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Clinical efficacy of metagenomic next-generation sequencing for the detection of pathogens in peritoneal dialysis-related peritonitis: a prospective cohort study

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Abstract

Background Metagenomic next-generation sequencing (mNGS) has been reported to improve pathogen identification in infectious diseases. This prospective cohort study aimed to explore the etiological diagnostic value of mNGS in peritoneal dialysis (PD)-related peritonitis.

Methods Patients with PD-related peritonitis were consecutively recruited at the Nephrology Department of Nanjing Drum Tower Hospital. PD effluent samples for mNGS and culture were collected simultaneously. The positive rate, detection time, and consistency of mNGS and culture were compared.

Results From August 1, 2021 to August 31, 2022, 38 patients with 41 episodes of PD-related peritonitis were enrolled. The positive rate of mNGS was higher than that of culture, although not statistically significant (92.7% vs 78.0%, $P=0.109$). The average reporting time of mNGS was significantly shorter than that of culture (30.4 ± 10.5 vs 86.9 ± 22.2 h, $P<0.001$). mNGS identified more co-pathogens and unusual pathogens than culture, with multiple pathogens being detected in nearly half of the samples. Among the 30 samples that tested positive by both methods, 27 (90%) showed completely (13 cases) or partly (14 cases) matched results between mNGS and culture. Fourteen patients (with 14 episodes of peritonitis) had used antibiotics within 2 weeks before specimen collection. Antibiotic usage led to a significant decrease in the culture-positive rate (57.1% vs 88.9%, $P=0.042$), while the mNGS-positive rate remained unaffected (92.9% vs 92.6%, $P=1.000$).

Conclusions This study revealed that mNGS exhibited higher sensitivity and shorter reporting time compared to culture in detecting pathogens in PD-related peritonitis. For samples that yielded positive results by both methods, the consistency between mNGS and culture was substantial. mNGS may offer a novel approach for the etiological diagnosis of PD-associated peritonitis, particularly in cases involving prior antibiotic use and unusual pathogens.

Keywords Metagenomics next-generation sequencing, Peritoneal dialysis-related peritonitis, Etiology, Diagnosis

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Background

Peritoneal dialysis (PD)-associated peritonitis is a common complication and the main cause of technique failure in PD patients. Timely and accurate pathogen identification is crucial for implementing precise antimicrobial therapy. Currently, conventional microbial cultivation is the most widely used method for identifying pathogens, but it is time consuming and the positive rates remain unsatisfying in many centers, as a result of which patients need to receive prolonged empirical antibiotic therapy, leading to increased risk of antibiotics-related complications. Moreover, for some special pathogens that are difficult to cultivate, the preferred drugs are rarely included in the initial treatment plan for peritonitis, leading to a high rate of treatment failure. Thus, it is of great importance to establish a rapid and more sensitive pathogen identification method in PD-associated peritonitis.

Metagenomic next-generation sequencing (mNGS) has emerged as a promising pathogen detection approach for various infectious diseases [1–4], owing to its high-throughput, sensitivity, and broad coverage. However, the utility of mNGS in pathogen recognition of PD-related peritonitis remains limited. Several case reports confirmed the value of mNGS in identifying rare pathogen microorganisms, such as Ureaplasma urealyticum [5], non-tuberculous mycobacteria [6], and Gordonia aichiensis [7]. Our previous retrospective study with a small sample showed that mNGS using PD effluent could improve the detection rate of pathogenic microorganisms [8]. Ye et al. reported a comparison of mNGS with microbial cultivation in 30 PD effluent samples from PD-related peritonitis, demonstrating a significantly higher detection rate of mNGS than cultivation [9]. However, all patients were not treated with antibiotics before sample collection in their study. In the present prospective study, we aimed to comprehensively examine the clinical impact of mNGS in identifying pathogens in dialysis effluent of peritonitis patients including those who have taken antibiotics.

Methods

Patients selection and study design

The rates of peritonitis in 2021 and 2022 were 0.16 and 0.14 episodes per patient-year in our center. This is a prospective cohort study. From August 1, 2021 to August 31, 2022, patients with PD-related peritonitis were consecutively recruited in the department of Nephrology, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University. The inclusion criteria were as follows: (1) age ≥ 18 years old; (2) met the diagnostic criteria of PD-related peritonitis recommended by International Peritoneal Dialysis Association (ISPD) [10]. Exclusion criteria were peritonitis caused by intestinal perforation and aseptic peritonitis including chemical peritonitis and eosinophilic peritonitis. Patients with combined infections of other organs were also excluded from this study. Patients who have received antibiotics for treating peritonitis were not excluded unless the condition eased. All patients were informed about the aim of this study and gave their consent. The protocol was approved by the local ethics committee.

