Group of non-co-infection patients in average of CDI, DDI,and LOH. Different background colors represented different comparison content. TraE Group, traditional examination group. mNGS Group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

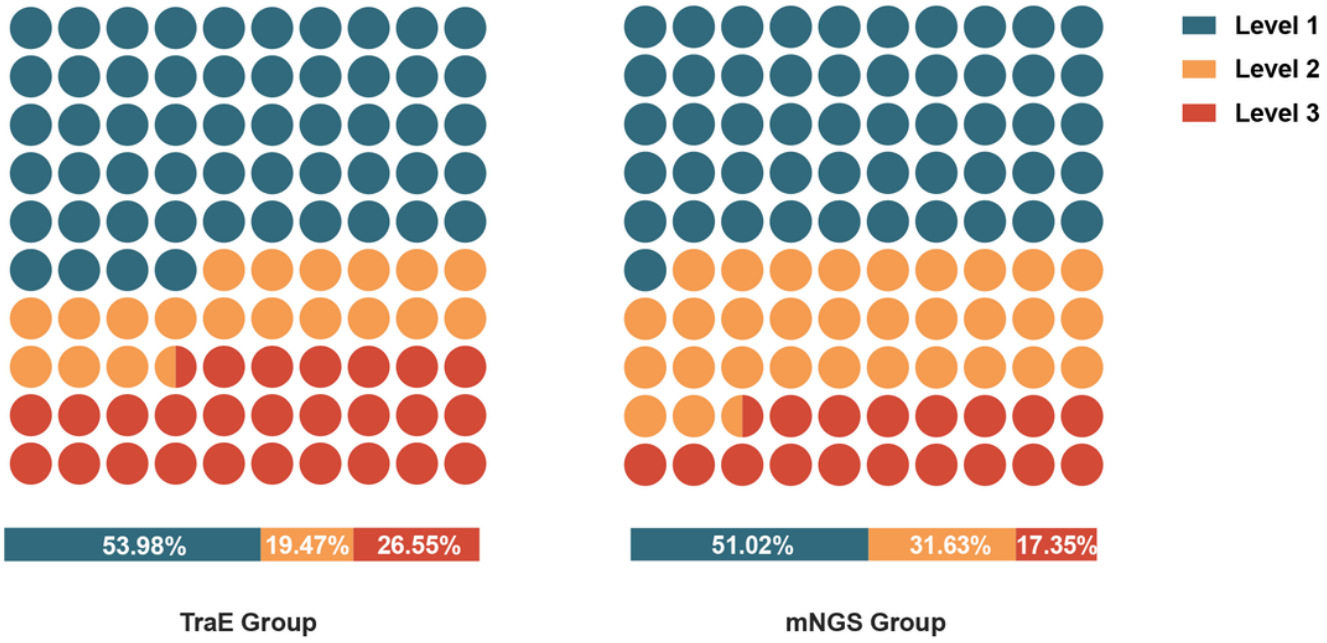

Figure 8

mRS Grades of patients in the TraE Group and the mNGS Group at discharge. Patients were divided into different levels according to their mRS Grades at discharge. We compared the composition of Level 1, 2 and 3 patients in the TraE Group and mNGS Group, and the percentages were listed above. TraE Group, traditional examination group. mNGS Group, metagenomic next-generation sequencing group. mRS, modified Rankin Scale. Level 1: Good outcome. Level 2: Intermediate outcome. Level 3: Poor outcome.

