Am J Transl Res 2024;16(12):7707-7715
www.ajtr.org /ISSN:1943-8141/AJTR0159395

Original Article

Clinical value of metagenomic sequencing in system evaluation of potential donors and donor-derived infection in kidney transplantation

Lian Tan[1], Bangsheng Chen[2], Yudong Xu[1], Zhengfeng Wen[1], Bo Feng[1], Danqi Chen[1], Xiongxiong Wang[1], Xuena Cui[1], Dongjun Hu[1]

[1]*Intensive Care Medicine Department, Ningbo Urology and Kidney Disease Hospital, Ningbo 315100, Zhejiang, China;* [2]*Emergency Medical Center, Ningbo Urology and Kidney Disease Hospital, Ningbo 315100, Zhejiang, China*

Received July 23, 2024; Accepted October 31, 2024; Epub December 15, 2024; Published December 30, 2024

Abstract: Objective: To explore the application and the clinical value of metagenomic sequencing in system evaluation of potential kidney donors, along with donor-derived infection in kidney transplantation. Methods: A prospective study was conducted on 40 voluntary renal donors in Ningbo Urology and Kidney Disease hospital from January 2021 to August 2023. The results of donor pathogen fed back by metagenomic sequencing were analyzed to understand the clinical significance of metagenomic sequencing in donor evaluation. Results: (1) Detection rate of pathogens. The probability of pathogens detected by traditional laboratories and metagenomic sequencing was 72.50% and 90.00%, respectively. Compared with traditional laboratory tests, metagenomic sequencing detected significantly more pathogens ($P < 0.05$). The percentage of co-infection of multiple pathogens detected by traditional laboratory tests (31.03%) in donors was significantly lower than that detected by metagenomic sequencing (88.89%) ($P < 0.001$). Traditional laboratory tests detected bacteria in 20 donors and fungi in 9 donors, but its performance on detecting viruses and mycoplasmas was limited. Metagenomic sequencing detected bacteria in 30 donors, fungi in 12 donors, viruses in 9 donors, and mycoplasmas in 9 donors. The positive rates of bacteria, viruses and mycoplasmas detected by metagenomic sequencing were significantly higher than those detected by traditional laboratory tests ($P < 0.05$). (2) Predictive value. The sensitivity, specificity, positive predictive value, and negative predictive value of metagenomic sequencing were 97.30%, 100.00%, 100% and 75.00%, respectively, while those of traditional laboratory tests were 78.39%, 100.00%, 100.00% and 27.27%, respectively. (3) The diagnostic efficiency of metagenomic sequencing was superior to that of traditional laboratory tests. (4) Time needed for result feedback. From specimen collection to the result feedback given to the clinician, the time required for traditional laboratory tests was longer than that for metagenomic sequencing, with significant differences ($P < 0.001$). In addition, the required time for traditional laboratory tests in detecting bacterial positivity was longer than that for metagenomic sequencing, with a statistically significant difference ($P < 0.001$). Conclusion: This study probes into the application of metagenomic sequencing in the evaluation of donor pathogens, especially in negative samples detected by traditional laboratory tests. Our findings suggest that metagenomic sequencing can improve the sensitivity and specificity of diagnosis, increase the detection rate of pathogens, and minimize the turnover time.

Keywords: Metagenomic sequencing, kidney transplantation, tissue donors

Introduction

Kidney transplantation is the preferred treatment for end-stage renal disease as it significantly enhances patient survival rates and quality of life compared to maintenance dialysis [1]. While transplantation extends the lifespan of patients with renal failure, it also elevates the risk of donor-derived infections, thereby increasing the potential for donor-derived diseases in recipients. Recent reports indicate a rise in unexpected donor-derived diseases among solid organ recipients, which has led to higher mortality rates [2]. Consequently, precise monitoring of pathogenic microorganisms associated with donor infections is essential for improving patient outcomes and reducing morbidity and mortality.