Clinical characteristics of the patients were recorded upon enrollment, including age, gender, dialysis vintage, and the use of antibiotics within 2 weeks prior to the study. Upon enrollment, two samples of dialysis effluent (at least 2 h of dwell time) of each patient were collected simultaneously by trained PD nurses according to the method recommended by guidelines. One sample was used for cultivation and the other for mNGS.

Conventional culture

One of the dialysis effluent sample of each patient was injected into a BACT/ALERT FA Plus (Aerobic) bottle (bioMerieux, Inc., Durham, USA) and a BACT/ALERT FN Plus (Anaerobic) bottle (bioMerieux, Inc., Durham, USA) (10 ml each) and immediately transported to the laboratory of the microbiology department of our hospital within 30 min after collection. The microbiology department conducted bacterial culture using a fully automated blood culture instrument (BacT/ALERT 3D, BioMerieux, French), according to the standard operating procedures. The drug sensitivity was determined using the VITEK2 System (BioMerieux, French).

mNGS detection and analysis

The other dialysis effluent sample was placed in a disposable container that was sterile, sealed, and nuclease free, and was sent for PMSeq-DNA analysis, a commercially available mNGS service provided by BGI-Shenzhen. Fresh samples were promptly transported to the laboratory via a cold chain with dry ice for testing. Samples that could not be transported immediately were stored in a − 80 °C freezer until transportation.

To extract nucleic acid, a 3–5 mL dialysate sample was centrifuged at 4000 r/min for 10 min at 4 °C. Subsequently, DNA extraction was performed using the TIANamp Micro DNA Kit (DP316, Tiangen Biotech, Beijing, China) following the manufacturer's instruction. DNA libraries were then prepared through processes including DNA-fragmentation, end-repair, adapter ligation, and PCR amplification. Library fragment size was assessed using a 2100 Bioanalyzer (Agilent, Technologies, CA, USA). Libraries were then sequenced on the MGISEQ-2000 sequencing platform at BGI Co. After removing adapter sequences, low-quality sequences, and

human host reads, the remaining data were aligned to pathogenic metagenomics database (PMDB) microbial genome databases, which includes 6350 bacteria, 1064 fungi, 1798 DNA viruses, and 234 parasites associated with human diseases, and can be downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/).

A bacterial or fungal specie was considered as positively detected if (1) it was among the top 5 genera with the highest number of stringently mapped reads (SMRN); (2) it ranked top within its genus; (3) it had a SMRN ≥ 3; and (4) it was a commonly reported pathogen of PD-related peritonitis. For the detection of Mycobacterium spp., given the challenges of DNA extraction and the low possibility of contamination, the pathogen was considered detected if (1) it belonged to the top 20 genera with the highest SMRN; (2) it ranked top within its genus; and (3) it had a SMRN ≥ 1.

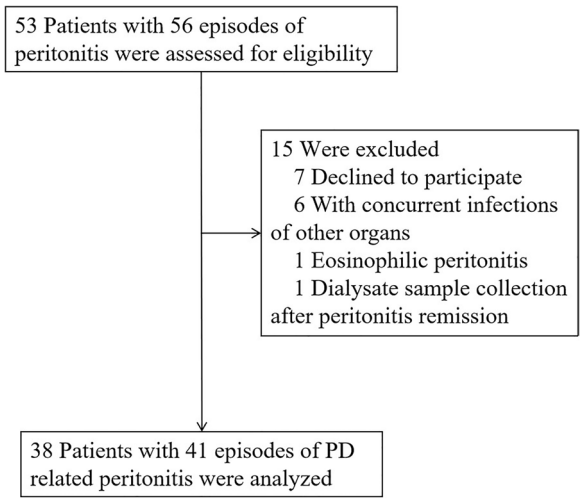

Fig. 1 Flow chart of patients enrollment

Statistical analyses

Analyses were performed using IBM SPSS Statistics 22. Data are expressed as mean ± standard deviation or median (25–75th percentile) for continuous variables and percentage for categorical data unless otherwise specified. Differences between groups were assessed by Paired samples *t* test, related samples Wilcoxon signed-rank test, Fisher exact test, or McNemar test when applicable. Statistical significance was assumed at a two-tailed value of $P < 0.05$.

