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Application of metagenomic next-generation sequencing in treatment guidance for deep neck space abscess

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Abstract

Background Infectious etiologies of deep neck space abscess (DNSA) by conventional culture tests can be challenging, which also leads to frequent irrational antibiotic usage. Metagenomic next-generation sequencing (mNGS), as a novel method for analyzing the complex microbial ecosystem from clinical samples, has been utilized in clinical research and practice of various infectious diseases but deep neck space abscess. We here aimed to explore the clinical value of mNGS for pathogen detection and treatment guidance in DNSA patients compared with conventional culture tests.

Methods One hundred six patients diagnosed with DNSA were retrospectively enrolled and allocated into mNGS group and culture group according to whether mNGS was conducted. The pathogen detection effectiveness was of mNGS was compared with conventional culture. Effectiveness of mNGS-modified antimicrobial therapy was evaluated by comparing the treatment outcomes between two groups.

Results mNGS showed a significantly higher detection rate than conventional culture ($p < 0.05$) with faster result acquisition. Treatment success rate of patients in the mNGS group was significantly higher than in the culture group (RR: 1.22, 95%CI: 1.07–1.82, $p = 0.033$). Besides, patients in the mNGS group had shorter duration of irrational antimicrobial therapy, shorter hospital stay and less medical costs ($p < 0.05$).

Conclusions mNGS is an effective technology for facilitating pathogen detection and improving treatment outcomes of DNSA patients.

Keywords Deep neck space abscess, Metagenomic next-generation sequencing, Microbiological tests, Pathogen detection, Antibiotic administration

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Introduction

A deep neck space abscess (DNSA) arises from the breakdown of cervical fascia due to bacterial invasion and the subsequent inflammatory response. This condition can lead to life-threatening complications like septic shock, thrombophlebitis, and descending necrotizing mediastinitis [1]. Management of DNSA primarily involves drainage procedures, supportive care, and antibiotic therapy [2, 3]. In recent years, advancements in treatment modalities have contributed to reducing complication rates and mortality. However, DNSA is still a global public health burden because of its complexity and high-cost [4, 5].

Presently, in the treatment of DNSA, microbiological results are predominantly obtained through conventional pus cultures, which suffer from drawbacks such as time consumption and low positivity rates [3]. Consequently, empirical antimicrobial therapy remains the primary approach in the initial and sometimes the entire course of DNSA treatment. A previous study has reported that the majority of microorganisms in DNSA were *Streptococcus pyogenes* (*S. pyogenes*) and *Streptococcus aureus* (*S. aureus*), and co-infection of anaerobic bacteria was frequently found [6]. Several other studies have recommended different empirical antimicrobial therapy based on local epidemiology and disease severity [6, 7]. However, these recommendations lack validation from prospective studies, thereby limiting their effectiveness and generalizability. In this situation, antimicrobial therapy for patients with DNSA remains heterogenic and sometimes irrational.

As a comprehensive tool for analyzing microbial material in samples derived from patients, metagenomic next-generation sequencing (mNGS) has the capacity to detect all potential pathogens in one sample [8]. Therefore, it helps greatly to promote the research of microbiome and has great potential clinical utility in the diagnosis and treatment of infectious diseases [9–11]. Previous studies have demonstrated excellent performance of mNGS in guiding diagnosis and treatment of various infectious diseases like pneumonia, central nervous system infection and sepsis [12–14]. In the field of DNSA, although our previous study had preliminarily reported the pathogen detection performance of mNGS, no further analysis had been carried to compare the performance of mNGS and conventional culture on pathogen detection and treatment guidance [7].

Based on the situation mentioned above, this study was carried out to inspect the clinical value of mNGS in rationalizing antimicrobial therapy and improving treatment compared with conventional culture tests, with the ultimate goal of enhancing prognosis and reducing the economic burden for patients afflicted with DNSA.

Methods

Study design and data collection

This was a retrospective cohort study conducted in First Affiliated Hospital and Sixth Affiliated Hospital of Sun Yat-sen University. It was approved by Ethics Committee for Research and Publication of First Affiliated Hospital and Sixth Affiliated Hospital of Sun Yat-sen University (NO. [2023]445–1). The study design was summarized in Fig. 1. A total of 124 deep neck space abscess adult patients who met the diagnosis criteria of DNSA according to the International Classification of Diseases, Tenth Revision, Clinical Modifications (ICD-10-CM) diagnosis codes J39.002, J39.004, and L02.051 between January 2022 and August 2023 were evaluated for inclusion. Exclusion criteria included: (1) No mNGS nor culture results. (2) Quit treatment. (3) Combined with malignant tumors. (4) Incomplete medical records. After exclusion, 106 patients were enrolled into the study.

All the patients received appropriate drainage operation including ultrasound-guided aspiration or surgical incision according to the size and distribution of abscess area. Pus sample were collected during drainage operation in aseptic conditions and then transported to relevant labs for culture and mNGS tests within 2 h.

Outcome

To evaluate the effect of mNGS guided antibiotic administration on clinical outcome, patients underwent both mNGS and conventional culture were allocated into the mNGS group, while patients who only received conventional culture tests were assigned to the culture group.

The treatment outcomes of the study were classified into 3 categories: 1) treatment success, which was defined as remission of clinical symptoms, restoration of inflammatory markers, complete remission of abscess by imaging and negative microbiological results after 14 days follow-up. 2) Stable condition, defined by the improvement of clinical symptoms, absence of severe complications such as sepsis and partial remission of abscess by imaging. 3) Treatment failure, defined as progression of clinical symptoms, development of severe complications or expansion of abscess area after treatment.

