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Detection of potential pathogen in pancreatic fluid aspiration with metagenomic next-generation sequencing in patients with suspected infected pancreatic necrosis

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ABSTRACT

Background: Timely and accurate microbial diagnosis is important in managing patients with infected pancreatic necrosis (IPN).
Aims: To evaluate the diagnostic performance of Metagenomic next-generation sequencing (mNGS) in patients with suspected IPN.
Methods: The clinical data of 40 patients with suspected IPN who underwent CT-guided pancreatic fluid aspiration were retrospectively analyzed. Microbial culture and mNGS were simultaneously applied to identify the potential pathogens. The diagnostic performance of the mNGS was assessed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).
Results: The mNGS report can be obtained significantly earlier than culture methods (42 (36–62 h) vs. 60 (42–124 h), $P = 0.032$). Across all the study samples, seven species of bacteria and two species of fungi were reported accordingly to the culture results, while 22 species of bacteria and two species of fungi were detected by mNGS. The sensitivity, specificity, NPV, and PPV of mNGS were 88.0%, 100%, 83.33%, and 100%, respectively.
Conclusions: The diagnostic accuracy of mNGS in patients with suspected IPN is satisfactory. Moreover, mNGS may broaden the range of identifiable infectious pathogens and provide a more timely diagnosis.

1. Introduction

Infected pancreatic necrosis (IPN) is a complex local complication of acute pancreatitis, with substantial morbidity and mortality [1,2]. Early and accurate diagnosis of IPN is of great clinical relevance since it would help decision-making regarding the following treatment and the choice of antibiotics [3–5]. However, conventional microbial culture, which is currently the gold standard, takes at least 48 h to obtain results, and the sensitivity is not satisfying [6].

Metagenomic next-generation sequencing (mNGS) is a potential alternative or supplement to the conventional culture-based method. It directly studies the genetic composition and function of all microorganisms contained in samples and can widely identify known and unknown pathogens. This technology is renowned for its short detection cycle and high sensitivity [7,8]. Another advantage of mNGS is that it can semi-quantitatively determine the microbial concentration by counting the sequence reads and can detect potential drug resistance genes to assist the choice of antibiotics [9,10]. At present, mNGS has been widely applied in the diagnosis and treatment of central nervous system infection, unexplained pneumonia, bone, and joint infection, local abscess, and other diseases [8,11–13].

The role mNGS may play in diagnosing IPN remains to be explored. In this study, we assessed the diagnostic performance of mNGS in patients with suspected IPN, taking confirmatory diagnosis of IPN within a week as the reference.

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2. Methods

2.1. Study setting

This is a retrospective database-based cohort study conducted in Jinling Hospital, Nanjing University. The study protocol was approved by the institutional ethics committee of Jinling Hospital (2019NZKY009–01). Clinical and laboratory data were extracted from a web-based electronic database (Acute Pancreatitis Database). All the study participants signed broad informed consent and gave permission to use of the clinical and laboratory data for academic research.

2.2. Study participants

We retrospectively screened AP patients admitted to the center of severe acute pancreatitis(CSAP), Jinling Hospital, from September 2020 to May 2021. The diagnosis of AP was made according to the revised Atlanta 2012 classification and based on the presence of at least two of the three following features [14]: (1) acute onset of persistent, severe, epigastric pain often radiating to the back; (2) serum lipase activity (or amylase activity) at least three times greater than the upper limit of normal; and (3) characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT).

Study inclusion criteria were as follows: 1. Age $>$ 18 years old; 2. CECT showed pancreatic parenchymal and/or peripancreatic necrosis; 3. Computed Tomography (CT)-guided percutaneous catheter drainage (PCD) or percutaneous fine-needle aspiration (FNA) was applied due to suspected IPN based on clinical manifestations like fever with elevated inflammatory indexes, like C-reactive protein and procalcitonin. The decision of drainage or FNA was made by the treating physician; 4. The samples obtained from the index PCD or FNA procedure were simultaneously sent for mNGS and microbial culture, which was also decided by the treating physicians. Patients who were pregnant or had incomplete data were then excluded.

