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ORIGINAL RESEARCH

Positive Metagenomic Next-Generation Sequencing of Renal Lavage Fluid Associates with Delayed Graft Function in Kidney Transplants from Donors After Circulatory Death: A Retrospective Study

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Background: Delayed graft function (DGF) is a major complication in kidney transplants from donation after circulatory death (DCD). This study assessed the association between metagenomic next-generation sequencing (mNGS) results and the occurrence of DGF during the perioperative period in DCD kidney transplant recipients.

Methods: We analyzed 191 DCD kidney transplant recipients in this single-center retrospective cohort study. All recipients underwent routine mNGS testing of renal lavage fluid between July 2021 and July 2024. Demographic, clinical, and microbial data were collected. Associations between mNGS results and DGF were evaluated using logistic regression models adjusted for covariates.

Results: The study revealed a strong association between mNGS positivity and DGF development. mNGS-positive recipients (n=97/191) showed significantly higher DGF incidence than mNGS-negative cases (30.9% vs 6.4%, $p<0.001$), highlighting the potential clinical utility of mNGS in predicting DGF. Multivariate analysis confirmed this association after adjusting for confounders (aOR=7.90, 95% CI 1.63–38.24). Bacterial pathogens constituted the majority of detected microorganisms (62.7%), with prevalent isolates including Enterococcus (37 cases) and Staphylococcus (29 cases) that harbored clinically relevant resistance genes.

Conclusion: Our findings demonstrate a significant association between mNGS positivity in renal lavage fluid and DGF development in DCD kidney recipients (aOR 7.90, 95% CI 1.63–38.24), These findings support further investigation into mNGS as a tool for early risk stratification and targeted antimicrobial therapy in DCD kidney recipients.

Keywords: kidney transplantation, antimicrobial resistance, delayed graft function, metagenomic next-generation sequencing, perioperative infections, transplant-related complications

Introduction

Kidney transplantation (KT) is the optimal treatment for end-stage renal disease (ESRD) but remains limited by donor organ shortages, prompting increased use of donation after circulatory death (DCD) kidneys.[1–3] In China, DCD has emerged as the primary source of transplant organs, reflecting both its potential to expand the donor pool and its growing acceptance in clinical practice.[4] However, research indicates that the occurrence of DGF is substantially higher in deceased donor (DD) transplants, with rates between 20% and 50%, compared to living donor (LD) transplants, which have incidence rates of 4% to 10%.[5,6] This undoubtedly increases surgeons' concerns about the quality and function of DCD transplanted kidneys. DGF can

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cause oliguria and anuria in the early postoperative period, resulting in increased allograft immunogenicity and heightened the risk of acute rejection, and DGF complicates perioperative management and is linked to long-term adverse outcomes, including reduced graft survival, increased patient morbidity, and elevated healthcare costs.[7–9]

Evaluating the risk factors of DGF from DCD is an important research topic. Studies have shown that many risk factors lead to DGF in DCD recipients.[10] The KT recipients (KTRs) need long-term immunosuppressive therapy after surgery and belongs to the immune deficient population. Immunosuppressed recipients are vulnerable to bacterial (most common), viral, and fungal infections, which contribute to poor outcomes.[11] Early infection after KT is often associated with poor prognosis.[12] Therefore, early detection and intervention are extremely important for controlling infection after KT, and fast and accurate detection methods need to be adopted in clinical practice. Nowadays, Metagenomic next-generation sequencing (mNGS) has emerged as an innovative and powerful tool for pathogen detection in clinical practice, offering a noninvasive, rapid, and highly sensitive approach for identifying infectious agents. Unlike conventional microbiological test (CMT), including nucleic acid testing, serology detection and culture of different body fluids and tissues, mNGS directly targets nucleic acids in clinical samples, enabling unbiased and precise detection of a broad spectrum of pathogens, including bacteria, fungi, viruses, and parasites.[13,14] This culture-independent technique demonstrates significant potential for the rapid and accurate diagnosis of infections, with promising applications in the field of renal transplantation.[15,16]

However, there is no detailed study and analysis on the correlation between the results of pathogen detection by mNGS during the perioperative period of recipients and DGF. This study examines the correlation between mNGS results and DGF occurrence, and considers potential mechanisms underlying this association in DCD KTRs during the perioperative period. Using mNGS, we analyzed renal lavage fluid from recipients and characterized the pathogen spectrum. The aim was to provide clinicians with valuable information and to provide potential antibacterial strategies for the occurrence of infection-related DGF.

Materials and Methods

Patient Population

We obtained ethical approval from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University. A retrospective review of the electronic medical records of 191 KTRs was conducted between July 1, 2021, and July 31, 2024, and all data were anonymised prior to analysis. The specific screening process is shown in Figure 1 (flowchart). Among these, 16 received a second kidney transplant, while 175 underwent their first, originating from various provinces in China. All kidneys were from DCD donors. Since DGF typically manifests within the first days to week post-transplant, the three-year study window (2021–2024) was appropriate for capturing this early postoperative outcome.

All surgical procedures and postoperative care were performed by the same transplant team. Routine standard of care and post-transplant medication including immunosuppressive drug therapy was administered in accordance to center standard. The standard immunosuppressive regimen consisted of induction therapy with anti-thymocyte globulin (ATG) followed by triple maintenance therapy with tacrolimus (FK506), mycophenolate mofetil (MMF), and prednisone (Pred). Induction was initiated intraoperatively with 25 mg ATG, followed by 25 mg daily on postoperative days (POD) 1 and 2. High-dose methylprednisolone was administered as pulse therapy at 500 mg during surgery and on POD 1 and 2. The dose was then tapered to 120 mg on POD 3, 80 mg on POD 4, and 40 mg on POD 5. From POD 6 onward, oral prednisone was given at 10 mg once daily. Oral MMF (1.0 g/day, divided into two doses) was initiated on POD 1. On POD 2, tacrolimus was added at an initial dose of 0.1 mg/(kg·day), divided into two doses. From POD 6 onward, maintenance immunosuppression consisted of the triple-drug regimen of MMF + tacrolimus + prednisone. MMF dosing was adjusted to maintain a mycophenolic acid area under the concentration–time curve (AUC) of 30–60 mg·h/L, and tacrolimus dosing was modified to achieve target trough concentrations. Figure 2 summarizes the dosing timeline.