Other outcomes included duration of irrational empirical antimicrobial therapy, length of hospital and ICU stay and medical costs. The duration of irrational empirical antimicrobial therapy was defined as time from empirical therapy initiation to change of rational antimicrobial therapy based on the following reasons: 1) Adjustment according to mNGS or culture results; 2) Modification of therapy due to poor response to previous empirical therapy, including patients with negative mNGS or culture results. Notably, Patients receiving constant empirical antimicrobial therapy according to standard

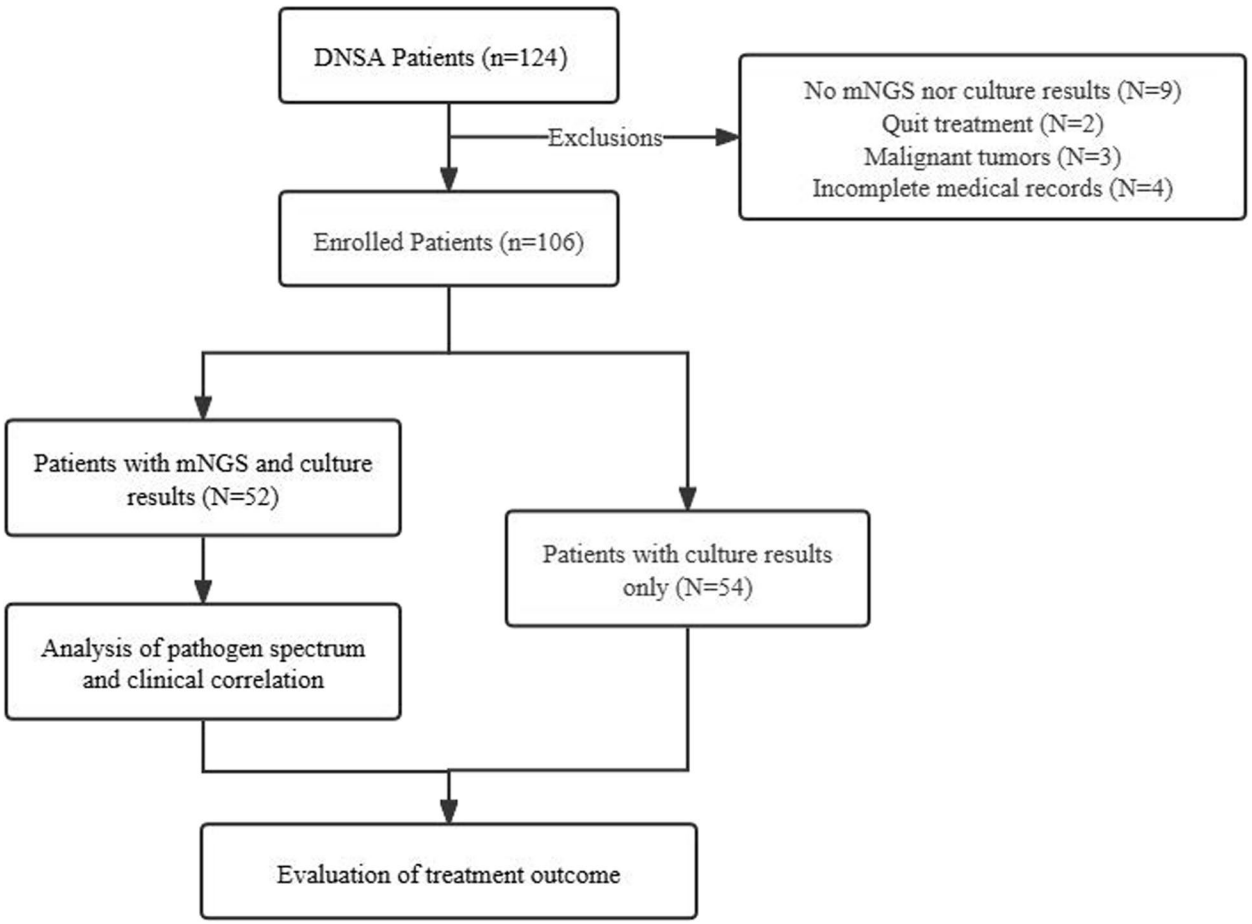

Fig. 1 Diagram of patient enrollment and study design

antimicrobial de-escalation and had a good outcome were considered to be effective, rather than rational or irrational, and therefore not included for analysis. Moreover, the changing trends of inflammatory markers and organ function indicators between the two groups were recorded and analyzed. Treatment outcomes were independently evaluated by two senior otorhinolaryngologists for reducing subjective bias.

Conventional culture tests

Pus specimens were aseptically collected during aspiration or surgery. Specimens were then inoculated onto Columbia blood agar plates (Qingdao Haibo Biotechnology Co., Ltd., China), chocolate agar (Qingdao Haibo Biotechnology Co., Ltd., China) and MacConkey agar plates (Qingdao Haibo Biotechnology Co., Ltd., China) and cultured at 35 °C in 5% CO2 for at least 48 h. Blank control plates were also inoculated in the same condition to eliminate potential contamination. Plates were examined daily for bacterial growth. Colony morphology, size, color, and hemolytic patterns were recorded. Single colonies were subsequently cultured onto fresh media for purification and further identification. For culture-positive specimens, antimicrobial susceptibility testing was further performed to guide the formulation of appropriate antibiotic treatment regimens. Anaerobic culture was not routinely conducted due to low positive rate and lengthy time consumption.