2.3. Metagenomic next-generation sequencing and analysis

2.3.1. DNA extraction and sequencing

The drain samples were collected by PCD or percutaneous FNA and were sent to the clinical microbiology lab within 2 h after collection. Meanwhile, the remaining specimens (2 mL) were transported on dry ice for PACEseq mNGS analysis (Hugobiotech, Beijing, China). DNA was purified using TIANGEN DP316 kit according to its manual. NEBNext Ultra II DNA Library Prep Kit was then used to construct the DNA libraries according to the manufacturer's instructions. After construction, the quality of each library was assessed using Qubit (Thermo Fisher) and Agilent 2100 Bioanalyzer (Agilent Technologies). The qualified libraries were finally sequenced on a Nextseq 550 platform (Illumina). Internal control, negative control, and positive control were built during each test to track the whole process and guarantee the accuracy of the detection.

2.3.2. Bioinformation analysis in the mNGS pipeline

Adaptors, as well as low quality, low-complexity, and short reads (less than 35 bp) were removed from the raw data. SNAP and Burrow-Wheeler alignment was then applied to exclude human sequences by mapping the reads to the human reference genome. The screened sequences were finally mapped to the microbial genome database (NCBI: ftp://ftp.ncbi.nlm.nih.gov/genome). All parameters of the detected pathogenic microorganisms were calculated, including the sequence number, relative abundance, genome coverage, and depth.

2.3.3. Criteria for a positive mNGS result

The detected infectious pathogens (including bacteria and fungi) by mNGS that meet the following two thresholds are judged as positive [15]: 1. The relative abundance of microorganisms (bacteria, viruses, and fungi) is above 30%; 2. The coverage of bacteria is 10 times higher than that of any other microorganism, and the coverage of fungi is 5 times higher than that of any other fungi.

2.4. Microbial culture

The drain samples were placed in sterile containers and then transported to the microbiology lab. To identify pathogens, samples were stained with gram and amplified by microbial culture. The identification of pathogens was also carried out by Vitek-2compact automated microbial system (bioMerienx, version 1.7, France).

2.5. Diagnosis of confirmatory IPN

In this study, confirmatory diagnosis of IPN within a week after the index drainage was considered the reference standard. Infected pancreatic necrosis is defined as infection of either an acute necrotic collection (before 4 weeks) or walled-off necrosis (after 4 weeks). IPN was confirmed based on positive microbial culture results from samples obtained by FNA or during drainage procedures and/or operative necrosectomy within the one-week period. Otherwise, sterile pancreatic necrosis would be defined. The final diagnosis was made by two independent and experienced treating physicians.

2.6. Data collection

The demographic characteristics of the subjects (age, gender, etiology of pancreatitis), as well as the clinical data at the time of sampling, including organ function status, acute physiology and chronic health assessment II (APACHE II) score, laboratory biochemistry, etc., were extracted from the database. For identification of the pathogens, mNGS results were collected based on the standard reports, and culture results were retrieved from the electronic medical records. Investigators manually check the data before analysis to ensure the quality of the data.

2.7. Statistical analysis

Continuous variables were reported as median with interquartile range (25%, 75%). Categorical variables were expressed as frequencies and percentages. A Mann–Whitney *U test* was used for non-parametric comparison of continuous variables between two groups.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of the mNGS tests for the confirmatory diagnosis of IPN are presented in a 2×2 contingency table. The diagnostic accuracy was calculated as (sum of true negatives and true positives)/(total number of samples). SPSS 26 and Graphed Prism7 software have been applied for data validation and data analysis. All tests were two-tailed, and P-values of less than 0.05 were considered statistically significant.

3. Result

3.1. Baseline characteristics

During the study period, 462 patients with acute pancreatitis were admitted to our center. Of the 122 patients with suspected IPN, 82 were excluded (Fig. 1). Patients' baseline characteristics are listed in Table 1. Thirty-one patients (77.5%) were male with a mean age of 42 (31–57) years. Gallstone ($n = 21$, 52.5%) was the