Renal lavage fluid samples were collected intraoperatively during back-table donor kidney preparation. Each donor kidney was perfused with standard cold organ preservation solution under strict aseptic conditions. Approximately 50 mL of lavage fluid was collected from each graft, either stored at −20 °C or immediately transported on dry ice to Hugobiotech Co., Ltd. (Beijing, China) for metagenomic next-generation sequencing (mNGS) analysis. After collection, each sample was centrifuged at 12,000 × g for 10 minutes at 4 °C. The supernatant (200 μL) was used for DNA extraction using the QIAamp

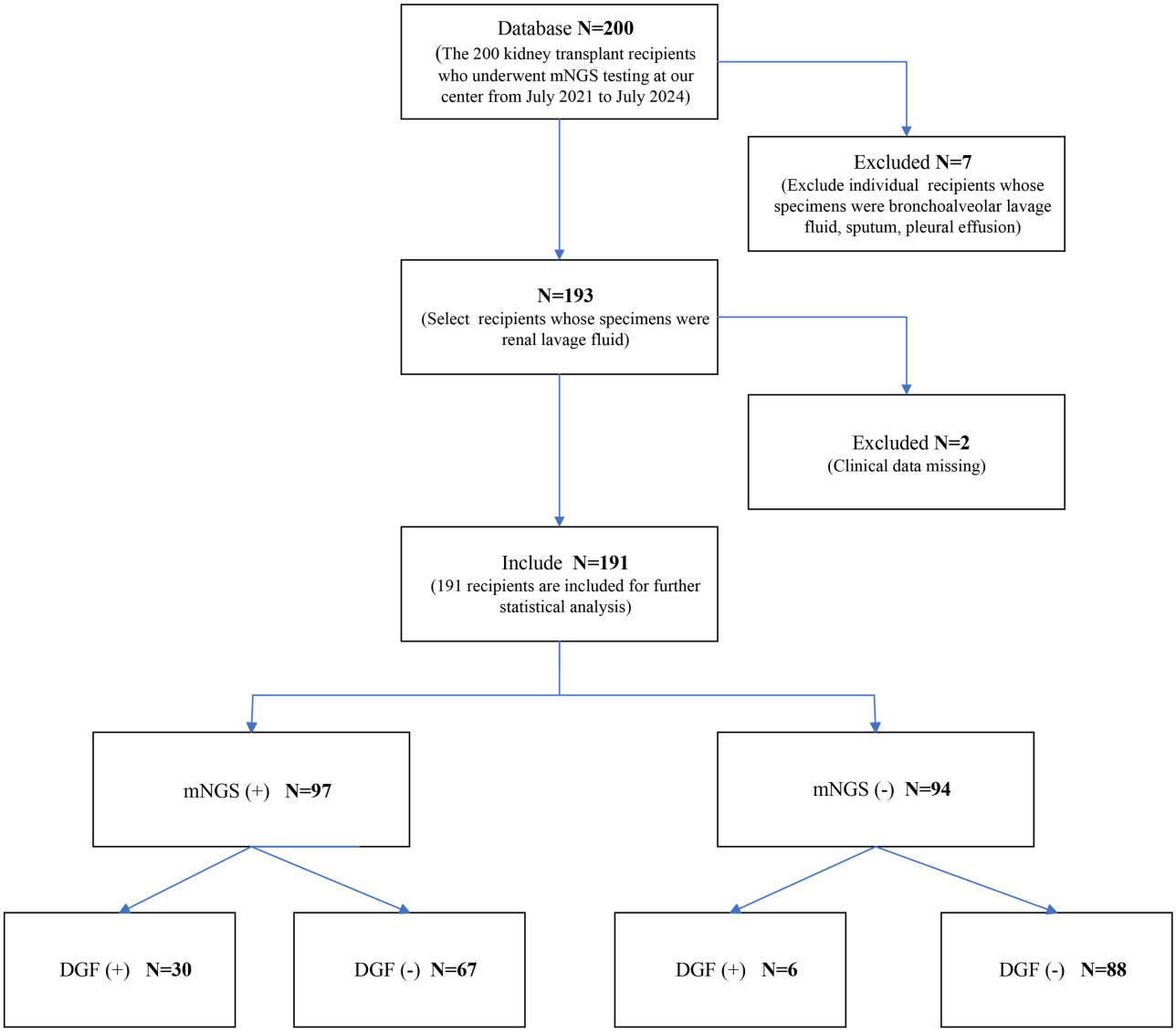

Figure 1 Schematic diagram illustrating the patient enrollment process, inclusion/exclusion criteria, and final cohort selection for the analysis of mNGS in renal transplant recipients. The flowchart details the number of participants at each stage of screening, exclusion, and final inclusion in the study.

DNA Micro Kit (QIAGEN, Hilden, Germany), and DNA concentration and quality were assessed with the Qubit 3.0 Fluorometer and agarose gel electrophoresis. DNA libraries were constructed using the QIAseq Ultralow Input Library Kit. Quality control was performed with the Qubit 3.0 Fluorometer and Agilent 2100 Bioanalyzer. Indexed libraries were pooled and sequenced on the Illumina NextSeq 550 platform using a 75-cycle single-end (SE75) strategy. Raw sequencing data were processed to remove low-quality reads, adapter sequences, low-complexity reads, and short fragments. Human-origin reads were subtracted by aligning to the human reference genome (hg38) using SNAP software. The remaining high-quality reads were mapped to a curated microbial genome database (NCBI) containing over 30,000 genomes spanning bacteria, viruses, fungi, and parasites, using the Burrows-Wheeler Aligner (BWA). To mitigate the impact of potential contamination, a no-template control (NTC) was included in each sequencing batch.

Clinical Data Collection

We included DGF occurs during the perioperative period as an outcome variable, Positive mNGS test result as an exposure variable, and the following variables as covariates in our analysis: gender, donor-recipient age, donor-recipient BMI (kg/cm2), type of dialysis (hemodialysis, peritoneal dialysis) and duration. Biochemical indexes: donor-recipient

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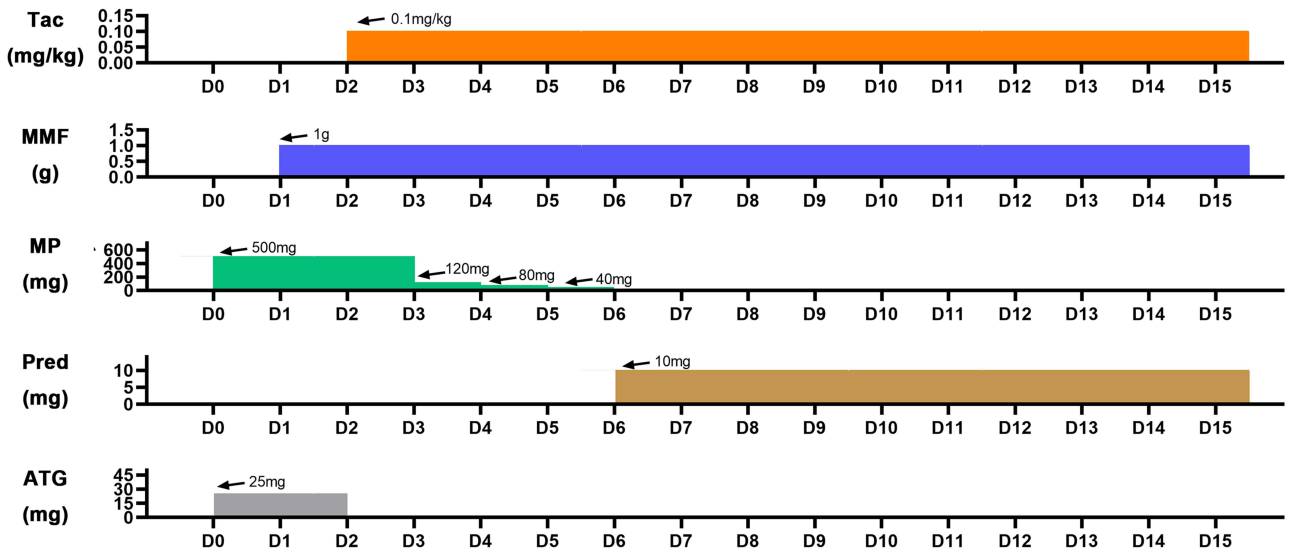