https://doi.org/10.62347/ZNKM3687

Traditional detection methods are time-consuming, have low positive detection rates, and may fail to identify specific pathogens [3]. Additionally, the complexity of donor infections, limited clinical evaluation windows, and the urgency of organ transplantation underscore the inadequacies of conventional methods such as serology in donor pathogen screening. In recent years, metagenomic sequencing has emerged as a promising technology in medical microbiology due to its cost-effectiveness, rapid turnaround, and ease of use [4, 5]. This non-targeted approach determines the DNA/RNA sequences of microorganisms across various sample types [6], allowing for the detection of transplantation-related infections and co-infections without prior assumption of the pathogens involved. It facilitates hypothetical and culture-independent pathogen detection directly from clinical specimens and even enables the discovery of novel organisms [7, 8]. However, there is limited research on the use of metagenomic sequencing for evaluating potential donors. This study aims to investigate the value of metagenomic sequencing in the systematic evaluation of potential donors and in addressing donor-derived infections in kidney transplantation.

Materials and methods

Research design

A prospective study was conducted on 40 voluntary renal donors admitted to Ningbo Urology and Kidney Disease Hospital from January 2021 to August 2023. All donors were informed about the study purpose and provided written informed consent prior to sample collection. The study adhered to the principles of the Declaration of Helsinki (2013) [9] and received approval from the Ethics Committee of Ningbo Urology and Kidney Disease Hospital (No. 2021-P-041). It was also registered at the Chinese Clinical Trial Registration Center (ChiCTR2400-8033).

Inclusion and exclusion criteria

Inclusion criteria: (1) donors who were aged 16-60 years old; (2) donors with a negative infectious disease screening, including tests for human immunodeficiency virus (HIV), hepatitis B and C, and syphilis; (3) donors with no evidence of active infections based on clinical presentation and preliminary laboratory tests; (4) donors with a body mass index (BMI) of 18.5 to 30 kg/m^2.

Exclusion criteria: (1) living donors for kidney transplantation; (2) those with tumor and other diseases; (3) those with severe mental disorders or other psychological problems; (4) those with dependence on drugs, illicit drugs or other substances.

Gold standard for infection

The gold standard for diagnosing infection in this study was based on a combination of clinical signs, symptoms, and confirmatory laboratory tests. Specifically, the presence of infection was confirmed by: (1) clinical signs and symptoms including fever and leukocytosis; (2) positive cultures from blood, urine, or other relevant bodily fluids; (3) radiological evidence consistent with infection, such as pneumonia or sepsis; (4) histopathological signs of infection, such as inflammation or abscess formation; (5) microbiological positive identification of pathogens through culture or molecular diagnostics, such as polymerase chain reaction (PCR).

Research methods

Data collection: Baseline data of donors were collected, including gender, age, history of antibiotic exposure, preoperative infection, infection sites, medical history, and hospitalization time in the intensive care unit. The bronchial alveolar lavage fluid (BALF) samples of donors and the results of traditional laboratory tests were collected before kidney transplantation.

Traditional laboratory tests: BALF was collected after the donors were admitted to the intensive care unit. The operation method was as follows. The end of the fiberoptic bronchoscope was placed into the opening of the bronchus at the site of pulmonary infiltration lesions under aseptic operation, and then 37°C sterile saline was injected into the biopsy port of the fiberoptic bronchoscope. Subsequently, 5 mL of BALF was retrieved and placed in a sterile container, stored at -20°C, and sent to the microbiology laboratory for the smear and culture of bacteria and fungi and the detection of viruses and mycoplasmas using PCR. The types and quantities of pathogens such as bacteria and fungi in

BALF were counted and identified by two experienced physicians.

Bacterial PCR: Primers targeting the 16S rRNA gene were used for bacterial detection. Specific primer sequences were: forward primer: 5'-AGAGTTTGATCMTGGCTCAG-3'; reverse primer: 5'-TACGGYTACCTTGTTACGACTT-3'.