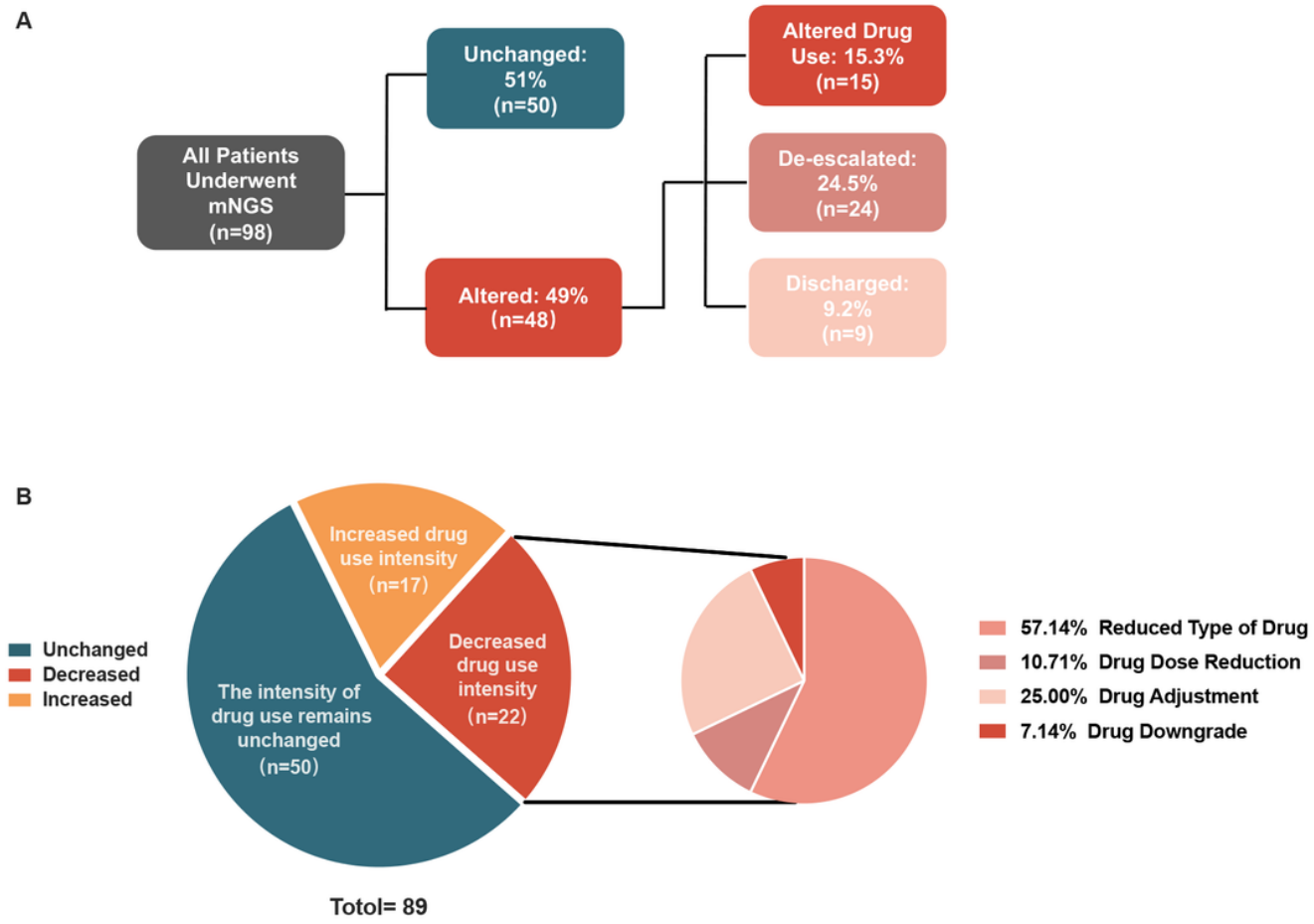

Figure 9

Changes of drug use intensity in the mNGS Group. (A) The number of patients who altered the medication regimens according to the mNGS results were listed and the percentages were calculated. We listed the specific types of regimens alterations. (B) The specific changes of medication decisions were listed. We further calculated the composition ratio of various detailed reasons for drug-use-intensity reduction. *Among the patients with decreased drug use intensity, 1 patient took both less dose and fewer types of drug and the medication was also adjusted. Another patient took both downgraded and fewer types of drug and the medication was also adjusted. 2 patients experienced reduction of drug types as well as the adjustment of medication.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 5.SupplementaryinformationNGSEN1015.docx