Figure 2 Timeline and dosing regimen of the immunosuppressive therapy used in the study, including induction (anti-thymocyte globulin, ATG) and maintenance therapy (tacrolimus, mycophenolate mofetil, and prednisone). Key phases: (1) Intraoperative methylprednisolone pulse therapy, (2) Postoperative tapering of corticosteroids, and (3) Long-term triple immunosuppression (MMF + Tac + Pred).
Abbreviations: Tac, tacrolimus; MMF, mycophenolate mofetil; MP, methyl prednisolone; Pred, prednisone; ATG, anti-thymocyte globulin.

creatinine, blood urea nitrogen (BUN), uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, blood glucose. Blood count: absolute red blood cell count (RBC), absolute white blood cell count (WBC), platelet count (PLT), lymphocyte count, hemoglobin level. And cold ischemia time (CIT) and warm ischemia time (WIT) of the ex vivo kidney. We collected the mNGS results, including positive results with detected pathogen types and their associated drug resistance gene information, as well as negative results. In this study, renal lavage fluid denotes the effluent collected from the renal vein after vascular perfusion of deceased donor kidneys with Celsior solution - a standardized preservation solution for both flushing and cold storage of solid organs. Comprising histidine, lactobionate, glutathione, mannitol, glutamate, and electrolytes, the solution yields effluent containing residual Celsior components, renal vascular endothelial biomarkers (including shed cells and inflammatory mediators), and potential microbial pathogens. This perfusate serves as a valuable sample for assessing the kidney's microbiological status. DGF was defined as the recipient still needs dialysis in the first week after KT.[17] The positive criteria for the mNGS result were set as follows: In the mNGS detection process, for bacteria, fungi, and atypical pathogens, a pathogen was considered positive if it had sequencing coverage ranking in the top 10 among all detected pathogens and was not present in the negative control (NTC), or if the sample-to-NTC ratio of reads per million (RPM) was greater than 10. For viruses, a pathogen was considered positive if at least one specific sequence was detected in the sample and not in the NTC, or if the sample-to-NTC RPM ratio exceeded 5. This threshold has been widely adopted and validated in previous clinical mNGS studies.[18,19] In this study, a recipient was classified as mNGS-positive during the perioperative period if the kidney lavage fluid sample tested positive. As a retrospective study, the risk of unmeasured confounding—such as undocumented donor infection history or variations in perioperative antimicrobial prophylaxis—cannot be fully excluded. To mitigate this limitation, we deliberately selected covariates that are well-established contributors to DGF—such as donor and recipient demographics, dialysis history, ischemia times, and key biochemical indicators—to ensure robust adjustment for known confounders in the multivariate models. Given the high sensitivity of mNGS, some detected organisms—particularly commensals like Enterococcus—may represent colonization rather than infection. We treated mNGS positivity as a microbiological signal for statistical modeling, without incorporating clinical symptoms or histopathology. This research did not involve clinical interventions based on mNGS findings. This approach aligns with our objective to explore pathogen-DGF risk associations, while highlighting that prospective validation of mNGS-guided clinical interventions (eg, preemptive antimicrobial therapy) remains a critical future direction.

Statistical Analysis

Data analysis and visualization were performed using SPSS software [Version 25.0; SPSS Inc., Chicago, IL, USA], Empower RCH software (version 4.1, Wuhan, China), GraphPad Prism version 8.0, and tables were produced by Microsoft® Word 2019. Continuous variables following a normal distribution were expressed as mean ± standard deviation (SD) and compared using independent sample *t*-tests or one-way ANOVA. Non-normally distributed data were presented as medians (P25, P75) and compared using the Mann–Whitney *U*-test or the Kruskal–Wallis *H*-test. Categorical variables were expressed as frequencies and percentages (%) and analyzed using the chi-square test. To quantify the achieved power for the primary between-group comparison, we additionally performed a post-hoc power analysis in G*Power 3.1 (two-sided $\alpha = 0.05$; chi-square test). To explore independent associations, we constructed three logistic regression models: (1) unadjusted; (2) Covariates included donor age and BMI, recipient age, sex, and BMI; and (3) adjusted for all covariates. Binary logistic regression analysis was employed for statistical evaluation. To mitigate confounding bias caused by potential confounders, participants were stratified into subgroups based on different levels of covariates. Stratified analyses were then conducted to investigate the impact of mNGS results on the incidence of DGF in different subgroups. Given the exploratory nature of these subgroup analyses and the increased risk of type I error due to multiple comparisons, we additionally applied false discovery rate (FDR) correction to obtain *q*-values for each subgroup comparison. The *q*-value represents the expected proportion of false positives among statistically significant findings after controlling for multiple testing. *q*-values < 0.05 were considered statistically significant after correction.

Results

Characteristics of the Study Population

Baseline demographic and clinical characteristics of donors and recipients are summarized in Table 1. The cohort comprised predominantly male recipients (74.87%, n=143), with a median age of 43 years (IQR: 35–49). Significant differences were observed between the NGS-positive (n=97) and NGS-negative (n=94) groups in sex distribution (male: 67.00% vs 83.00%, p=0.011), delayed graft function (DGF) incidence (30.90% vs 6.40%, $p<0.001$), and recipient age (median 45 vs 37 years, $p<0.001$). Additionally, NGS-positive recipients had higher preoperative glucose levels (median 6.23 vs 5.2 mmol/L, $p<0.001$) and white blood cell counts (median 7.56 vs 6.72 $\times 10^9$/L, p=0.009). Other variables, including donor age, creatinine, and dialysis duration, did not differ significantly between groups ($p>0.05$). A post-hoc power analysis based on the observed between-group difference in DGF incidence (chi-square test; two-sided $\alpha = 0.05$) indicated an achieved power of 96.83%, supporting the adequacy of our sample to detect the observed effect.