Viral PCR: Primers targeting conserved regions of viral genomes were used for viral detection. Specific primer sequences were: Influenza A: forward primer: 5'-GGGGAAATCCTGCTTAGTC-3', reverse primer: 5'-CAAGGTCAGGGACCATCTA-3'; respiratory syncytial virus (RSV): forward primer: 5'-AGGAAGAACCCGTGGAAC-3', reverse primer: 5'-CACCTTCTGCCCTCTTCTT-3'.

Metagenomic sequencing: The BALF samples of donors were collected before kidney transplantation and sent to Hangzhou Matridx Biotechnology Co., Ltd. for metagenomic next-generation sequencing. After inactivating the samples, the nucleic acid was extracted and compared with the existing gene database to obtain the sequence number of the report results.

Library preparation: DNA and RNA were extracted from BALF samples. Libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, USA) and TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA).

Sequencing: Sequencing was performed on the Illumina NovaSeq 6000 platform using 150 bp paired-end reads.

Data analysis: Raw sequencing data were processed using quality control filters, trimmed for adapters, and aligned to the human genome (GRCh38) to remove host-derived sequences. The remaining reads were aligned to a comprehensive database of microbial genomes (NCBI RefSeq and GenBank) using BLASTn and Bowtie 2. Specific primers and probes were designed for targeted pathogens: bacteria (primers targeting conserved regions of the 16S rRNA gene were used for initial screening); viruses (primers targeting conserved regions of viral genomes, including influenza, RSV, and others, were used); fungi and mycoplasmas (primers targeting specific conserved regions were used for fungal and mycoplasma detection).

Positive threshold and interpretation criteria for metagenomic sequencing: The positive threshold in the report was determined by the number of millions of molecular sequences. The determination of the positive threshold did not depend on a single indicator, including but not limited to the detected sequence number of specific microorganisms, the ratio of normalized reads per million, and the genome coverage of the detected species. Since viruses rarely survive outside the body, a small number of specific sequences could be detected as positive (e.g., specific sequences ≤ 3), avoiding the detection of clinically irrelevant environmental bacteria, symbiotic bacteria, and opportunistic pathogens. The larger the sequence number, the greater the likelihood of pathogenic microorganisms (dozens of specific sequences). The difficult-to-detect pathogens such as Mycobacterium tuberculosis, Yersinia pestis and Brucella could be detected using independent interpretation standards. If one specific sequence was detected, it could be judged as positive. Due to the complexity of parasite genomes and their similarity to human genomes, the interpretation of parasite genomes was performed after strict confirmation of sequence specificity.

Statistical methods

This study adopted SPSS software (version: 27.0; manufacturer: International Business Machines Corporation; origin: Armonk, New York, USA) to analyze and process the data. When $P < 0.5$, the differences were considered statistically significant. Categorical variables, such as detection rate of pathogens, were tested by chi-square test or Fisher's exact test and expressed as [n (%)]. Continuous variables, such as time of result feedback by different inspection methods, were tested for normal distribution by Shapiro-Wilk test. The continuous variables that did not conform to the normal distribution were tested by non-parametric test and represented by M (P_{25}, P_{75}). **Figure 1** was plotted using WPS Office Excel (version: 2021; manufacturer: Kingsoft; origin: Beijing, China).

Results

Baseline characteristics of donors

The baseline characteristics of the donors in this study are shown in **Table 1**. The median age of the 40 donors was 40.50 years old, and