DNA extraction, library preparation, and sequencing

DNA of each sample was extracted with Microbiome DNA Kit (Qiagen, Germany) following the manufacturer's instructions. The selected DNA was constructed to library with Nextera XT DNA Library Prep Kit (Illumina, USA) [15]. Library was quality controlled by Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, USA) and High Sensitivity DNA kit (Agilent, China) on an Agilent 2100 Bioanalyzer (Agilent, China). Library pools were then loaded onto an Illumina Nextseq 550 sequencer (Illumina, USA) for 75 cycles of single-end sequencing of 20 million reads for each library using shotgun metagenomic sequencing approach. This method involves the

random fragmentation and sequencing of all DNA in the sample, and therefore allows for the detection of all potential pathogens without targeting specific genomic regions. A whole blood sample from healthy donors prepared alongside each batch using this same protocol as negative control for eliminating potential contamination [16].

mNGS analysis

During the bioinformatics analysis process, Trimmomatic was used to remove low quality reads, and human sequence data were identified by mapping to a human reference (hg19) using Burrows-Wheeler Aligner software and excluded [17, 18]. The remaining sequence data were aligned to the current bacterial, virus, fungal, and protozoan databases (NCBI; ftp://ftp.ncbi. nlm.nih.gov/genome) using Kraken2 [19]. Sequencing parameters including mapping read number, relative abundance and coverage rate were used to interpretate pathogen data and present sequencing results.

Interpretation of mNGS results

Although there are now some consensuses regarding to interpretate mNGS results in respiratory tract infection, no relative studies or guideline have been reported about DNSA. Previous studies have developed different criteria to define whether a microbe detected by mNGS is a causative pathogen [20]. In our study, a microbe was defined as a causative pathogen and used for guide antimicrobial therapy if the mNGS results were concordant with culture results, or in cases that mNGS results inconcordant with culture results, met one of the followings: 1) The pathogens were clearly reported to be associated with DNSA according to previous studies; 2) Pathogens with high relative abundance (> 20%). Generally, the interpretation of mNGS results were reported independently by two senior otorhinolaryngologists.

Statistics

Descriptive and comparative analyses of the data were conducted using Mann–Whitney *U* test (for non-normal distribution continuous data) and Fisher's exact test (for categorical data). In the aspects of correlation tests, for categorical variables like clinical symptoms, we calculated the odds ratio and significance through Fisher's exact tests. For continuous variables such as laboratory test results, we computed the odds ratio by logistics regression and determined significance using Mann–Whitney *U* test. The results were then visualized and presented as heatmaps. Laboratory results on different time point between groups were compared by Friedman tests. *P*-values < 0.05 were considered to be statistically significant. All the statistical analyses were performed using R software (version 4.3.0).

Results

Demographic and baseline characteristics

There are 106 DNSA patients enrolled and the median age was 55 (IQR: 42–64 years old), of whom 72 (67.9%) were male. 17 (16.0%) patients had dyspnea on admission, and about one third of them had a history of smoking. The most common morbidity was hypertension, presenting in 33 (31.1%) patients. Enhanced computer tomography showed that the most primary region was retropharyngeal space (46, 43.4%), and more than half patients had multi-space involvement (61, 57.5%). Gas formation and descending necrotizing mediastinitis (DNM) was confirmed in 20 (18.9%) and 14 (13.2%) patients, respectively. The time from admission to mNGS results (2 days, IQR: 1–2 days) were significantly shorter than to culture results (5 days, IQR: 4–6 days), with a *p*-value < 0.05. Detailed laboratory test results on admission were summarized and presented clearly in Table 1.

Pathogen detection performance of mNGS

To investigate the diagnosis and pathogen detection performance of mNGS, the results from 54 patients who underwent both mNGS and culture tests were presented and analyzed in Fig. 2. Overall, a total of 57 microbes were detected, but only 23 (40.4%) of them were defined as putative pathogen according to the criteria mentioned above, including 10 aerobic bacteria, 9 anaerobic bacteria and 4 fungi. Other kinds of microbes like viruses and mycoplasma were not detected in our cohort. The median number of pathogens detected in the patients was 3 (IQR: 1.5–4). At the phylum level, the phylum Firmicutes was accounted for a majority of DNSA patient infections (detected in 44 of 54 patients, 81.5%), followed by bacteria of the phylum Bacteroidetes (48.1% patients), the phylum Proteobacteria (29.6% patients), the phylum Ascomycota (27.8% patients), the phylum Actinobacteria (5.6% patients) and the phylum Fusobacteria (3.7% patients). Notebly, co-infections involving different bacterial phyla frequently occured in DNSA patients. The most frequent pathogen was *Parvimonas micra* (*P. micra*), followed by *Prevotella* spp., including *Prevotella intermedia* (*P. intermedia*) and *Prevotella oris* (*P. oris*), which were all anaerobic bacteria. Among aerobic bacteria, *Streptococcus constellatus* (*S. constellatus*), *Streptococcus angiosus* (*S. angiosus*) and *Klebsilla pneumoniae* (*K. pneumoniae*) were the primary reason for infection (Fig. 2a). Culture methods was found to had a faint advantage in detecting *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*) while mNGS was absolutely more efficient in detecting anaerobic pathogens.