Table 1 Baseline Characteristics of DCD KTRs

Characteristic	Overall (n=191)	NGS Positive		*p*-value
		No (n=94)	Yes (n=97)	
R_sex				0.011
Male, n (%)	143 (74.87)	78 (83.00)	65 (67.00)	
Female, n (%)	48 (25.13)	16 (17.00)	32 (33.00)	
DGF				< 0.001
No, n(%)	155 (81.15)	88 (93.60)	67 (69.10)	
Yes, n(%)	36 (18.85)	6 (6.40)	30 (30.90)	
Recipient_Age (year)	43.0 (35.0, 49.0)	37.0 (29.0, 48.0)	45.0 (39.0, 51.0)	< 0.001
Donor_Age (year)	47.0 (38.0, 53.0)	47.0 (35.0, 51.0)	48 (40.5, 55.0)	0.033
R_Creatinine (μmol/L)	935.10 ± 236.35	925.70 ± 223.94	944.20 ± 248.61	0.588
D_Creatinine (μmol/L)	88.00 (60.00, 126.00)	88.00 (57.83, 122.20)	86.00 (60.35, 132.50)	0.464
R_BUN (mmol/L)	21.33 ± 7.19	21.42 ± 8.49	21.25 ± 5.70	0.874
R_Uric _Acid (μmol/L)	398.80 ± 104.14	391.40 ± 107.46	406.10 ± 100.85	0.331
R_ALT (U/L)	25 (19, 33)	24.5 (19, 34)	25 (19, 32)	0.913

(*Continued*)

Table 1 (Continued).

Characteristic	Overall (n=191)	NGS Positive		*p*-value
		No (n=94)	Yes (n=97)	
R_AST (U/L)	18 (14, 25)	17 (13, 22)	20 (14, 26)	0.078
R_Total _Protein (g/L)	74.12 ± 9.82	75.46 ± 10.06	72.81 ± 9.45	0.062
R_GLU (mmol/L)	5.38 (4.87, 7.05)	5.20 (4.79, 5.83)	6.23 (4.95, 8.59)	< 0.001
R_WBC (x10^9/L)	7.06 (5.74, 9.53)	6.72 (5.50, 8.72)	7.56 (5.91, 10.97)	0.009
R_LYMPH (x10^9/L)	1.24 (0.83, 1.70)	1.26 (0.93, 1.73)	1.20 (0.54, 1.57)	0.066
R_RBC (x10^12/L)	3.79 (3.45, 4.18)	3.80 (3.40, 4.19)	3.75 (3.46, 4.19)	0.964
R_HB (g/L)	115.00 (103.00, 127.00)	113.50 (103.75, 128.00)	115.00 (103.00, 126.00)	0.949
R_PLT (x10^9/L)	178.00 (138.00, 214.00)	179 (137.50, 218.25)	175.00 (138.00, 202.00)	0.358
R_BMI (kg / m^2)	22.11 ± 3.21	21.59 ± 3.11	22.60 ± 3.24	0.029
D_BMI (kg / m^2)	22.06 ± 3.26	21.75 ± 3.63	22.37 ± 2.85	0.191
Dialysis _Time (month)	37 months (12, 60)	36 months (12, 60)	38 months (24, 61)	0.151
WIT (h)	10 h (10, 15)	10 h (10, 15)	12 h (10, 15)	0.153
CIT (h)	8 h (6, 10)	8 h (6, 10)	8 h (8, 10)	0.059
Type of Dialysis				0.939
Hemodialysis, n (%)	154 (80.60)	76 (80.90)	78 (80.40)	
Peritoneal dialysis, n (%)	37 (19.40)	18 (19.10)	19 (19.60)	

Notes: Data are presented as n (%), median (interquartile range), or mean ± standard deviation, as appropriate. Recipient parameters are indicated with R_, donor parameters with D_. *p*-values <0.05 indicate statistically significant differences.
Abbreviations: DGF, delayed graft function; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GLU, glucose; WBC, white blood cells; LYMPH, lymphocytes; RBC, red blood cells; HB, hemoglobin; PLT, platelets; BMI, body mass index; WIT, warm ischemia time; CIT, cold ischemia time.

The Association Between Positive mNGS Results of Renal Lavage Fluid and DGF

Logistic regression analysis demonstrated that positive mNGS findings in renal lavage fluid were significantly associated with increased risk of DGF (Table 2). In the unadjusted model, recipients with positive mNGS results had 6.57-fold higher odds of developing DGF compared to those with negative findings (OR 6.57, 95% CI 2.59–16.68; $p<0.001$). After adjusting for donor age and BMI, recipient age, sex, and BMI (Model I), the association remained significant (aOR 4.12,

Table 2 Association Between mNGS Positivity and DGF in DCD KTRs: Unadjusted and Adjusted Logistic Regression Analyses

		OR (95% CI)		
		Non-Adjusted	Adjust I	Adjust II
NGS				
	Negative	Reference	Reference	Reference
	Positive	6.567 (2.585, 16.684)	4.124 (1.528, 11.132)	7.902 (1.633, 38.236)
	p-value	<0.001	0.005	0.010

Notes: This table presents odds ratios (OR) with 95% confidence intervals (CI) comparing outcomes between positive and negative mNGS test results across three analytical models: Non-adjusted model: Crude association without covariate adjustment; Adjust I model: Covariates included donor age and BMI, recipient age, sex, and BMI; Adjust II model: Fully adjusted for recipient factors (sex, age, creatinine, BUN, uric acid, ALT, AST, total protein, glucose, WBC, lymphocytes, RBC, hemoglobin, platelets, BMI), donor factors (age, creatinine, BMI), and transplantation-related factors (DGF status, dialysis duration and type, warm and cold ischemia times). Key Findings: A positive mNGS result was significantly associated with higher odds of the outcome in all models, with the strongest association in the Adjust II model (OR = 7.902, 95% CI: 1.633–38.236). *p*-values indicate statistical significance ($p < 0.05$) for all comparisons.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CIT, cold ischemia time; CI, confidence interval; DGF, delayed graft function; HB, hemoglobin; mNGS, metagenomic next-generation sequencing; OR, odds ratio; PLT, platelets; RBC, red blood cells; R/D, recipient/donor; WBC, white blood cells; WIT, warm ischemia time.

95% CI 1.53–11.13; p=0.005). In the fully adjusted model (Model II), which included comprehensive donor-, recipient-, and transplant-related variables (listed in Supplementary Table 1), positive mNGS results remained an independent risk factor for DGF (aOR 7.90, 95% CI 1.63–38.24; p=0.01).

Stratified analysis (Figure 3) revealed that male recipients with positive mNGS results were at higher risk of DGF. Recipients with shorter cold ischemia time, preoperative hemodialysis, and lower levels of creatinine, BUN, uric acid, AST, WBC, and glucose showed a more pronounced association between mNGS positivity and DGF. Similarly, higher donor age and creatinine, and elevated recipient ALT, total protein, lymphocyte count, RBC, and PLT levels were