Results

Clinical characteristics of the study patients

During the study period, 53 patients had 56 episodes of PD-related peritonitis. Fifteen patients were excluded from this study due to the combination of infections of other organs (6 patients with 6 episodes of peritonitis), unwillingness to participate in the study (7 patients with 7 episodes of peritonitis), eosinophilic peritonitis (1 patient with 1 episode of peritonitis), and dialysate sample collection after peritonitis remission (1 patients with 1 episodes of peritonitis). Ultimately, 38 patients with 41 episodes of PD-related peritonitis were included in this study (Fig. 1). Among them, 26 were males and 12 were females. All patients have hypertension, and nineteen of them (experienced 21 episodes of peritonitis) had diabetes mellitus. The primary diseases were diabetic kidney disease ($n = 16$), chronic glomerulonephritis ($n = 13$), hypertensive nephropathy ($n = 7$), autosomal dominant polycystic kidney disease ($n = 1$), and Henoch-Schonlein purpura nephritis ($n = 1$). During the study period, 35 patients experienced 1 episode of peritonitis each, while the remaining 3 male patients each had 2 episodes of peritonitis, 2 to 4 months apart. The median dialysis vintage was 22.0 (8.5, 49.0) months, and the median number of previous peritonitis episodes was 0 (0, 1). Twenty-five patients had a history of peritonitis before. Dialysis effluent samples for mNGS and culture were collected within 1 day to 13 days after peritonitis onset, with a median and mean collection time of 1.0 (1.0, 3.5) and 3.2 ± 3.5 days, respectively. Fourteen patients (with 14 episodes of peritonitis) used antibiotics within 2 weeks prior to dialysate specimen collection. The clinical characteristics of the patients are detailed in Table 1.

Positive rates and reporting time of mNGS and conventional culture

Of the 41 PD effluent mNGS tests, 3 yielded negative results and 38 reported at least one pathogen with a positive rate of 92.7%. In comparison, the positive detection rate of conventional culture was 78.0%, which is lower than that of mNGS, although not statistically significant ($P = 0.109$). Among the 41 PD effluent samples analyzed, 30 were positive by both mNGS and culture, 8 were mNGS positive but culture negative, 2 were mNGS negative but culture positive, and only 1 was negative by both methods. Overall, the combination of mNGS and culture provided a total positive rate of 97.6% (40/41) in PD-related peritonitis patients included in this study (Fig. 2A, B). The average reporting time of mNGS was 30.4 ± 10.5 h, which was significantly shorter than that of culture (86.9 ± 22.2 h) ($P < 0.001$) (Fig. 2C). The positive rates for mNGS and culture were 95.2% and 76.2%, respectively, in patients with diabetes, while the rates were 90.0% and 80.0% in patients without diabetes. However, these differences were not statistically significant. The positive rates for mNGS and culture were 87.5% and 81.3%, respectively, in patients with prior peritonitis history, while the rates were 96.0% and 76.0% in patients

Table 1 Clinical characteristics of the enrolled patients during the occurrence of peritonitis ($n=41$)

Age (years)	**54.5 ± 14.7**
Sex (Male/Female)	26/12
Primary disease	
Diabetic kidney disease (*n*)	16
Chronic glomerulonephritis (*n*)	13
Hypertensive nephropathy (*n*)	7
Autosomal dominant polycystic kidney disease (*n*)	1
Henoch-Schonlein purpura nephritis (*n*)	1
Comorbidities	
Diabetes mellitus (*n*)	19
Hypertension (*n*)	38
Dialysis vintage (months)	22.0 (8.5, 49.0)
Previous peritonitis episodes	0 (0, 1)
Leukocytes in dialysis effluent (*10^6/L)	2475.0 (971.5, 3900.5)
Polymorphonuclear cells in dialysis effluent (%)	86.1 (77.1–92.4)
White blood cell (*10^9/L)	8.3 ± 5.6
Percentage of neutrophil (%)	76.1 ± 9.9
Hemoglobin (g/ L)	92.6 ± 21.2
Serum creatinine (μmol/L)	813.0 (621.4, 1109.4)
Serum albumin (g/L)	28.9 ± 5.8
C- reactive protein (mg/ L)	57.2 (11.8, 87.4)
Procalcitonin (ng/mL)	0.6 (0.3, 4.7)
Dialysate sample collection time (day)	1.0 (1.0, 3.5)
Patients using antibiotics within 2 weeks prior to dialysate specimen collection (*n*/%)	14 (34.1%)

Data are presented based on number of samples ($n=41$) except for sex, primary disease, and comorbidities

without. Still, these differences were not statistically significant. Thus, the presence of diabetes and prior peritonitis history did not appear to influence the positive rates of the two detection methods.