Table 1 Baseline characteristics of enrolled patients

Characteristics	*N* = 106
Sex, male, n (%)	72 (67.9)
Age, median (IQR), years	55 (42, 64)
Medical history	
Dyspnea, n (%)	17 (16.0)
Temperature, median (IQR), ℃	36.6 (36.4, 36.8)
Heart rate, median (IQR), bpm	85.0 (76.0, 98.0)
Diabetes, n (%)	27 (25.5)
Hypertension, n (%)	33 (31.1)
Cardiovascular diseases, n (%)	5 (4.7)
Chronic pulmonary diseases, n (%)	11 (10.4)
Smoking, n (%)	33 (31.1)
Alcoholism, n (%)	29 (27.4)
Imaging results	
Primary region	
Suprahyoid, n (%)	25 (23.6)
Infrahyoid, n (%)	35 (33.0)
Retropharyngeal, n (%)	46 (43.4)
Multispace involvement, n (%)	61 (57.5)
Gas formation, n (%)	20 (18.9)
DNM, n (%)	14 (13.2)
Laboratory results	
CRP, median (IQR), mg/L	152.4 (131.2, 176.7)
WBC, median (IQR), 10^9	20.5 (17.8, 23.6)
Hemoglobin, median (IQR), g/L	136.5 (124.2, 147.5)
Platelet, median (IQR), 10^9	262.0 (207.0, 336.0)
ALT, median (IQR), mmol/L	44.0(36.0, 46.0)
AST, median (IQR), mmol/L	38.0 (31.0, 42.0)
Total protein, median (IQR), g/L	69.6 (64.1, 74.0)
Albumin, median (IQR), g/L	38.4 (34.0, 42.1)
Time from admission to results	
mNGS, median (IQR), days	2 (1, 3)
CMT, median (IQR), days	5 (4, 6)
Cost of tests	
mNGS, median (IQR), USD	428.6 (428.6, 428.6)
CMT, median (IQR), USD	69.2 (31.0, 100.6)

IQR interquartile range, *DNM* descending necrotizing mediastinitis, *CRP* C-reactive protein, *WBC* white blood cell, *ALT* Alanine aminotransferase, *AST* Aspartate aminotransferase, *mNGS* metagenomic next-generation sequencing, *CMT* conventional microbiological tests

Comparing the two methods, the positive rate of mNGS was significantly higher than culture methods (50, 92.6% vs 23, 42.6%, $p < 0.05$).

Among the 21 patients who had both mNGS and culture positive results, 14 were partial concordant, which was mainly caused by detection of co-infection of aerobic bacteria and anaerobic bacteria by mNGS (Fig. 2b). Therefore, we analyzed the co-infection feature of DNSA patients. A total of 44 (81.5%) DNSA patients had co-infection by two or more microbes. The most common type of co-infection detected by mNGS was aerobic-anaerobic co-infection (22, 40.7%), followed by anaerobic-anaerobic co-infection (17, 31.5%), aerobic-aerobic co-infection (3, 5.6%), aerobic-fungi co-infection (1, 1.9%) and anaerobic-fungi co-infection (1, 1.9%) (Fig. 2c). Culture methods was only able to detect aerobic-aerobic and aerobic-fungi co-infection, while with higher positive rate in detecting aerobic-aerobic co-infections (6, 11.8%). For the detection rate of anaerobic bacteria, conventional culture is almost powerless. To further analyze the co-infection feature of DNSA pathogens, we calculated the proportion of co-infection between each two microbes and created a clustered heatmap (Fig. 2d). The results showed that *Prevotella* spp., *P. micra* and streptococci tended to co-infect with each other, while *S. aureus, K. pneumoniae*, *Candida albicans* (*C. albicans*) and *Escherichia coli* (*E. coli*) tended to cause sole infections.

Correlation between mNGS results and clinical characteristics.

Since mNGS results provided an accurate and comprehensive understanding of pathogen spectrum of DNSA patients, we attempted to analyze the association between various pathogens and clinical characteristics. We selected 10 most common pathogenic bacteria and analyzed their correlation with clinical symptoms and laboratory test results. The results were visualized in heatmaps and presented in Fig. 3. *S. constellatus* and *K. pneumoniae* were more likely to cause dyspnea and pleural effusion in DNSA patients, and patients infected by the latter tended to suffer from diabetes and receive surgical drainage rather than ultrasound guided aspiration (Fisher's exact test, all $p < 0.05$). While those Gram-positive cocci tended to be related to gas formation and multispace involvement, no significant difference was found (Fisher's exact test, $p > 0.05$, Fig. 3a). *S. aureus* was found to be significantly associated with a high neutrophil-to-lymphocyte ratio (NLR) ($p < 0.05$), while no significant associations were observed between other bacterial species and laboratory test results (Fig. 3b).

Impact of mNGS results on treatment

To investigate whether mNGS results can guide antibiotic therapy decision and benefit patients, we divided the patients into the mNGS group and the culture group. The mNGS group included patients who underwent both mNGS and conventional culture testing, while patients who only received conventional culture tests were assigned to the culture group. Overall, 30 (57.7%) patients in the mNGS group received adjusted antibiotic therapy within 2 days after admission, while other