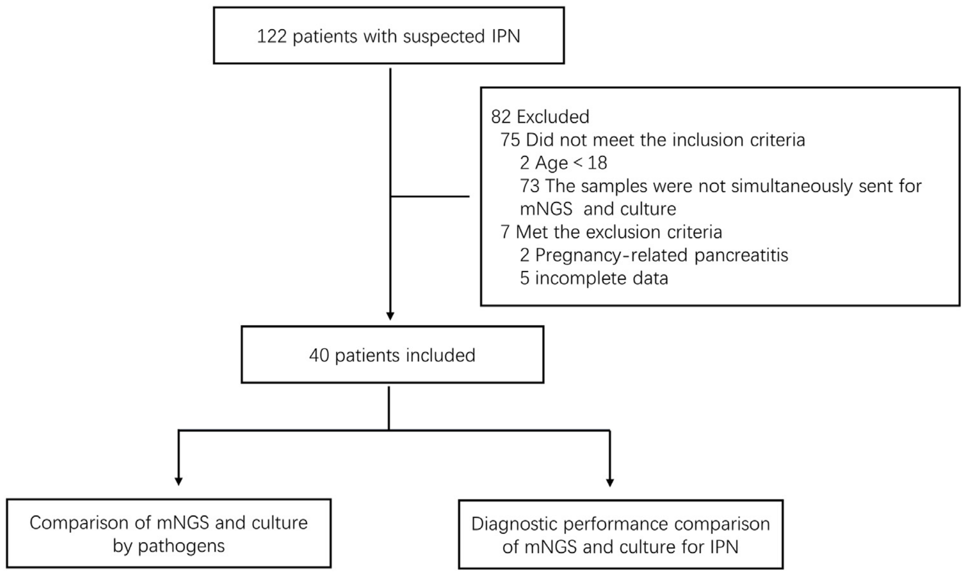

Fig. 1. flowchart of participant inclusion in the trial. IPN: infected pancreatic necrosis; mNGS: metagenomic next-generation sequencing.

Table 1
Baseline characteristics.

Characteristics	Value
mNGS Positive	22 (55.0%)
Age (years)	42 (31, 57)
Gender (male,%)	31 (77.5%)
APACHEII Score	11 (7, 15)
SOFA Score	3 (1, 5)
CTSI Score	8 (6, 10)
SIRS	32 (80%)
Sepsis	11 (27.5%)
Shock	10 (25%)
AKI	9 (22.5%)
ARDS	12 (30%)
Days from onset (days)	26 (18, 32)
Length of hospital stay (days)	39.5 (18, 58)
Degree of severity	
mild AP	2 (5%)
moderately severe	5 (12.5%)
severe	33 (82.5%)
Etiology	
Hypertriglyceridemia	16 (40%)
Gallstone	21 (52.5%)
Other	3 (7.5%)
Temperature (°C)	38.65 (38.10, 39.00)
PCT (ug/L)	0.55 (0.22, 3.37)
CRP (mg/L)	141.35 (86.90, 199.05)
Leukocyte ($\times 10^9$/L)	12.23 (7.79, 14.97)
Neutrophils ($\times 10^9$/L)	10.58 (6.29, 12.73)

Data are presented as n (%) or median (interquartile range). mNGS: metagenomic next-generation sequencing; APACHEII Score: acute physiology and chronic health assessment II score; SOFA Score: sequential organ failure assessment score; CTSI Score: computed tomography severity index score; SIRS: systemic inflammatory response syndrome; AKI: acute kidney injury; ARDS: acute respiratory distress syndrome; PCT: procalcitonin; CRP: C-reactive protein.

main etiology, followed by hypertriglyceridemia ($n = 16$, 40%) and others ($n = 3$, 7.5%). Median APACHE II scores and SOFA scores at admission were 11 (7–15) and 3 (1–5), respectively. The median time from symptom onset to the sampling was 26 (18–32) days. We also list the baseline data and microbial results of the excluded patients in **Table S1–2**.

3.2. Pathogen detection and mNGS results

The median time from sampling to report was 42 (36–62 h) for the mNGS approach and 60 (42–124 h) for the culture approach, respectively($P = 0.032$). Seven species of bacteria and two species of fungi (24 strains in total) were detected in 20 samples by the microbial culture. Meanwhile, 22 species of bacteria and two species of fungi (38 strains in total) were found in 22 samples by mNGS tests. Among the 38 strains detected in total, *Enterococcus faecium* was the most common (six stains, 15.79%), followed by *Acinetobacter baumannii* (four strains, 10.53%) and *Escherichia coli* (four strains, 10.53%), and seven obligate anaerobic bacteria were also detected (Fig. 2**a, c**). There was no significant difference between the two methods in positive rate (55% vs. 50%, $P = 0.823$). Culture methods detected four cases of polymicrobial infection, while mNGS results showed eight cases, but there was no significant difference (20% vs. 10%, $P = 0.348$) (Fig. 2**b**). In the polymicrobial infection detected by mNGS, up to 6 different strains were detected in one sample.