Pathogen spectrum detected by mNGS and conventional culture

Among the 38 mNGS-positive samples, 19 reported only 1 pathogen, while mixed pathogens were identified in the remaining 19 samples. In total, 59 records of pathogenic microorganisms encompassing 22 different pathogen species were reported by mNGS reports. Of these, 55 were bacteria and 4 were fungi. In contrast, a total of 34 pathogen records from 18 different pathogen species were isolated by culture. Most culture-positive samples (30 out of 32) reported 1 pathogen, with only 2 samples detecting mixed pathogens. Gram-positive bacteria were the most frequently detected pathogens by both methods, followed by Gram-negative bacteria and fungus. Notably, mNGS also detected Mycobacterium tuberculosis in one sample (Fig. 3A, B).

Comparison analysis at pathogen-type level

Of the 22 pathogen species detected by dialysate mNGS, *Staphylococcus* epidermidis was the most common one, being positive in 14 (34.1%) of the 41 dialysate samples. The next seven common pathogens were Staphylococcus hominis, Klebsiella pneumoniae, Corynebacterium striatum, Stenotrophomonas maltophilia, Staphylococcus lugdunensis, Candida parapsilosis, and Candida tropicalis, which were detected in 12 (29.3%), 6 (14.6%), 4 (9.8%), 3 (7.3%), 2 (4.9%), 2 (4.9%), and 2 (4.9%) of the 41 dialysate samples, respectively. The remaining 14 pathogen species were singletons, each detected in only one sample (2.4%). The proportion of each pathogens and numbers of sequence reads reported by mNGS are illustrated in Fig. 4A, B. Of note, mNGS also detected viruses in 10 samples, predominantly Torque teno virus and Human betaherpesvirus 5, which were not listed as pathogenic microorganisms, since they are not common pathogens of PD-related peritonitis (Supplementary Table 1). The most frequently pathogen identified

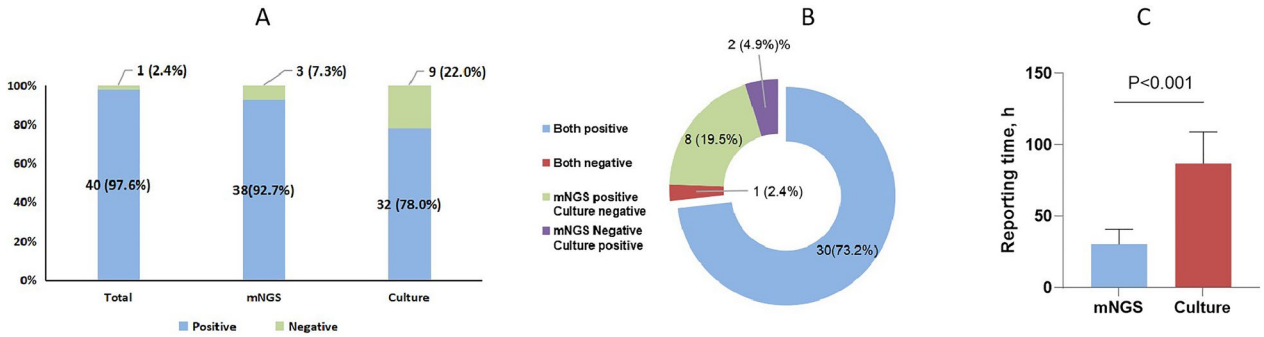

Fig. 2 Comparison of detection rate and reporting time of mNGS and culture. **A** Positive rates of mNGS and conventional culture ($n=41$); **B** Pie chart demonstrated the positivity distribution of mNGS and culture for all samples ($n=41$); C Reporting time of mNGS and conventional culture ($n=41$)