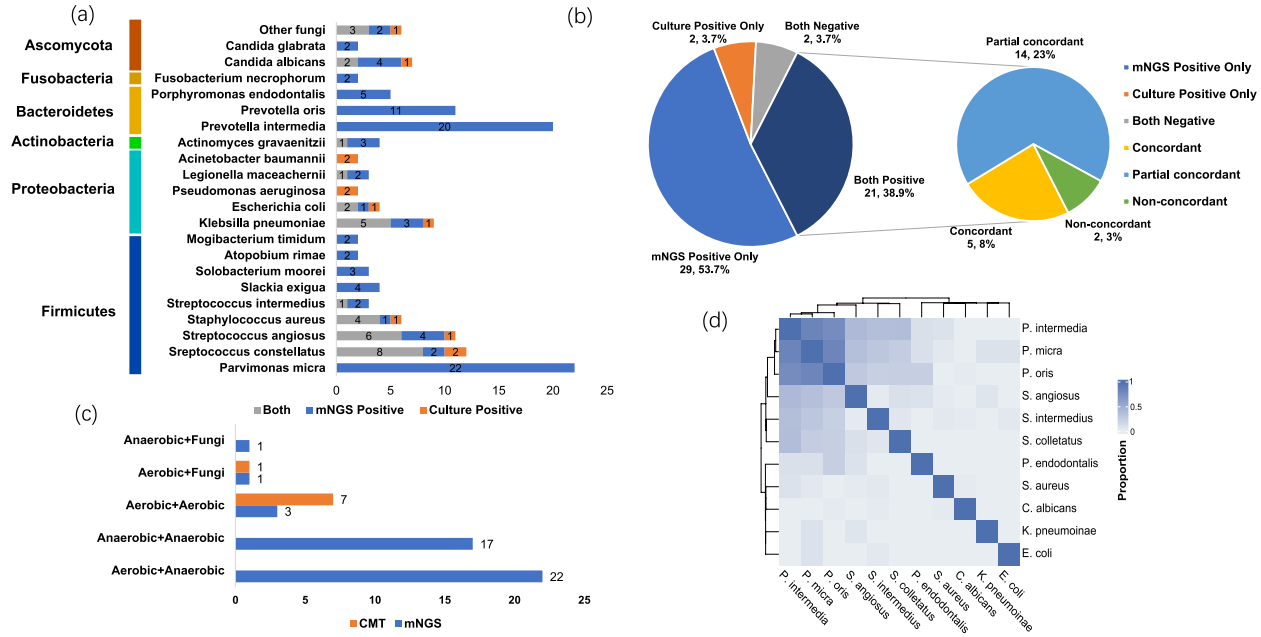

Fig. 2 Microbiological spectrum analysis based on mNGS and culture methods. **a** The proportion of pathogens detected. **b** Comparison of detection results. **c** Comparison of each co-infection type detected by culture and mNGS. **d** Co-infection feature between specific pathogens

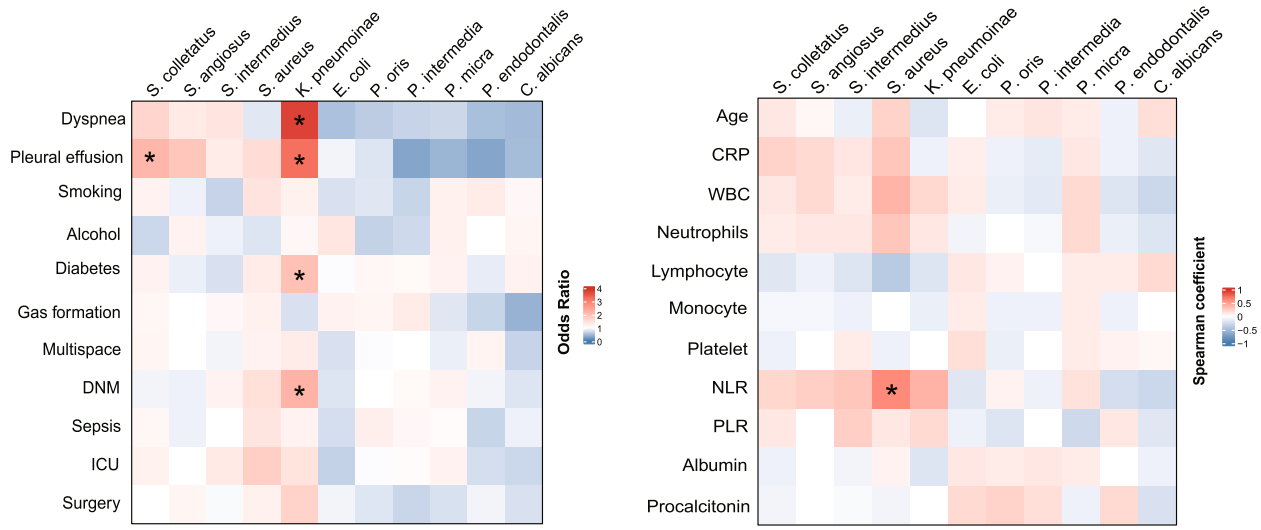

Fig. 3 Correlation analysis of curative pathogens and clinical characteristics. **a**, **b** Heatmaps showing the odds ratio between each clinical manifestation and pathogen. DNM, descending necrotizing mediastinitis; CRP, C-reactive protein; NLR, neutrophil–lymphocyte ratio; PLR, platelet-lymphocyte ratio

patients continued the original empirical antibiotic therapy as it had already covered the identified pathogens. There was no difference of the baseline characteristics between the two groups (Table 2).The duration of irrational empirical antimicrobial therapy of the mNGS group was significantly shorter than that of the culture group (2 vs 5 days, $p=0.017$). Therapy adjustment was presented in Fig. 4, and detailed information about the adjustment was shown in Supplementary Table 1.