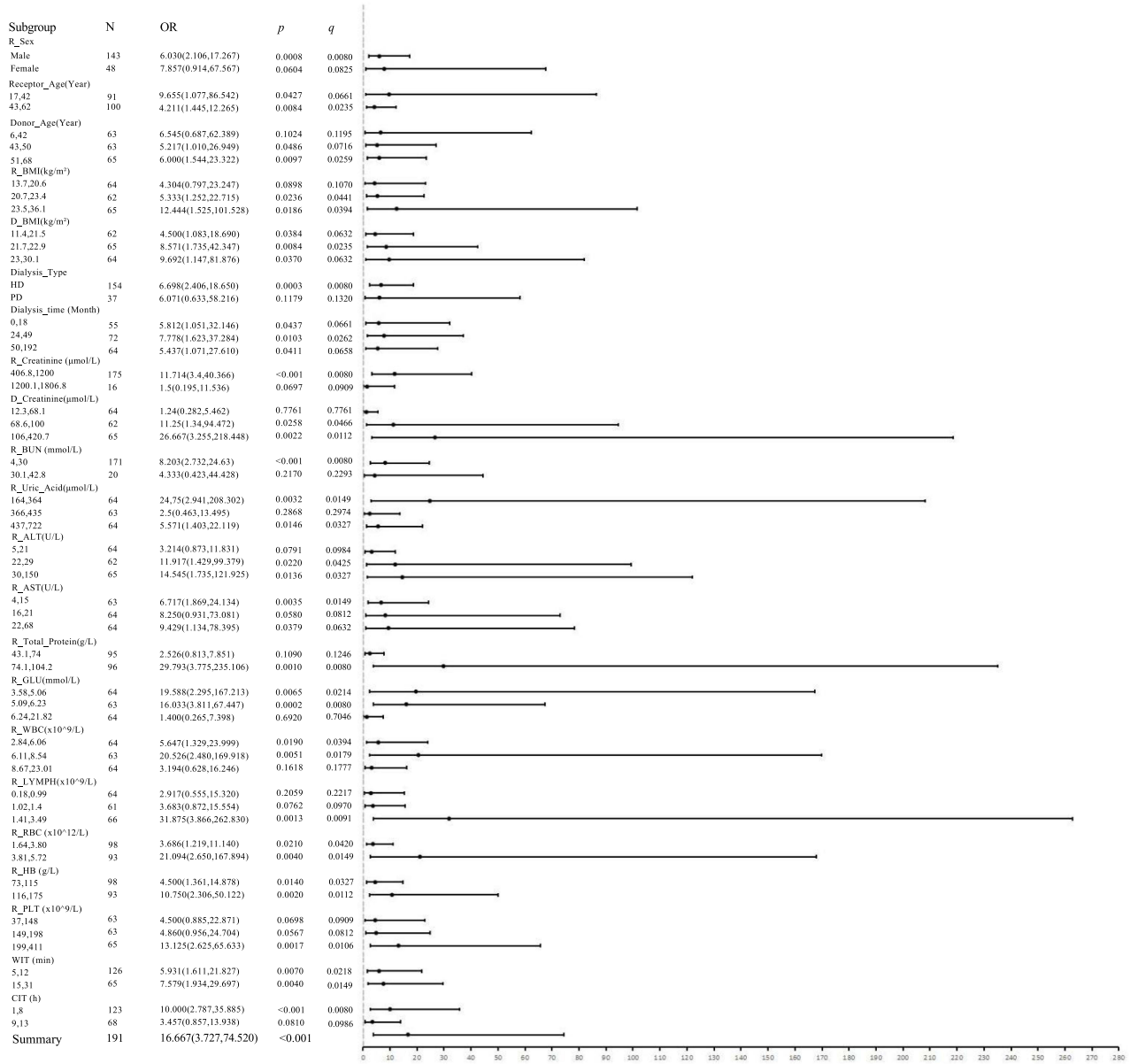

Figure 3 Forest plot illustrating the association between mNGS-positive results and delayed graft function (DGF) across various clinical subgroups. Odds ratios (ORs) with 95% confidence intervals (CIs) were derived from multivariable logistic regression models adjusted for potential confounders. *q*-values (FDR-adjusted *p*-values) are presented to account for multiple comparisons. Subgroups include recipient/donor demographics, dialysis parameters, preoperative biochemical markers, hematologic indices, and ischemia times (CIT/WIT). The vertical dashed line (OR=1.0) indicates null association. The "Summary" row (displayed in larger font) displays the pooled effect across the cohort.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CIT, cold ischemia time; HB, hemoglobin; PLT, platelets; RBC, red blood cells; R/D, recipient/donor; WBC, white blood cells; WIT, warm ischemia time; GLU, Glucose; HD, Hemodialysis; PD, Peritoneal Dialysis.

associated with increased DGF risk when mNGS results were positive. After applying FDR correction for multiple subgroup comparisons, several associations remained statistically significant ($q < 0.05$), including male sex, older donor age, higher donor BMI, preoperative hemodialysis, lower recipient creatinine, lower uric acid, higher ALT, higher lymphocyte count, higher RBC count, higher platelet count, and shorter cold ischemia time. These findings suggest that the observed associations are unlikely to be explained solely by chance.

Detection of DGF Related Pathogens and Antimicrobial Resistance Genes by mNGS

Metagenomic sequencing revealed distinct microbial patterns associated with DGF. Bacterial organisms accounted for the majority of identified microbes (62.7%), with Enterococcus (37 cases) and Staphylococcus (29 cases) being most common. Notably, Acinetobacter baumannii and Enterobacteriaceae species frequently carried multiple resistance genes, including β-lactamases (VEB, KPC, TEM, SHV, OXA, CTX-M) and macrolide/methicillin resistance markers (ermB, mecA) (Figure 4). Pseudomonas aeruginosa and Candida species were less frequently detected and showed fewer resistance associations. Viruses were detected in 24.1% of positive samples, with human herpesviruses (23 cases) and cytomegalovirus (11 cases) being the most prevalent (Figure 5). Metagenomic sequencing revealed distinct microbial patterns associated with DGF. Bacterial organisms accounted for the majority of identified microbes (62.7%), with Enterococcus (37 cases) and Staphylococcus (29 cases) being most common. Notably, Acinetobacter baumannii and Enterobacteriaceae species frequently carried multiple resistance genes, including β-lactamases (VEB, KPC, TEM, SHV, OXA, CTX-M) and macrolide/methicillin resistance markers (ermB, mecA) (Figure 4). Pseudomonas aeruginosa and Candida species were less frequently detected and showed fewer resistance associations. Viruses were detected in 24.1% of positive samples, with human herpesviruses (23 cases) and cytomegalovirus (11 cases) being the most prevalent (Figure 5).

Although all intraoperative sampling followed strict aseptic protocols to minimize contamination, differentiating true infection from colonization or environmental contamination remains challenging in mNGS diagnostics - particularly without supporting clinical symptoms or histopathology. This limitation applies to various pathogens, including herpesviruses that may persist latently yet be detected during reactivation in immunocompromised hosts. Notably, the observed association between mNGS positivity and DGF suggests these findings may represent biologically relevant signals. This supports the potential value of mNGS for perioperative risk stratification in renal transplantation. Future prospective studies should validate the clinical utility of mNGS-guided interventions and extend these investigations to other solid organ transplant populations, including liver and lung recipients.