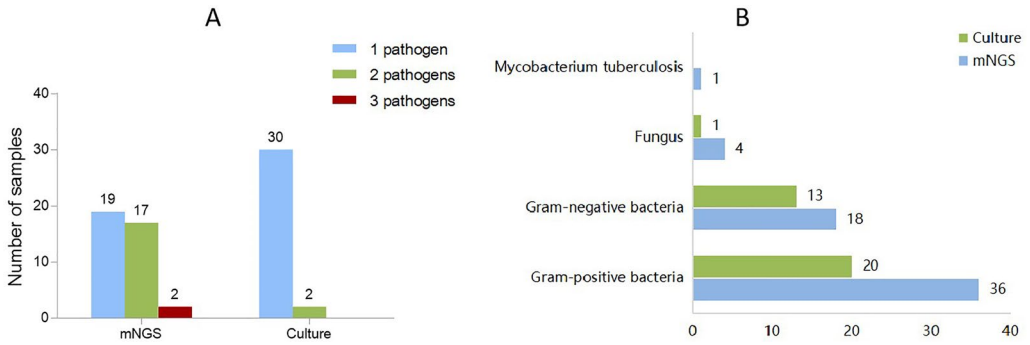

Fig. 3 Pathogens reported by mNGS and culture. **A** Comparison of numbers of pathogens detected by mNGS and culture; **B** Comparison of pathogen distribution by mNGS and culture

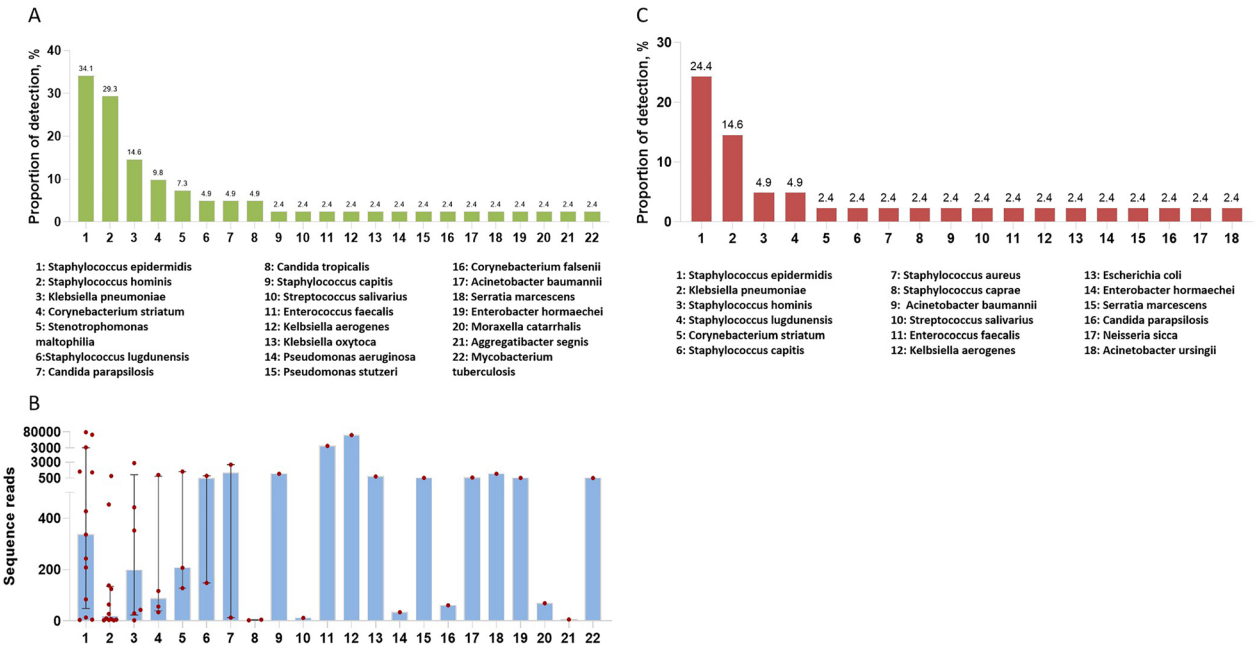

Fig. 4 Pathogens species detected by mNGS and culture. **A** The proportions of each species reported by mNGS. **B** Sequence reads of different pathogens reported by mNGS. **C** The proportions of each species reported by culture

by dialysate culture was also Staphylococcus epidermidis ($n=10$, 24.4%), followed by Klebsiella pneumoniae ($n=6$, 14.6%), Staphylococcus hominis ($n=2$, 4.9%), and Staphylococcus lugdunensis ($n=2$, 4.9%). There were 14 pathogen species detected in only one sample (Fig. 4C).