Regarding the overall results, mNGS and culture were in agreement for most samples (34/40, 85%; 18 mutual positives and 16 mutual negatives), as shown in Fig. 3. Four samples were found to be positive by mNGS exclusively(10.0%) and two by culture (5.0%). For mutual positive samples, 12 of 18 cases (66.67%) were completely consistent, showing the same pathogens. The microbial culture detected more pathogens in three mutual positive samples. Two fungal strains were undetected by mNGS approach. See Table 2 for the description of the inconsistency between the two methods.

3.3. Diagnostic performance of mNGS

A total of 25 patients were diagnosed with confirmatory IPN within a week after the index sampling, and the diagnostic details are shown in **Table S3**. Based on the reference, the sensitivity, specificity, NPV, and PPV of mNGS in the diagnosis of IPN were 88.0%, 100%, 83.33%, and 100%, respectively (Fig. 4). The mNGS tests produced three false negatives. We showed the diagnostic performance of the index culture in supplement files (**Fig. S1**).

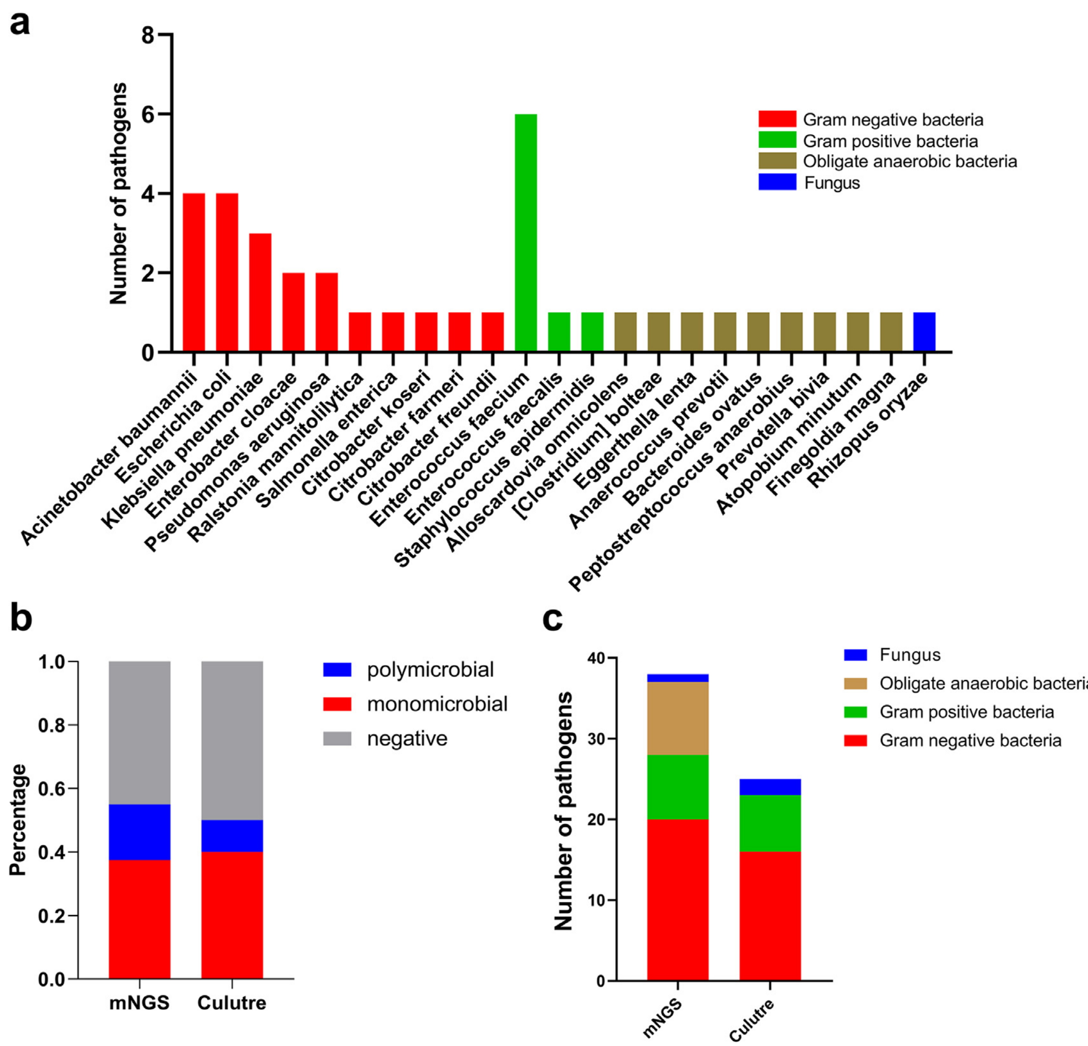

Fig. 2. Pathogen Detection by mNGS and culture methods. a. Pathogens detected by mNGS; b. Comparison of positive rates of two methods c. Comparison of the number of pathogens detected of two methods.

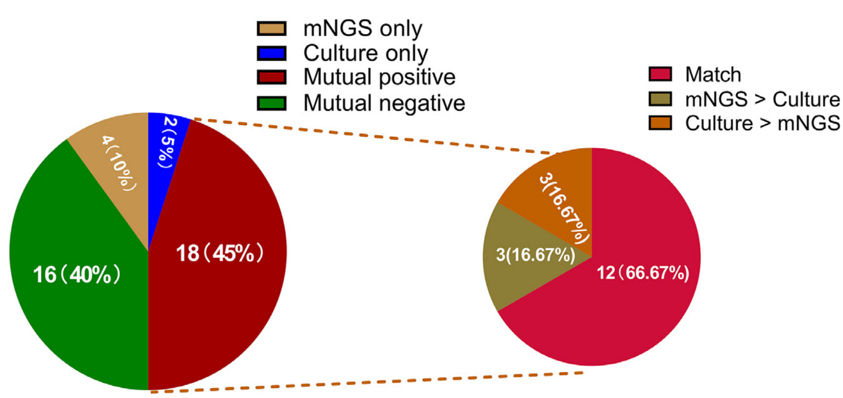

Fig. 3. Consistency between mNGS tests and culture methods. mNGS > Culture: mNGS detected more pathogens than culture in one sample. Culture >mNGS: culture detected more pathogens than mNGS in one sample. mNGS: metagenomic next-generation sequencing.

4. Discussion

In this study, we assessed the diagnostic performance of mNGS in patients with suspected IPN. The diagnostic accuracy of mNGS is overall satisfactory. Moreover, our results suggested that mNGS has advantages in the timeliness of diagnosing IPN and discovering more microbial information, which may provide helpful information for clinical decision-making.