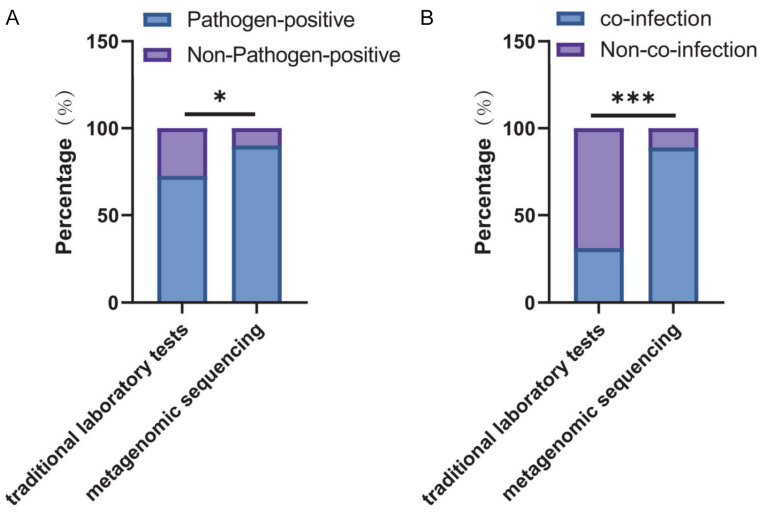

Figure 1. Detecting results in donors. A: Results of pathogen-positive; B: Results of co-infection. *: $P < 0.05$; ***: $P < 0.001$.

male donors accounted for a larger proportion of samples (75.00%). The average hospitalization time in the intensive care unit was 63.00 hours. Moreover, 92.50% of donors had a history of invasive procedures and were diagnosed with infection, and only a few donors (7.50%) had no history of antibiotic exposure.

Pathogen rates of donors

The pathogen rates detected by traditional laboratory tests and metagenomic sequencing were 72.50% (29/40) and 90.00% (36/40), respectively. Compared with the traditional laboratory tests, the metagenomic sequencing detected significantly more pathogens (χ^2 = 4.021, P = 0.045) (**Figure 1A**). The percentage of co-infection of multiple pathogens detected by metagenomic sequencing (88.89%, 32/36) was significantly higher than that detected by traditional laboratory tests (31.03%, 9/29) (χ^2 = 23.083, $P < 0.001$), as shown in **Figure 1B**.

Types of pathogens in donors

Traditional laboratory tests detected bacteria in 20 donors and fungi in 9 donors, but its performance on detecting viruses and mycoplasmas was limited. Metagenomic sequencing detected bacteria in 30 donors, fungi in 12 donors, viruses in 9 donors, and mycoplasmas in 9 donors. In comparison, the positive rates of bacteria, virus, and mycoplasmas detected by metagenomic sequencing were significantly higher than those detected by traditional laboratory tests ($P < 0.05$), as shown in **Table 2**.

Predictive value of different detection methods for infections in donors

A total of 40 samples were detected in this study. The results of gold standard examinations showed that there were 37 infected donors and 3 uninfected ones. Pathogens were detected in 36 donors by metagenomic sequencing, with a sensitivity of 97.30%, a specificity of 100.00%, a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 75.00%. Pathogens were detected in 29 donors by traditional laboratory tests, and the sensitivity, specificity, PPV and NPV were 78.39%, 100.00%, 100.00% and 27.27%, respectively. See **Table 3**.

Diagnostic efficacy of different examination methods of infections in donor patients

The receiver-operating characteristic curve showed that the diagnostic efficiency of metagenomic sequencing was better than that of traditional laboratory tests, as shown **Figure 2** and **Table 4**.

Time needed for result feedback by different detection methods

From specimen collection to the result feedback given to the clinician (T1), the time required for traditional laboratory tests was longer than that for metagenomic sequencing, with a significant difference ($P < 0.001$). In addition, the feedback time of traditional laboratory tests in detecting bacterial positive (T2) was longer than that of metagenomic sequencing, with statistically significant difference ($P < 0.001$), as shown in **Table 5**.