Concordance between mNGS and culture for pathogen detection

Among the 30 specimens that tested positive by both methods, the pathogens detected were completely matched (overlapped of all pathogens) in 13 samples, partly matched (overlapped of at least one but not all pathogens) in 14 samples, and totally mismatched (overlapped of no pathogen) in 3 samples. In the 13 samples with consistent pathogens, both detection methods identified only one pathogen per sample. In 13 of the 14 samples with partially consistent pathogens, conventional culture detected only one pathogen, while mNGS identified two or more pathogens per sample, with the pathogen having the highest sequence number being consistent with the pathogen detected by culture. Among the 9 culture-negative samples, mNGS detected pathogens in 8, the most common of which was Gram-positive bacteria, including Staphylococcus (detected in 5 samples) and Corynebacterium (detected in 2 samples). Moreover, mNGS also identified atypical or difficult-to-culture pathogens such as Mycobacterium tuberculosis (Table 2). It is worth mentioning that among the 4

Table 2 Concordance between mNGS and culture for pathogen detection

Samples (n)	mNGS		Culture
	Pathogens	Sequence reads[a]	
4	*Staphylococcus epidermidis*	3406 (208, 66,746)	*Staphylococcus epidermidis*
3	*Klebsiella pneumoniae*	442 (3, 2864)	*Klebsiella pneumoniae*
2	*Staphylococcus lugdunensis*	518 (148, 888)	*Staphylococcus lugdunensis*
1	*Staphylococcus hominis*	852	*Staphylococcus hominis*
1	*Serratia marcescens*	1199	*Serratia marcescens*
1	*Streptococcus salivarius*	12	*Streptococcus salivarius*
1	*Enterobacter hormaechei*	569	*Enterobacter hormaechei*
3	*Klebsiella pneumoniae* *Staphylococcus hominis*	43 (30, 352) 5 (3, 7)	*Klebsiella pneumoniae*
2	*Staphylococcus epidermidis* *Corynebacterium striatum*	40,325 (1420, 79,229) 76 (34, 117)	*Staphylococcus epidermidis*
1	*Staphylococcus epidermidis* *Stenotrophomonas maltophilia*	1541 128	*Staphylococcus epidermidis*
1	*Staphylococcus epidermidis* *Moraxella catarrhalis* *Candida tropicalis*	336 69 3	*Staphylococcus epidermidis*
1	*Staphylococcus epidermidis*	427	*Staphylococcus epidermidis* *Staphylococcus hominis*
1	*Staphylococcus capitis* *Pseudomonas stutzeri*	1197 552	*Staphylococcus capitis*
1	*Enterococcus faecalis* *Staphylococcus epidermidis*	13,338 4244	*Enterococcus faecalis*
1	*Klebsiella aerogenes* *Staphylococcus hominis*	65,286 3	*Klebsiella aerogenes*
1	*Corynebacterium striatum* *Staphylococcus epidermidis*	56 5	*Corynebacterium striatum*
1	*Acinetobacter baumannii* *Klebsiella oxytoca* *Staphylococcus hominis*	610 581 64	*Acinetobacter baumannii*
1	*Candida parapsilosis* *Staphylococcus hominis*	2611 4	*Candida parapsilosis*
1	*Staphylococcus epidermidis* *Candida parapsilosis*	84 13	*Staphylococcus caprae*
1	*Stenotrophomonas maltophilia* *Staphylococcus epidermidis*	1539 14	*Neisseria sicca*
1	*Stenotrophomonas maltophilia* *Staphylococcus hominis*	207 21	*Staphylococcus epidermidis* *Acinetobacter ursingii*
2	*Staphylococcus hominis*	74 (10, 138)	Negative
1	*Staphylococcus hominis* *Pseudomonas aeruginosa*	453 34	Negative
1	*Corynebacterium falsenii* *Staphylococcus epidermidis*	61 4	Negative
1	*Staphylococcus hominis* *Candida tropicalis*	125 5	Negative
1	*Aggregatibacter segnis*	6	Negative
1	*Corynebacterium striatum*	1025	Negative
1	*Mycobacterium tuberculosis*	543	Negative
1	Negative	–	*Escherichia coli*
1	Negative	–	*Staphylococcus aureus*
1	Negative	–	Negative

a: Data are presented as median (min, max) for samples ≥ 2

samples indicated as fungi positive by mNGS, only the one with simultaneous fungi-positive culture experienced PD technique failure, while the other three patients (the sequence reads of fungi were significantly lower than those of bacteria detected at the same time in these samples) all showed improvement after treatment.