Compared with microbial culture, mNGS tests have advantages in diagnosing polymicrobial and rare pathogens infections. A study of 109 adults with infectious diseases, including respiratory system infections, bloodstream infections, and central nervous system infections, showed that the sensitivity of mNGS was significantly higher than that of the culture method (67.4% vs. 23.6%, $P<0.001$), especially in bronchoalveolar lavage fluid ($P = 0.002$), blood ($P<0.001$) and sputum specimens ($P = 0.037$) [16]. Similarly,

Table 2
The details of inconsistency between mNGS tests and culture methods.

ID	mNGS Results	Culture Results	Probable Cause
2	Negative	Acinetobacter baumannii	The reads of pathogen sequences are below the threshold;
47	Negative	Klebsiella pneumoniae	The reads of pathogen sequences are below the threshold;
15	Rhizopus oryzae, Rhizopus delemar	Negative	The sensitivity of microbial culture is low;
16	Pseudomonas aeruginosa	Negative	The sensitivity of microbial culture is low;
21	Enterobacter cloacae,Citrobacter farmeri	Negative	The sensitivity of microbial culture is low;
41	Bacteroides ovatus, Eggerthella lenta, Alloscardovia omnicolens, [Clostridium] bolteae	Negative	Anaerobic bacteria are difficult to be detected by culture
9	E. coli,Enterococcus faecalis	E. coli	The sensitivity of microbial culture is low;
29	E. coli, Citrobacter koseri, Citrobacter freundii, Enterobacter cloacae, Salmonella enterica	E. coli	Anaerobic bacteria are difficult to be detected by culture
30	Peptostreptococcus anaerobius, Anaerococcus prevotii , Prevotella bivia, Atopobium minutum, Enterococcus faecium , Finegoldia magna	Enterococcus faecium	Anaerobic bacteria are difficult to be detected by culture
7	Klebsiella pneumoniae	Klebsiella pneumoniae, Candida tropicalis	The sensitivity of mNGS detection to fungi was low
36	Klebsiella pneumoniae, Pseudomonas aeruginosa	Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida glabrata	The sensitivity of mNGS detection to fungi was low
49	Enterococcus faecium	Acinetobacter baumannii;Enterococcus faecium;	The reads of pathogen sequences are below the threshold; mNGS detection error

mNGS: metagenomic next-generation sequencing;.