Discussion

With the advancement of DCD KT in China, an increasing number of patients now have access to transplantation. However, this progress has also been accompanied by an increase in the incidence of postoperative complications, among which DGF stands out as a critical and severe concern. DGF was reported to occur at rate of 20%-40% for most centers with 3.9% indicating their incidence to be >60%.[20] DGF has remained a major research focus in KT due to its significant impact on both short-term management and long-term graft outcomes. Therefore, Assessing the risk of DGF has become an integral part of clinical decision-making, aiding in the development of treatment protocols and the allocation of healthcare resources.[21] Therefore, using mNGS data from DCD KTRs at our center to assess the risk of DGF is beneficial for clinicians, enabling them to diagnose infections promptly, identify pathogenic microorganisms, and apply sensitive drugs, implement early interventions for patients with pathogenic microbial infections (including subclinical cases), and ultimately reduce the risk of DGF while improving the quality of life and survival rates of transplant recipients.

In this study, we investigated the relationship between mNGS results and the occurrence of DGF during the perioperative period among 191 DCD KTRs. As an advanced technological tool, mNGS enables comprehensive pathogen profiling, particularly for microorganisms undetectable by conventional microbiological methods.[22] Its unique capability to simultaneously identify bacterial, viral and fungal without culture dependence provides significant clinical advantages for rapid diagnosis and treatment strategy formulation in post-transplant infection management. Our findings provide evidence that positive mNGS results are correlated with an increased risk of DGF, highlighting the clinical significance of mNGS testing in the perioperative management of these patients. Our results demonstrated that recipients with positive mNGS results had a significantly higher incidence of DGF compared to those with negative results, with an

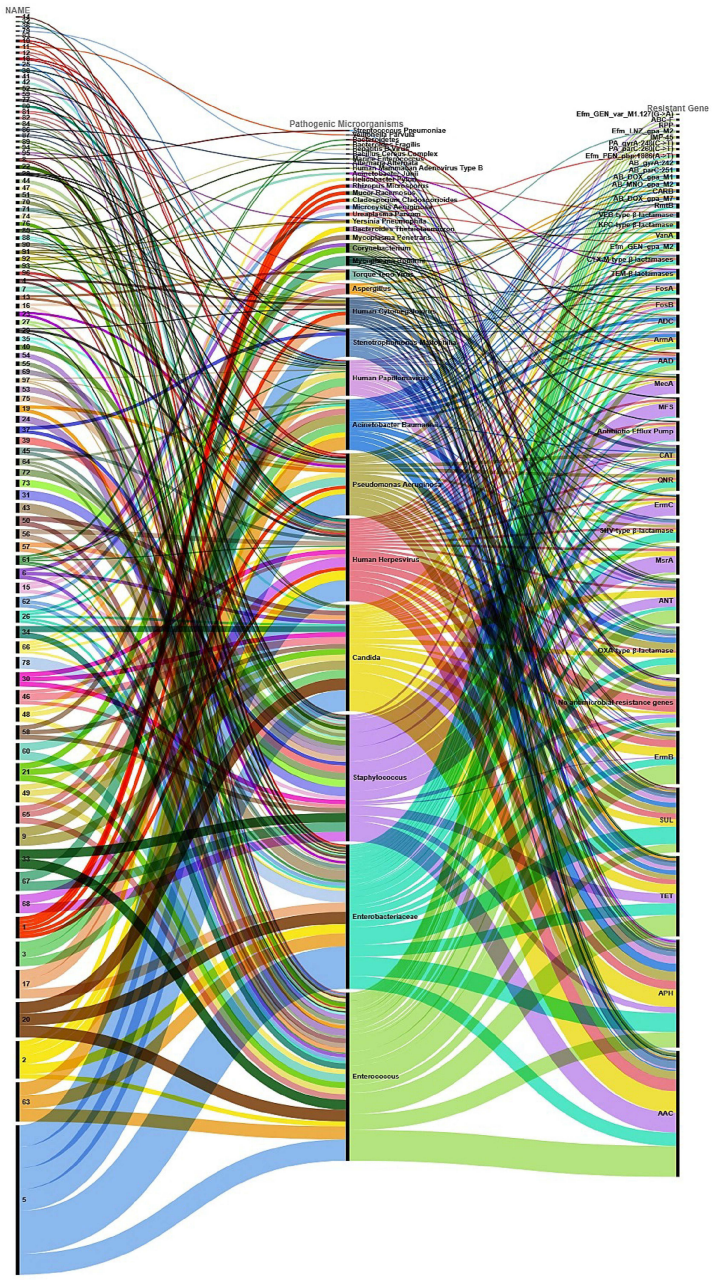

Figure 4 Sankey diagram depicting the relationships between detected pathogens (left) and their associated antimicrobial resistance (AMR) genes (right). Stream width corresponds to detection frequency, with thicker lines indicating stronger associations. Key findings include: (1) Frequent co-occurrence of Acinetobacter baumannii and Enterobacteriaceae with multiple AMR genes; (2) Staphylococcus isolates predominantly linked to mecA (methicillin resistance) and ermB (macrolide resistance); (3) Limited resistance gene associations for Pseudomonas aeruginosa and Candida species. β-lactamase genes (eg, OXA-type) are prominently represented, underscoring the complexity of resistance patterns in DGF-related infections.

Abbreviations: Efm_GEN_var_M1.127(G>A), Enterococcus faecium gentamicin resistance variant M1.127 with G>A mutation; ABC-F, ATP-binding cassette F-type protein (ribosomal protection); RPP, ribosomal protection protein; Efm_LNZ_gpa_M2, Enterococcus faecium linezolid resistance gene group M2; IMP_45, IMP-type metallo-β-lactamase variant 45; PA_gyrA:248(C>T), Pseudomonas aeruginosa DNA gyrase subunit A mutation at position 248 (C>T); PA_parC:260(C>T), Pseudomonas aeruginosa topoisomerase IV subunit C mutation at position 260 (C>T); Efm_PEN_pbp:1886(A>T), Enterococcus faecium penicillin-binding protein mutation at position 1886 (A>T); AB_gyrA:242, Acinetobacter baumannii DNA gyrase subunit A mutation at position 242; AB_parC:251, Acinetobacter baumannii topoisomerase IV subunit C mutation at position 251; AB_DOX_gpa_M1, Acinetobacter baumannii doxycycline resistance gene group M1; AB_MNO_gpa_M2, Acinetobacter baumannii minocycline resistance gene group M2; CARB, carbapenem resistance gene (general); AB_DOX_gpa_M7, Acinetobacter baumannii doxycycline resistance gene group M7; RmtB, 16S rRNA methyltransferase conferring aminoglycoside resistance; VEB-type-β-lactamase, Vietnamese extended-spectrum β-lactamase; KPC-type-β-lactamase, Klebsiella pneumoniae carbapenemase; VanA, vancomycin resistance gene A; Efm_GEN_gpa_M2, Enterococcus faecium gentamicin resistance gene group M2; CTX-M-type-β-lactamase, cefotaximase-type extended-spectrum β-lactamase; TEM-type-β-lactamase, Temoniera-type β-lactamase; FosA, fosfomycin resistance protein A; FosB, fosfomycin resistance protein B; ADC, Acinetobacter-derived cephalosporinase; ArmA, 16S rRNA methyltransferase; AAD, aminoglycoside adenylyltransferase; MecA, methicillin resistance gene A; MFS, major facilitator superfamily efflux transporter; Antibiotic Efflux Pump, general multidrug efflux pump mechanism; CAT, chloramphenicol acetyltransferase; QNR, quinolone resistance protein; ErmC, erythromycin ribosome methylase C; SHV-type-β-lactamase, sulfhydryl variable β-lactamase; MsrA, macrolide-streptogramin resistance protein A; ANT, aminoglycoside nucleotidyltransferase; OXA-type-β-lactamase, oxacillinase-type β-lactamase; ErmB, erythromycin ribosome methylase B; SUL, sulfonamide resistance gene; TET, tetracycline resistance gene; APH, aminoglycoside phosphotransferase; AAC, aminoglycoside acetyltransferase.