Impact of antibiotics exposure on the results of mNGS and culture

An additional analysis was conducted to evaluate the impact of antibiotics on the results of mNGS and culture. As aforementioned, 14 patients (with 14 episodes of peritonitis) used antibiotics within 2 weeks prior to dialysate specimen collection. There was no significant difference regarding positive rates of mNGS between these samples and samples collected from patients not receiving antibiotics (92.9% vs 92.6%, $P=1.000$). However, the positive rate of culture in these antibiotic-exposed samples was notably lower than in samples from patients who did not receive antibiotics (57.1% vs 88.9%, $P=0.042$). Although not statistically significant, there was a trend toward a difference in positive rates of mNGS and culture in these 14 samples (92.9% vs 57.1%, $P=0.063$) (Fig. 5).

Discussion

The 2022 ISPD Guidelines for PD-associated peritonitis suggests the use of innovative diagnostic technologies for detecting pathogens in PD-related peritonitis [11]. mNGS offers a wider range of pathogen identification, making it valuable in diagnosing and treating various infectious diseases. This study investigated the clinical efficacy of mNGS for the detection of pathogens in PD-related peritonitis and found that mNGS had a higher positive rate and shorter reporting time compared to culture. For samples that yielded positive results by both methods, mNGS had good consistency with culture. Antibiotics treatment significantly reduced the detection rate of culture, while that of mNGS was not affected.

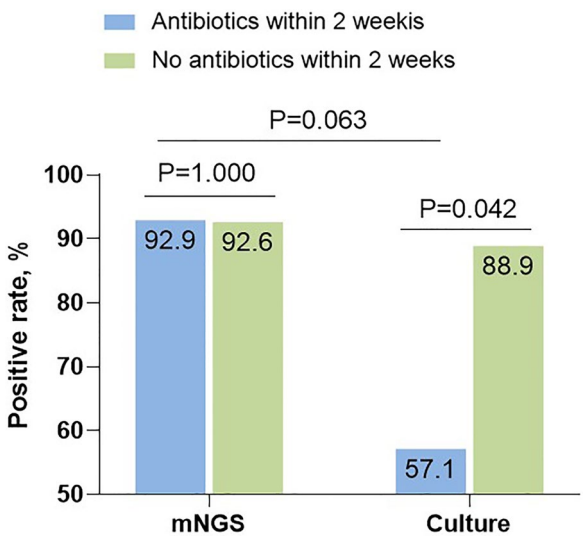

Fig. 5 Positive rates of mNGS and culture in samples of patients receiving or not receiving antibiotics within 2 weeks before collection

mNGS was reported to have higher positive rates in pathogens identification in numerous diseases [12–14]. However, data regarding PD-associated peritonitis were relatively rare. In the present study, we prospectively recruited 38 patients with 41 episodes of PD-related peritonitis and compared the detection rate of mNGS and culture. The result showed that mNGS yielded a positive rate of 92.7%, higher than that of culture, though not statistically different, which might be due to the small sample size investigated. Future studies with larger sample sizes are needed to further confirm the superiority of mNGS over culture in pathogen detection rate in these patients. In addition, we found that the reporting time of mNGS was significantly shorter than that of culture, providing a substantial advantage in terms of timeliness, which could help facilitate the early administration of precise antibiotics, thereby reducing the risk of secondary infections and other side effects caused by prolonged broad-spectrum antibiotics use.

mNGS provides a wide range of microbial profiles and can detect many potential infectious agents in a single assay [15]. Previous studies in various infectious diseases revealed that mNGS identified more co-pathogens than culture [4, 9, 14, 16]. Likewise, the types and quantities of pathogens identified by mNGS in our study were much more than those identified by culture, with multiple pathogens being detected in nearly half of the samples. In addition to the number of pathogens detected, most studies on mNGS focused on the diagnostic value of specific pathogens. In Ye's study [9], mNGS detected pathogens that were difficult to be identified by culture, including viruses and Mycobacterium tuberculosis, which were also detected by mNGS in the present study, although viruses were all seen in samples that showed co-infection of bacteria and were not interpreted as pathogenic microorganisms since their role as pathogens for PD-related peritonitis had not yet been proved. mNGS also detected more fungi in our study, but only the one with simultaneous fungi-positive culture experienced PD technique failure, while the other three patients all showed improvement after antibiotics therapy. Since fungal peritonitis is usually associated with treatment failure, pathogenicity of fungi in these patients remains to be determined. These together suggest that mNGS is a more sensitive method for pathogen identification; however, the results should be interpreted with caution.