Treatment success rate of the mNGS group was significantly higher than that of culture group (Relative risk, RR: 1.22, 95%CI: 1.07–1.82, $p=0.033$, Table 3). The hospital and ICU stay of patients in the mNGS group was shorter than that of patients in the culture group, but with no

Table 2 Comparison of baseline characteristics of patients between targeted group and empiric group

Characteristics	mNGS *N* = 52	Culture *N* = 54	*p* value
Sex, male, n (%)	35 (67.3)	37 (68.5)	1
Age, median (IQR), years	58.0 (33.0–64.0)	63.0 (44.0–67.0)	0.328
Medical history			
Dyspnea, n (%)	8 (15.4)	9 (16.7)	1
Diabetes, n (%)	12 (23.1)	15 (28.8)	0.658
Hypertension, n (%)	12 (23.1)	21 (40.4)	0.095
Smoking, n (%)	16 (30.8)	17 (31.5)	0.968
Alcoholism, n (%)	12 (23.1)	17 (31.5)	0.387
Imaging results			
Multispace involvement, n (%)	32 (61.5)	29 (53.7)	0.438
Gas formation, n (%)	10 (19.2)	10 (18.5)	1
DNM, n (%)	8 (15.4)	6 (11.1)	0.576
Operation			
Surgical drainage, n (%)	17 (32.7)	20 (34.2)	0.687
Ultrasound aspiration, n (%)	35 (67.3)	34 (65.8)	
Laboratory results			
CRP, median (IQR), mg/L	158.7 (134.8, 170.4)	159.6 (136.4, 165.2)	0.691
WBC, median (IQR), 10^9	21.6 (16.0, 26.7)	20.0 (17.3, 21.1)	0.327
NLR, median (IQR)	13.9 (12.0, 16.2)	14.0 (8.8, 18.0)	0.744
Platelet, median (IQR), 10^9	254.0 (200.0, 327.0)	221.0 (199.0, 322.0)	0.305
Glucose, median (IQR), mmol/L	6.0 (5.0, 9.1)	7.0 (6.4, 11.5)	0.232
ALT, median (IQR), IU/L	42.0 (36.0, 46.0)	40.0 (35.0, 43.0)	0.292
AST, median (IQR), IU/L	36.0 (30.0, 41.0)	36.0 (28.0, 48.0)	0.726
Total protein, median (IQR), g/L	67. 5(64.4, 73.2)	62.0 (56.2, 67.6)	0.100
Albumin, median (IQR), g/L	32.7 (32.5, 38.0)	31.2 (26.0, 35.0)	0.147

IQR interquartile range, *DNM* descending necrotizing mediastinitis, *mNGS* metagenomic next-generation sequencing, *CRP* C-reactive protein, *WBC* white blood cell, *NLR* neutrophil-to-lymphocyte ratio, *ALT* Alanine aminotransferase, *AST* Aspartate aminotransferase

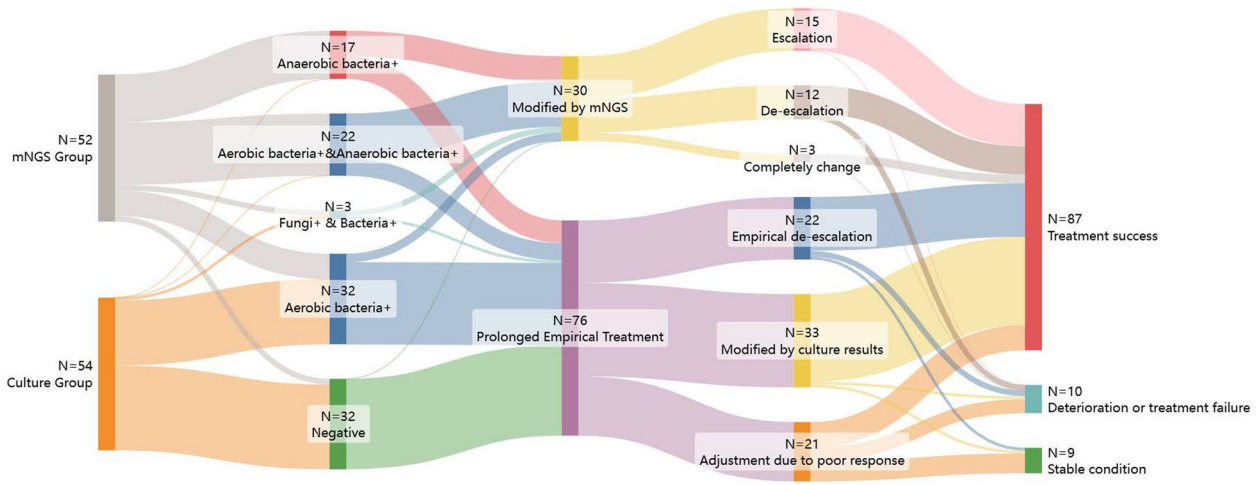

Fig. 4 Sankey diagram demonstrating the therapy adjustment in the mNGS and culture group. mNGS, metagenomic next-generation sequencing

Table 3 Comparison of outcomes between two groups

	mNGS group *N* = 52	Culture group *N* = 54	*p* value
Treatment outcome			
Treatment success, n (%)	46 (88.5)	41 (75.9)	0.033
Other outcomes			
Deterioration and complication, n (%)	3 (5.8)	7 (13.0)	< 0.001
ICU stay[a], median (IQR), days	5 (3, 10)	7 (4, 17)	0.062
Hospital stay, median (IQR), days	13 (7, 16)	15 (12,19)	0.081
Hospitalization costs, median (IQR), USD	3544.1 (2600.1, 4214.9)	4822.6 (3662.7, 5148.8)	0.020

[a] The comparison of ICU stay only included the subgroup of patients who were transferred to the ICU for monitoring and treatment, with 16 patients in the mNGS group and 19 patients in the culture group. *IQR* interquartile range, *ICU* intensive care unit, *mNGS* metagenomic next-generation sequencing

statistical significance ($p > 0.05$), while the medical costs of the mNGS group were significantly lower (Test cost of mNGS included, 3544.1 USD vs 4822.6 USD, $p = 0.020$, Table 3). Moreover, laboratory results of the two groups were visualized and presented in Fig. 5, demonstrating that the levels of inflammatory markers including CRP, WBC and neutrophil were decreasing more rapidly in the mNGS group (Friedman test, p all < 0.05).