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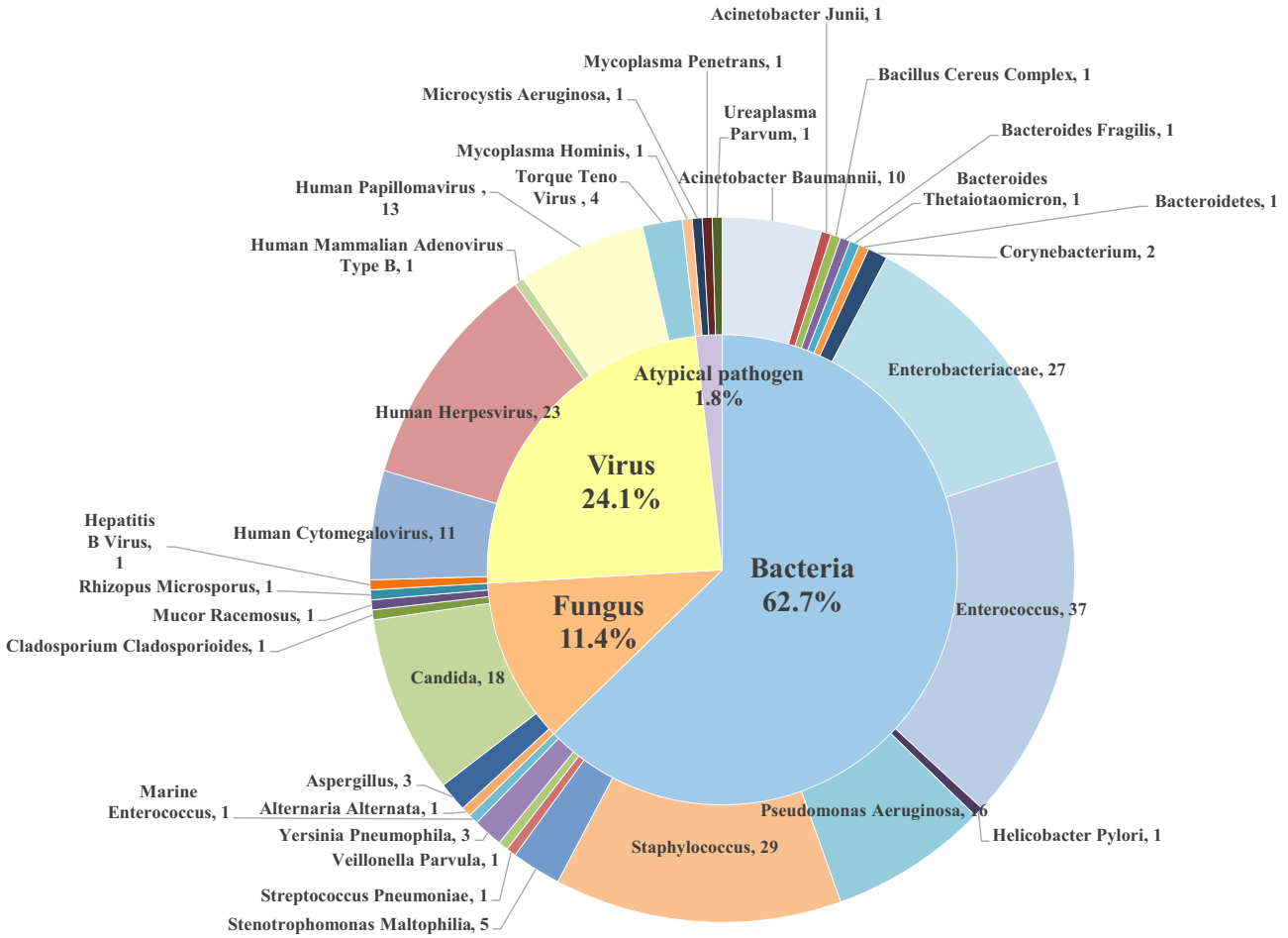

Figure 5 Pie chart illustrating the taxonomic composition of pathogens detected via metagenomic next-generation sequencing (mNGS) from renal lavage fluid samples of DCD kidney transplant recipients. The inner ring shows the proportion of major pathogen categories among mNGS-positive cases (n=97): bacteria (62.7%), viruses (24.1%), fungi (11.4%), and atypical pathogens (1.8%). The outer ring lists representative species with detection counts: Enterococcus (37), Staphylococcus (29), human herpesviruses (25), Candida (18), and cytomegalovirus (11). Percentages represent the relative frequency of each pathogen category among positive samples, while numbers indicate the total number of detections per species.

odds ratio of 6.567 (95% CI: 2.585–16.684, $p< 0.001$). Importantly, this association persisted after adjusting for donor and recipient demographic variables, such as age, sex, and BMI, as well as a full range of clinical covariates, this supports the notion that microbial factors play a critical role in the pathogenesis of DGF, either through direct effects on graft tissue or by triggering systemic inflammatory responses.[23]

Consistent with established clinical observations, bacterial infections predominated in the early post-transplant period, with mixed bacterial infections being equally common.[24] Our mNGS analysis of renal lavage fluid confirmed this pattern, revealing bacterial pathogens in 62.7% of cases. Enterococcus (37 cases) and Staphylococcus (29 cases) constituted the most prevalent isolates, followed by Enterobacteriaceae (27 cases), Pseudomonas aeruginosa (16 cases), and Acinetobacter baumannii (10 cases). Bacterial infections, particularly those caused by multidrug-resistant or opportunistic organisms, threaten graft function in kidney transplant recipients. While our study focused on the association between microbial presence and DGF, the underlying mechanisms warrant further exploration. One plausible pathway involves lipopolysaccharide (LPS) released by Gram-negative bacteria, which activates innate immune responses, promotes persistent inflammation, and contributes to ischemia-reperfusion injury, thereby impairing graft recovery.[25] In addition, biofilm formation by pathogens such as Staphylococcus and Enterococcus may facilitate immune evasion and establish chronic low-grade infections; notably, Enterococcus has been shown to attenuate neutrophil extracellular trap formation, enhancing the survival of co-infecting bacteria like Staphylococcus and amplifying tissue damage.[26] Furthermore, secreted virulence factors from Staphylococcus—including pore-forming toxins, proteases, and nucleases—can disrupt

epithelial barriers, degrade host immune molecules, thereby exacerbating graft dysfunction.[27] These mechanisms may partially explain the observed correlation between positive mNGS results and increased DGF risk. Further experimental and translational studies are needed to validate these pathways. In addition to renal lavage fluid, urinary metagenomic next-generation sequencing has also shown promise in detecting urogenital infections and donor-derived pathogens post-transplantation.[28] As urine collection is non-invasive and repeatable, urinary mNGS may serve as a valuable complement for longitudinal monitoring, early detection of ascending infections, or verification of lavage findings.