The consistency between mNGS and culture has been a topic of discussion in numerous studies. Some researchers displayed poor concordance between mNGS and culture, often attributed to the low positive rate and narrow detection range of etiological culture [12, 16, 17]. Additional factors that might contribute to this discrepancy included the non-simultaneous collection of specimens [18], and low sensitivity of mNGS in detecting certain pathogens [19]. In the present study, we found that among the 30 samples that tested positive by both methods, 27 (90%) showed completely (13 cases) or partly (14 cases) matched results between mNGS and culture. Furthermore, in the 14 samples with partially consistent results, the pathogen with the highest sequence reads detected by mNGS matched the pathogen identified by culture. In agreement with our findings, Ye et al. reported a 72.2% concordance rate in pathogen detection between mNGS and culture in PD-related peritonitis. These results suggest that there is favorable consistency between mNGS and culture in PD-related peritonitis, and selecting antibiotics based on the pathogens with the highest number of mNGS detection sequences might be appropriate in subsequent treatment for peritonitis, which needs further study.

Previous studies indicate that culture-negative peritonitis is often caused by gram-positive bacteria that fail to reach the threshold of detection [20, 21]. Our result showed that among the 9 cases of culture-negative peritonitis, 6 had positive bacteria detected by mNGS, primarily Staphylococcus, with relatively low sequence reads. This supports the views of previous literatures, although the pathogenicity of these bacteria remains to be elucidated. Apart from gram-positive bacteria, we also identified Mycobacterium tuberculosis. This highlights the importance of considering atypical pathogens in cases of culture-negative peritonitis when empirical treatment is ineffective.

Although many studies have reported the impact of prior antibiotic exposure on mNGS testing results, studies focusing on patients with PD-related peritonitis are rare. To the best of our knowledge, only one study to date has investigated the influence of antibiotics on mNGS outcomes in this population, involving 26 individuals, half of whom had previously used antibiotics. The findings indicated that antibiotic usage led to a significant decrease in the culture-positive rate, while the mNGS-positive rate remained unaffected. Our findings align with these results, suggesting that mNGS can enhance pathogen detection in PD-related peritonitis patients with a history of antibiotic use, thereby facilitating the targeted prescription of antibiotics.

The present study has several limitations. Firstly, being a single-center study with a relatively small sample size, there may be inherent bias. Secondly, the lack of a negative control makes it challenging to validate the false-positive rate of mNGS. Additionally, while mNGS shows high sensitivity in identifying pathogens, there is a need to enhance its specificity in distinguishing contamination or colonization from infection during data analysis and interpretation. Lastly, it is important to note that mNGS does not provide information on drug susceptibility. Whether treatment decisions can be made based on mNGS results needs to be further investigated.

Conclusions

In summary, this study revealed that mNGS exhibited higher sensitivity and a shorter reporting time compared to culture in detecting pathogens in PD-related peritonitis. Moreover, the consistency between mNGS and culture was substantial for double-positive samples. Thus, mNGS may offer a novel approach for the etiological diagnosis of PD-associated peritonitis, particularly in cases involving prior antibiotic use and unusual pathogens. Nevertheless, the feasibility of guiding peritonitis treatment based on mNGS results requires validation through further studies.

Abbreviations

mNGS	Metagenomic next-generation sequencing
PD	Peritoneal dialysis
ISPD	International peritoneal dialysis association
SMRN	Number of stringently mapped reads

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-02322-y.

Supplementary material 1. Table 1. Viruses detected by mNGS

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Not applicable

Author contributions

QYZ conceived of the presented idea, collected the data and wrote the manuscript. YF, YYX, MZ and PFX conceived of the presented idea and collected the data. TFT verified the analytical methods. QYS and JL collected data. BJ, SXL and CMJ conceived of the presented idea and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The protocol of this study is in accordance with the Helsinki Declaration. All subjects were informed about the aim of this study and agreed to participate. The protocol was approved by the Ethics Committee of Nanjing Drum Tower Hospital (Approval number 2021-365-01).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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