Discussion

This prospective study examines the advantages of metagenomic sequencing in infection status assessment and pathogen identification among kidney transplant donors. Metagenomic

Table 1. Baseline characteristics of donors

Items (n = 40)		n (%)/M (P_{25}, P_{75})
Gender	Male	30 (75.00)
	Female	10 (25.00)
Age (years)		40.50 (27.25, 46.00)
History of antibiotic exposure	Tigecycline	4 (10.00)
	Cefoperazone sulbactam	5 (12.50)
	Piperacillin and tazobactam	11 (27.50)
	Biapenem	1 (2.50)
	Vancomycin	3 (7.50)
	Polymyxin E	3 (7.50)
	Voriconazole	26 (65.00)
	Meropenem	12 (30.00)
	Caspofungin	9 (22.50)
	Linezolid	6 (15.00)
	Imipenem	3 (7.50)
	Ceftazidime/Avibactam	4 (10.00)
	Fosfomycin	2 (5.00)
	No	3 (7.50)
History of invasive procedures	Deep venous catheterization	37 (92.50)
	Tracheal intubation	37 (92.50)
	Gastric tube	37 (92.50)
	Urethral catheter	37 (92.50)
	Arterial puncture	37 (92.50)
	No	3 (7.50)
Preoperative infection	Yes	37 (92.50)
	No	3 (7.50)
Infection sites	Lung	36 (90.00)
	No	4 (10.00)
Medical history	Hypertension	8 (20.00)
	Thyroid hyperfunction	3 (7.50)
	Asthma	1 (2.50)
	No	28 (70.00)
Hospitalization time in intensive care unit (h)		63.00 (49.50, 71.75)
Body mass index (kg/m^2)		22.92 (22.54, 23.85)
Blood groups	A	9 (22.50)
	B	12 (30.00)
	AB	9 (22.50)
	O	10 (25.00)

Table 2. Detection rate of different pathogens in donors (%)

Pathogens	Traditional laboratory tests	Metagenomic sequencing	Statistical value	P
Bacteria	68.97 (20/29)	91.67 (33/36)	5.499	0.019
Fungi	31.03 (9/29)	33.33 (12/36)	0.039	0.844
Viruses	0.00 (0/29)	25.00 (9/36)	-#	0.003#
Mycoplasmas	0.00 (0/29)	25.00 (9/36)	-#	0.003#

Notes: # indicated Fisher's exact test results, - indicated no data.

Table 3. Predictive value of traditional laboratory tests and metagenomic sequencing of infections in donors (cases)

Detection methods	Gold standard		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Infected (37)	Uninfected (3)				
Traditional laboratory tests						
+	29	0	78.39	100.00	100.00	27.27
-	8	3				
Metagenomic sequencing						
+	36	0	97.30	100.00	100.00	75.00
-	1	3				

PPV: positive predictive value, NPV: negative predictive value.

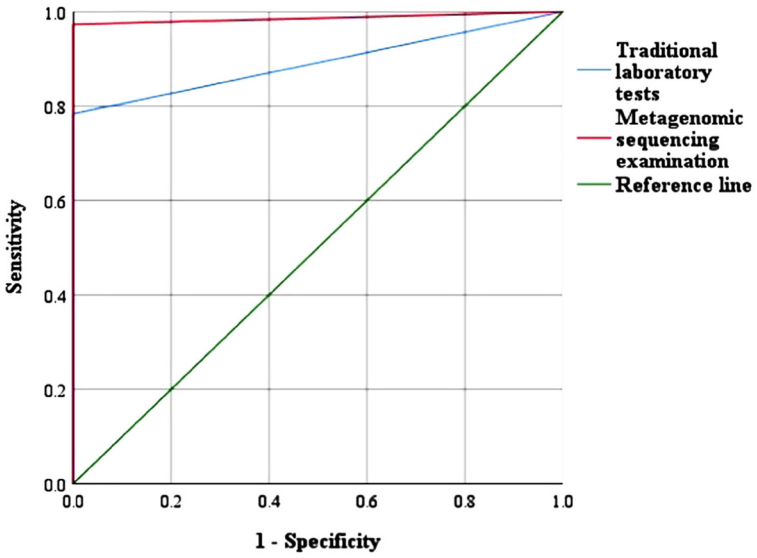

Figure 2. Receiver-operating characteristic curve.