Our mNGS analysis identified human herpesviruses as the predominant viral pathogens, with human herpesvirus (n=23), human Papillomavirus (n=13) and cytomegalovirus (CMV, n=11) being the most frequently detected. Although CMV infection is well-documented in the literature to affect 8.8–63.2% of kidney transplant recipients and has been associated with serious complications such as pneumonitis, hepatitis, and allograft rejection,[29,30] its contribution to DGF in our cohort appears limited based on its relatively low detection rate (5.8%). Nevertheless, the early detection capability of mNGS facilitates timely initiation of targeted antiviral regimens.[31] This proactive approach may help reduce infection severity, decrease mortality risk, and potentially prevent subsequent complications, including DGF.

Fungal infections represent a significant concern in kidney transplant recipients, with Pneumocystis jiroveci pneumonia (PJP), invasive pulmonary aspergillosis (IPA), and candidiasis being the most commonly reported types in the literature.[32] Consistent with these established patterns, our mNGS analysis identified fungal pathogens in renal lavage fluid, with Candida species predominating (18 cases), followed by occasional detections of Aspergillus and Mucor racemosus. Although atypical pathogens were rare, organisms such as Mycoplasma hominis and Ureaplasma parvum were identified. Regarding antimicrobial resistance, Acinetobacter baumannii and Enterobacteriaceae were strongly associated with multiple resistance genes, particularly β-lactamase genes such as OXA-type β-lactamases. Additionally, Staphylococcus exhibited common resistance mechanisms linked to mecA and ermB genes, indicative of methicillin-resistant Staphylococcus aureus (MRSA) and macrolide resistance. In contrast, pathogens like Pseudomonas aeruginosa and Candida showed fewer associations with resistance genes. The high frequency of detection of these prevalent bacterial species and resistance genes suggests their potential role in the development of DGF. Analyzing these pathogens and their resistance profiles provides insights for improving clinical diagnostics and optimizing antimicrobial treatment strategies, ultimately supporting better long-term outcomes in KT.

The stratified analysis provided deeper insights into specific recipient and donor characteristics that exacerbate the risk of DGF in the context of positive mNGS findings. Male recipients with positive mNGS results were found to have a high risk of DGF, suggesting a potential sex-specific susceptibility. Additionally, several preoperative and perioperative factors, including routine preoperative hemodialysis and lower preoperative levels of biomarkers such as creatinine, BUN, uric acid, and AST, were associated with increased DGF risk in mNGS-positive recipients. Recipients of kidneys from older donors and those with higher BMI are more susceptible to DGF when positive mNGS results are present. The influence of recipient age and recipient BMI on DGF occurrence has been widely reported in the literature.[33] Both recipient age and recipient BMI showed positive correlations with DGF risk, which aligns with our findings. Elevated donor creatinine levels and recipient laboratory parameters, such as higher ALT, total protein, lymphocyte count, RBC, and PLT, were similarly associated with higher risk. Meanwhile, these findings emphasize the need for comprehensive preoperative risk assessment and individualized management strategies based on both donor and recipient characteristics.

The findings of this study have important implications for clinical practice. Incorporating mNGS results into perioperative workflows may support individualized interventions based on pathogen profiles and resistance data. For example, recipients with multidrug-resistant bacterial infections may benefit from targeted antimicrobial therapy,[34] while viral or fungal detections could inform prophylactic strategies. Additionally, mNGS is well-suited for perioperative microbial screening in kidney transplant recipients, enabling timely identification of infection sources and guiding treatment decisions.[35] mNGS-guided risk stratification facilitates optimization of immunosuppressive regimens and intensified infection monitoring, ultimately reducing DGF risk, and apply not only to first-time DCD kidney recipients but also to those undergoing repeat transplantation. Prior studies in solid organ transplantation reported similar findings, in liver transplant recipients, mNGS has demonstrated significant advantages over traditional microbiological methods in terms of rapid and precise pathogen detection, facilitating early identification and management of donor-derived or perioperative infections.[36,37] Studies have also shown that mNGS results contribute to timely therapeutic decisions,

especially in patients with multidrug-resistant organisms or elevated infection-related biomarkers such as CRP and CMV viremia, which are closely associated with post-transplant mortality and prognosis.[38] Similarly, in lung transplantation, early identification of multidrug-resistant bacteria through mNGS has facilitated antimicrobial strategy optimization, contributing to improved graft function recovery and postoperative prognosis.[39] These observations from liver and lung transplantation reinforce the clinical utility and generalizability of our findings in the context of DCD KT.

Although our analysis provides valuable insights into the association between mNGS-positive results and the incidence of DGF in recipients following DCD KT, several limitations of the study must be acknowledged. First, as a single-center, retrospective study, the generalizability of our findings is inherently limited, and the relatively small sample size restricts the statistical power, thereby reducing the potential for high-level clinical evidence. Moreover, another major limitation is the interpretive challenge of mNGS data. The technology generates massive sequencing outputs that are difficult to analyze precisely. Host DNA and commensal microbes can obscure pathogenic organisms, potentially leading to misinterpretation. This requires sophisticated bioinformatics tools and expertise that many clinical labs lack.[40] This retrospective study demonstrates an association between perioperative mNGS results and DGF. While mNGS shows predictive potential, the clinical utility of mNGS-guided interventions (eg, preemptive antimicrobial therapy) requires prospective validation. We recommend the design and execution of large-scale, multi-center prospective studies to strengthen the reliability of our conclusions.

Conclusion

In summary, our findings suggest that mNGS analysis of renal lavage fluid may offer clinical value in KT by providing faster and more comprehensive pathogen detection compared to conventional methods. This approach could help identify patients at higher risk for DGF and guide appropriate antimicrobial therapy, particularly for donor-derived infections. These results redefine DGF risk assessment by implicating graft-specific microbial burden as a modifiable factor, urging integration of mNGS into perioperative transplant algorithms and warrant additional investigation into optimal clinical applications.

Data Sharing Statement

The datasets generated and analyzed during this study are available from the corresponding author (Guiyi Liao) upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Approval No: PJ 2025-01-90). The ethics committee granted a waiver of informed consent as this retrospective study only involved analysis of anonymized laboratory test results collected from electronic medical records during patients' routine hospitalization, with no additional interventions performed. All patient data were handled confidentially and analyzed in aggregate form to protect individual privacy. All kidney donations were obtained voluntarily with written informed consent in accordance with the Declaration of Istanbul. The study complied with the ethical principles of the Declaration of Helsinki (1964) and its later amendments. No organs were obtained from vulnerable populations, and all transplant procedures followed standard institutional ethical guidelines.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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