Discussion

DNSA is an infectious disease caused by various kind of bacteria and fungi. Although previous studies have explored the microbiological features of DNSA based on conventional microbiology tests, not all pathogens that cause DNSA are known and only a limited number of pathogens can be identified by conventional culture tests [6, 21, 22]. However, limitations of these tests, including time consumption and low positivity rates, led to a prevalent reliance on prolonged empirical antibiotic therapy for a majority of DNSA patients, which may induce side effects like drug resistance, exacerbated organ function and increased economic burden [23, 24]. In this context, our previous study has reported the fast detection and high positive rate of mNGS [7]. In this study, we supplemented a certain sample size and emphasized on further analyzing the guidance and benefits that mNGS provides for rationalizing antibiotic usage and improving outcome in DNSA patients.

In our study, mNGS exhibited significantly higher detection rates and quicker results compared to culture methods. The identified prevalent pathogens, including *Streptococcus* spp., *K. pneumoniae*, and *P. micra*, were consistent with findings from previous researches [6, 25]. A majority of DNSA involve co-infections with

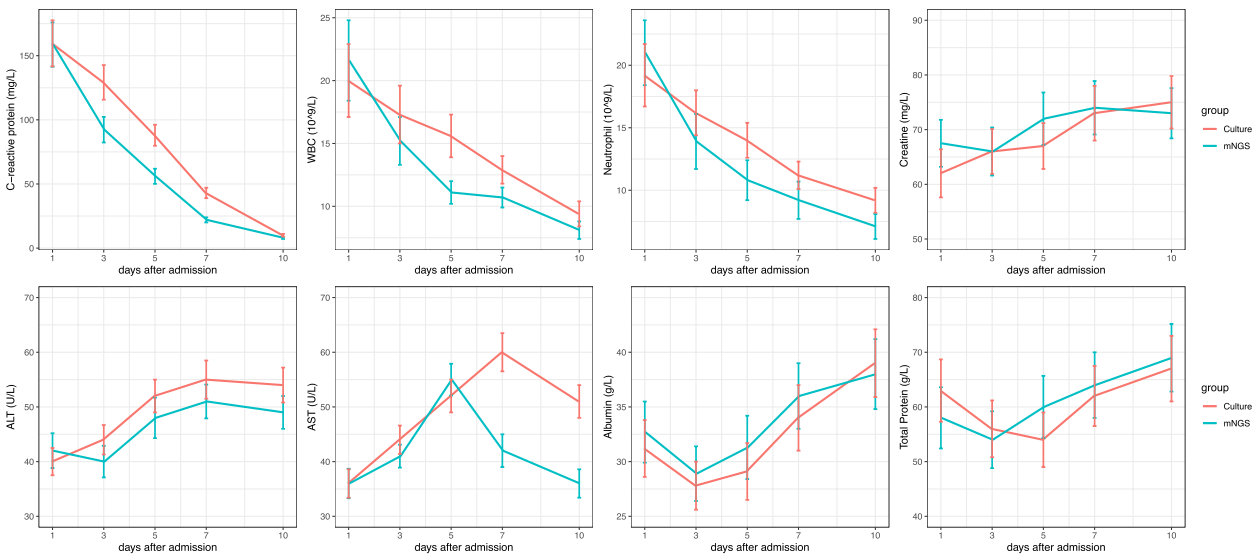

Fig. 5 Line graphs presenting the trends over time in inflammatory markers and organ function between the targeted group and the empirical group after sampling

two or more types of bacteria, with the highest proportion being co-infections of aerobic and anaerobic bacteria. This suggested the possibility of concurrent upper respiratory tract infections and odontogenic factors in the occurrence and development of DNSA. Besides, our study identified the prevalence of *Prevotella* spp. and *P. micra* in a considerable portion of DNSA patients, whether as single infections or co-infections, which contradicts prior research findings [6, 26, 27]. This discrepancy may be due to the lower detection rate of anaerobic bacteria by conventional culture methods used in previous microbiology studies. The conclusive evidence from our use of mNGS for anaerobic bacterial infection in most patients provides a good basis for us to combine anti-anaerobic bacterial regimens for treating DNSA. Conclusively, we recommend the empirical use of first- or second-generation cephalosporins combined with nitroimidazole antibiotics to cover both aerobic and anaerobic pathogens before acquisition of microbiological results. Additionally, we found that in critically ill patients, the causative pathogens are more likely to be Gram-negative bacteria, such as Klebsiella pneumoniae and Clostridium perfringens. Therefore, for critical patients with concurrent DNM or sepsis, broad-spectrum antibiotics, such as third- or fourth-generation cephalosporins or β-lactamase inhibitors, were recommended as empirical therapy for better therapeutic outcomes.