sequencing is widely employed for detecting various infectious pathogens [10]. This study introduces several innovations: it demonstrates metagenomic sequencing's superior detection capabilities compared to traditional labo ratory tests, particularly for viruses and mycoplasmas often overlooked by conventional methods. It also provides faster turnaround time, facilitating quicker clinical decision-making and timely interventions. Moreover, it underscores the significance of comprehensive pathogen detection in organ transplantation, potentially enhancing recipient outcomes. The application of metagenomic sequencing in donor evaluation marks a significant advancement, offering a more robust and reliable method to ensure the safety of organ transplantation.

Organ transplantation is the preferred treatment for end-stage organ failure, with kidney transplantation being particularly favored for patients with advanced or end-stage conditions [11]. Since January 1, 2015, community organ donation has been the sole legitimate source of transplantation in China. Donors in this model often experience severe traumatic brain injury, neurosurgery, or prolonged ICU stays, thereby increasing infection risks prior to organ acquisition and potentially elevating transmission risks to the recipient post-transplant [12].

Although both short-term and long-term clinical outcomes for kidney transplant patients have improved, post-transplant infections remain a serious complication [13]. In many transplant centers, donors with bacteremia or organ infections are considered as contraindications for transplantation; however, some infections can be managed, necessitating rapid pathogen identification and proactive treatment to expand donor pools. Early and accurate pathogen detection in donors can optimize targeted antibiotic and immunosuppressant use, improving post-transplant outcomes. Traditional laboratory methods such as cell culture, serological assays, and nucleic acid detection are time-consuming and may not identify all potential pathogens, thus affecting prevention and treatment efforts. In contrast, metagenomic se-

Table 4. Areas under curve

Variables	Areas under curve	Standard error	Progressive significance	Progressive 95% CI
Traditional laboratory tests	0.892	0.057	0.026	0.781-1.000
Metagenomic sequencing	0.986	0.017	0.006	0.953-1.000

Table 5. Comparison of time needed for result feedback by different detection methods [M (P_{25}, P_{75}), h]

Detection methods	Number of donors	T1	T2
Traditional laboratory tests	40	101.65 (98.06, 105.20)	68.18 (82.84, 99.03)
Metagenomic sequencing	40	39.79 (38.53, 41.01)	30.36 (28.84, 31.55)
Z	-	-7.698	-7.698
P	-	< 0.001	< 0.001

quencing, a novel diagnostic approach, effectively sequences and identifies microbial RNA and DNA in clinical samples [14]. Its low cost, rapid data analysis, and comprehensive databases have broadened its application in detecting donor pathogens [7, 15].

This study utilized metagenomic sequencing for pathogen detection in donors, revealing significantly higher positive detection rates compared to traditional tests ($P < 0.05$), which is consistent with Zhao et al.'s findings [4]. Metagenomic sequencing can directly extract and sequence all nucleic fragments in specimens, comparing them to reference sequences from a microbiology-specific database and analyzing them with intelligent algorithms, reducing false negatives [16, 17]. Unlike traditional tests, which struggle to detect viruses and mycoplasmas, metagenomic sequencing effectively detected bacteria, fungi, viruses, and mycoplasmas in 24 donors. The detection rates for these pathogens were significantly higher than those obtained via traditional methods ($P < 0.05$), underscoring metagenomic sequencing's advantages. Zhang et al. [18] applied this method to lung transplant recipients and found similar results, supporting our findings.