	IPN	SPN	Total
mNGS(+)	22	0	22
mNGS(-)	3	15	18
Total	25	15	40

Sensitivity	88.00%
Specificity	100.00%
PPV	100.00%
NPV	83.33%

Fig. 4. The diagnostic performance of the mNGS methods. IPN: infected pancreatic necrosis; SPN: sterile pancreatic necrosis; PPV: positive predictive value; NPV: negative predictive value.

in a prospective study of community-acquired pneumonia [15,17], compared with traditional techniques, mNGS approach found significantly more pathogens (29 of 59, 49.2% vs. 47 of 59, 79.7%, $P < 0.01$). For IPN, previous studies have shown that *E. coli, Klebsiella pneumoniae, Acinetobacter baumannii and Enterococcus faecium* were the most common pathogens of IPN [18,19]. With the application of mNGS tests, our results suggested that a broader range of rare pathogens and coinfection microbes should be considered, which may help deepen the understanding of IPN development and facilitate potential treatment [20,21]. A possible explanation for this advantage is due to antibiotic treatment before sampling. Compared with culture methods, pathogenic DNA may survive longer, thus mNGS is less affected by previous antibiotic use [22].

Although the mNGS approach may have a higher positivity rate and satisfactory diagnostic performance, it cannot yet replace the microbial culture method in the diagnosis of IPN. First, it is widely accepted that the pathogen of IPN is mainly related to bacterial translocation [23–25], and the pathogen spectrum includes common microorganisms such as *Enterobacter* and *Enterococcus,* which are easy to grow in microbial mediums (such as blood agar plates). Moreover, many studies have shown that the fungal cell wall is an important obstacle to nucleic acid extraction, and the effectiveness of cell lysis and DNA extraction seriously affects the sensitivity of mNGS detection [26,27]. In our study, two cases of fungal infection were undetected by mNGS. In addition, mNGS will report negative when the reads of pathogen sequences do not reach the threshold due to a low pathogen load, which may lead to false negatives. Therefore, in terms of the detection ability of common entheogenic pathogens, mNGS is similar to routine culture. But mNGS could detect the potential pathogen in a more timely manner, which may be helpful for facilitating appropriate antimicrobial treatments. Timely and adequate antibiotics may improve the clinical outcomes of IPN, including reducing the need for additional invasive interventions and avoiding secondary fungal infections [28–30].

On the other hand, the mNGS approach is naturally flawed due to the lack of antibiotic resistance profile that traditional culture methods can provide. Peng JM et al. [27] retrospectively analyzed 60 critically ill immunocompromised patients with suspected pneumonia and found that the combination of mNGS and conventional microbiological tests may be the best diagnostic strategy. Therefore, mNGS combined with traditional microbial culture needs to be further assessed in future studies.

This study had several limitations. First of all, the relatively small sample size may cause a bias. Second, 40% of the included patients have hypertriglyceridemic acute pancreatitis (HTG-AP). This high proportion of HTG-AP is similar to previously published studies in the Chinese AP population and may be related to altered dietary habits and genetic backgroud [31–33]. However, it may impact the generalizability of our results. Third, the drains were from either FNA or drainage procedure, which may also bring in some uncertainty due to sampling methods. Moreover, we excluded patients with suspected IPN who did not undergo mNGS and microbial culture simultaneously during the study period, which may have resulted in selection bias. Finally, due to local preference and technical availability, all the study subjects were managed with a surgical approach, which may potentially increase morbidity [34,35].

5. Conclusions

The application of mNGS in patients with suspected IPN may facilitate the identification of potential pathogens with satisfactory diagnostic accuracy. However, the results from mNGS cannot yet be considered as diagnostic criteria for IPN. Further prospective research is necessary to evaluate the cost-effectiveness of mNGS method in this population and the clinical impact it may incur.

Conflicts of Interest

None declared.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2022.07.014.

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