It was found that different physicians may have different habits regarding to the initial use of empirical antibiotic regimens, leading to irrational empirical antibiotic usage in the treatment of different DNSA patients. Following the acquisition of mNGS results (typically within 1 day), a considerable part of patients had their antibiotic regimens adjusted accordingly. This adjustment can shorten the duration of irrational empirical antimicrobial therapy and promote the standardization and normalization of antibiotic administration, thereby bringing potential clinical benefits to DNSA patients. We found that mNGS-guided antibiotic use in the early stages can comprehensively cover the suspected pathogens and achieve optimal anti-infective effects, thus expedite the recovery of patients' inflammatory status and improve treatment outcome. Besides, despite the relatively high cost of mNGS, the hospitalization cost of patients receiving mNGS-guided therapy was significantly lower than those who underwent prolonged empirical medication, which may be attribute to the lower rate of deterioration and shorter hospital stay. Previous studies have also reported that mNGS results can guide antibiotic use and achieve better therapeutic outcomes in conditions such as spinal infections, lower respiratory tract infections, soft tissue infections and sepsis [12, 14, 28]. To our best knowledge, this is the first study evaluating the performance of utilizing mNGS to guide precise antibiotic therapy for DNSA patients, which also indicates promising results.

Among all the pathogens contributing to DNSA, *K. pneumoniae* stands out as a rather distinct presence. It tended to cause solitary infections and exhibits a strong association with diabetes, which was consistent with a previous research conducted in Taiwan [29]. In DNSA cases induced by *K. pneumoniae*, our data showed that patients frequently present with symptoms such as dyspnea, pleural effusion, and descending mediastinitis. We will call it '*Klebsiella pneumoniae* triad'. This might be attributed to its tendency to not only cause deep neck infections but also to readily affect the lower respiratory tract and even the mediastinum. Therefore, in cases where a patient presents with '*Klebsiella pneumoniae* triad' clinicians should be vigilant about the possibility of *K. pneumoniae* infection and consider employing appropriate antibiotic regimens such as carbapenems and aminoglycosides. It is also worth noting that *K. pneumoniae* not only causes DNSA but is also closely associated with infections in other sites, such as pneumoniae, liver abscesses and bloodstream infections [30–32]. Therefore, DNSA caused by *K. pneumoniae* may also result from infection in other sites spreading to the deep neck tissues through the bloodstream. However, given that most DNSA patients present to the ENT department with high fever, sore throat, and painful neck swelling, we believe that in these patients, the condition is likely caused by the spread of infection by *K. pneumoniae* originating in the neck, leading to complications such as pleural effusion and DNM. The specific relationship between the primary focus and infection spreading may require further research in the future for clarification.

Additionally, although our study did not include patients with concurrent malignant tumors of the neck, literatures have reported cases where DNSA develops due to necrotic infection caused by primary or metastatic neck malignancies [33]. For these patients, treatment should not only focus on drainage and antimicrobial therapy to control abscess progression and systemic symptoms but, more importantly, address the primary disease after abscess resolution.

This study has several limitations. Firstly, this was a retrospective cohort study, which may be subject to potential selection bias. Secondly, in this study, only pus specimens were used as samples for mNGS analysis, without considering the diagnostic value of other specimen types such as throat swabs or blood. Combining these sample types for testing may achieve better diagnostic efficacy. Moreover, while our investigation into the effectiveness of mNGS for detecting pathogens and treatment guidance in DNSA has yielded valuable insights, the restricted sample size remains a notable constraint

that potentially affects the robustness of our statistical analyses. Therefore, to further enhance the clinical applicability of mNGS in guiding the diagnosis and treatment of DNSA patients, a well-designed controlled randomized trial with a proper sample size was required to overcome these limitations.

Conclusion

This study compared the application of mNGS and conventional culture tests in DNSA patients, demonstrating that mNGS exhibits a higher detection rate, sensitivity, and comprehensiveness in identifying pathogens associated with DNSA. Based on the mNGS results, we analyzed the correlation between pathogenic bacteria in DNSA patients and clinical manifestations. Most importantly, mNGS may help early rationalize antimicrobial therapy, therefore facilitate patient recovery, improve prognosis, and reduce the burden on healthcare resources.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03890-z.

Supplementary Material 1.

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Not applicable.

Clinical trial number
Not applicable.

Authors' contributions
H. L.: study design, data management, statistical analysis, drafting and revising manuscript, final approval; J. L.: data collection and management, statistical analysis; Y. L.: data collection and management, revising manuscript; T. L.: data collection and management; W. L.: study design, funding support, revising manuscript, final approval; W. G.: study design, funding support, revising manuscript, final approval.

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Data availability
The datasets generated and/or analyzed during the current study are available in the NCBI SRA repository (https://dataview.ncbi.nlm.nih.gov/object/PRJNA810198?reviewer=3hf38iat1ruji12tfur8b263po). 20 of the biosamples have not been released for confidentiality, and will be released immediately after publication of this study.

Declarations

Ethics approval and consent to participate
This study was conducted in full accordance with the ethical principles of the World Medical Association's Declaration of Helsinki, and it was approved by Ethics Committee for Research and Publication of First Affiliated Hospital of Sun Yat-sen University and Sixth Affiliated Hospital of Sun Yat-sen University (NO. [2023]445–1). Informed consent was waved for use of retrospective unidentified information by Ethics Committee for Research and Publication of First Affiliated Hospital and Sixth Affiliated Hospital of Sun Yat-sen University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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