The study results exhibited metagenomic sequencing's sensitivity, specificity, PPV, and NPV at 97.30%, 100.00%, 100%, and 75.00%, respectively. In contrast, traditional laboratory tests yielded values of 78.39%, 100.00%, 100.00%, and 27.27%, respectively. Meng et al. [19] studied metagenomic sequencing in organ transplant patients with pulmonary infections, reporting comparable sensitivity and specificity, corroborating this study's results. These findings demonstrate metagenomic sequencing's higher diagnostic sensitivity and specificity in detecting donor infections. The time from specimen collection to result feedback was also found to be significantly shorter for metagenomic sequencing than for traditional laboratory tests ($P < 0.001$). Ju et al. [20] reported similar findings, highlighting metagenomic sequencing's efficiency in data collection and analysis.

This single-center study has some limitations, including a small donor sample size and potential susceptibility of metagenomic sequencing results to various factors. Future large, multicenter studies are necessary to further validate the application of metagenomic sequencing in evaluating potential donors and donor-derived infections in kidney transplantation.

Conclusion

This study highlights the application of metagenomic sequencing in evaluating donor pathogens, particularly in cases where traditional laboratory tests yield negative results. Metagenomic sequencing can enhance pathogen detection rates, confirm the absence of infections, and reduce turnover time.

Acknowledgements

This study received support from the 2021 Ningbo Science and Technology Plan Project (2021S119) and the 2020 Ningbo Yinzhou District Science and Technology Plan Project.

Disclosure of conflict of interest

None.

Address correspondence to: Dongjun Hu, Intensive Care Medicine Department, Ningbo Urology and Kidney Disease Hospital, No. 998 Qianhe North Road, Yinzhou District, Ningbo 315100, Zhejiang, China. E-mail: tanlianfootball@163.com

References

[1] Rangaswami J, Mathew RO, Parasuraman R, Tantisattamo E, Lubetzky M, Rao S, Yaqub MS, Birdwell KA, Bennett W, Dalal P, Kapoor R, Lerma EV, Lerman M, McCormick N, Bangalore S, McCullough PA and Dadhania DM. Cardiovascular disease in the kidney transplant recipient: epidemiology, diagnosis and management strategies. Nephrol Dial Transplant 2019; 34: 760-773.

[2] Kaul DR, Vece G, Blumberg E, La Hoz RM, Ison MG, Green M, Pruett T, Nalesnik MA, Tlusty SM, Wilk AR, Wolfe CR and Michaels MG. Ten years of donor-derived disease: a report of the disease transmission advisory committee. Am J Transplant 2021; 21: 689-702.

[3] Zhou WY, Shen L, Shi JX, Gao XH, Yang J, Fu SJ, Pan XF, Zhu MF, Zhang S, Zhang C, Li F, Zhang H, Yao F, Tenover FC, Tang YW and Fang WT. Real-time, random-access organ screening for carbapenem-resistant organisms (CRO) reduces CRO-associated, donor-derived infection mortality in lung transplant recipients. Infection 2024; 52: 403-412.

[4] Zhao D, Guo L, Lian D, Gu Y, Yan X, Hu H and Yuan J. Diagnostic value and clinical application of mNGS for post-liver transplantation infection: a cross-sectional study with case reports. Front Microbiol 2022; 13: 919363.

[5] Li N, Cai Q, Miao Q, Song Z, Fang Y and Hu B. High-throughput metagenomics for identification of pathogens in the clinical settings. Small Methods 2021; 5: 2000792.

[6] de Vries JJC, Brown JR, Couto N, Beer M, Le Mercier P, Sidorov I, Papa A, Fischer N, Oude Munnink BB, Rodriquez C, Zaheri M, Sayiner A, Hönemann M, Cataluna AP, Carbo EC, Bachofen C, Kubacki J, Schmitz D, Tsioka K, Matamoros S, Höper D, Hernandez M, Puchhammer-Stöckl E, Lebrand A, Huber M, Simmonds P, Claas ECJ and López-Labrador FX; ESCV Network on Next-Generation Sequencing. Recommendations for the introduction of metagenomic next-generation sequencing in clinical virology, part II: bioinformatic analysis and reporting. J Clin Virol 2021; 138: 104812.

[7] Simner PJ, Miller S and Carroll KC. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis 2018; 66: 778-788.

[8] Spatz S and Afonso CL. Non-targeted RNA sequencing: towards the development of universal clinical diagnosis methods for human and veterinary infectious diseases. Vet Sci 2024; 11: 239.

[9] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310: 2191-2194.

[10] Qian YY, Wang HY, Zhou Y, Zhang HC, Zhu YM, Zhou X, Ying Y, Cui P, Wu HL, Zhang WH, Jin JL and Ai JW. Improving pulmonary infection diagnosis with metagenomic next generation sequencing. Front Cell Infect Microbiol 2021; 10: 567615.

[11] O'Connell K, Kennedy C, Skally M, Foley M, Alex S, Magee C, Davis NF, Humphreys H and Burns K. Surveillance of common infections in the early period after renal transplantation in a national center: 2014-2017. Transpl Infect Dis 2020; 22: e13261.

[12] Tong L, Hu XG, Huang F, Huang SW, Li LF, Tang ZX, Yao JY, Xu JH, Zhu YP, Chen YH, He XS, Guan XD and Cai C. Clinical impacts and outcomes with possible donor-derived infection in infected donor liver transplantation: a single-center retrospective study in China. J Infect Dis 2020; 221 Suppl 2: S164-S173.

[13] Kim JS, Jeong KH, Lee DW, Lee SY, Lee SH, Yang J, Ahn C and Hwang HS; Korean Organ Transplantation Registry Study Group. Epidemiology, risk factors, and clinical impact of early post-transplant infection in older kidney transplant recipients: the Korean organ transplantation registry study. BMC Geriatr 2020; 20: 519.

[14] Afshinnekoo E, Chou C, Alexander N, Ahsanuddin S, Schuetz AN and Mason CE. Precision metagenomics: rapid metagenomic analyses for infectious disease diagnostics and public health surveillance. J Biomol Tech 2017; 28: 40-45.

[15] Yao M, Zhou J, Zhu Y, Zhang Y, Lv X, Sun R, Shen A, Ren H, Cui L, Guan H and Wu H. Detection of Listeria monocytogenes in CSF from three patients with meningoencephalitis by next-generation sequencing. J Clin Neurol 2016; 12: 446-451.

[16] Miao Q, Ma Y, Wang Q, Pan J, Zhang Y, Jin W, Yao Y, Su Y, Huang Y, Wang M, Li B, Li H, Zhou C, Li C, Ye M, Xu X, Li Y and Hu B. Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical practice. Clin Infect Dis 2018; 67: S231-S240.

[17] Parize P, Muth E, Richaud C, Gratigny M, Pilmis B, Lamamy A, Mainardi JL, Cheval J, de Visser L, Jagorel F, Ben Yahia L, Bamba G, Dubois M, Join-Lambert O, Leruez-Ville M, Nassif X, Lefort

A, Lanternier F, Suarez F, Lortholary O, Lecuit M and Eloit M. Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. Clin Microbiol Infect 2017; 23: 574.e571-574.e576.

[18] Zhang X, Tang X, Yi X, Lei Y, Lu S, Li T, Yue R, Pan L, Feng G, Huang X, Wang Y and Cheng D. Etiologic characteristics revealed by mNGS-mediated ultra-early and early microbiological identification in airway secretions from lung transplant recipients. Front Immunol 2023; 14: 1271919.

[19] Meng X, Zhang L, Fan X, Fang X and Pan A. Diagnostic value of detection of pathogens in bronchoalveolar lavage fluid by metagenomics next-generation sequencing in organ transplant patients with pulmonary infection. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2021; 33: 1440-1446.

[20] Ju CR, Lian QY, Guan WJ, Chen A, Zhang JH, Xu X, Chen RC, Li SY and He JX. Metagenomic next-generation sequencing for diagnosing infections in lung transplant recipients: a retrospective study. Transpl Int 2022; 35: 10265.