Bacterial metagenomic analysis of patients with chronic kidney disease undergoing hemodialysis based on 16S rDNA amplicon sequencing

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Abstract. Chronic kidney disease (CKD) is a medical condition affecting >800 million patients globally, with end-stage kidney disease representing the most severe stage, usually requiring dialysis as a form of renal replacement therapy. As these patients have an increased risk of sepsis-associated mortality, and due to the limitations that arise from the use of traditional methods, prompt and accurate approaches in pathogen identification are required to ensure appropriate clinical management. The present study aimed to identify and analyze the bacterial profile of hemodialysis (HD) catheters obtained from patients with CKD who were undergoing hemodialysis using 16S ribosomal DNA (rDNA) amplicon sequencing. The present study proposed the use of the metagenomic approach in clinical laboratory settings. The results obtained in the present study revealed that the bacterial profile between site A (from the patient to the dialysis machine) and site V (from the machine back into the patient) had notable differences, with α- and β-diversity indices suggesting an increased diversity at site V. In addition, analyses of the relative abundance and linear discriminant analysis effect size revealed the presence of known pathogens, including *Klebsiella pneumoniae, Gardnerella vaginalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Corynebacterium striatum* and *Stenotrophomonas maltophilia*. In summary, the findings of the present study highlighted the potential use of 16S rDNA amplicon sequencing as a culture-independent alternative for determining pathogens in patients undergoing HD.

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Introduction

Chronic kidney disease (CKD) is characterized by a decline in kidney function, and its presence is indicated by a ≥3 month persistence of either a glomerular filtration rate (GFR) of <60 ml/min/1.73 m^2, or the presence of kidney damage (1). CKD is classified into five stages according to the estimated glomerular filtration rate (eGFR) and other signs of kidney damage such as albuminuria, urine sediment abnormalities, or structural abnormalities detected by imaging (2): Stage 1 (eGFR ≥90 ml/min/1.73 m^2), Stage 2 (eGFR 60-89 ml/min/1.73 m^2), Stage 3 (eGFR 30-59 ml/min/1.73 m^2), Stage 4 (eGFR 15-29 ml/min/1.73 m^2) and Stage 5, also referred to as end-stage kidney disease (ESKD), defined by an eGFR of <15 ml/min/1.73 m^2 or the requirement for kidney replacement therapy (1). CKD is a progressive disease characterized by a gradual decline in kidney function over time and affects >10% of the global population, accounting for >800 million individuals worldwide in 2017 (1). The disease is more prevalent among adults aged ≥65 years, women, Black individuals and those with diabetes mellitus or hypertension (1). According to the Global Burden of Disease Study 2023, CKD was the ninth leading cause of death worldwide,

accounting for approximately 1.48 million deaths in 2023 (3). The management and treatment of CKD focuses on slowing disease progression and reducing cardiovascular risk through interventions such as statin therapy or blood pressure control; however, in patients with ESKD, kidney transplantation or dialysis, including hemodialysis (HD) and peritoneal dialysis (PD), is required (4).

Despite these preventative measures, patients undergoing dialysis are highly susceptible to infections, with catheter-related dialysis increasing the risk of sepsis-associated mortality in patients compared with the general population. Patients with ESKD are further characterized by a high prevalence of comorbidities, regardless of the renal replacement modality. The study by Tzanakaki *et al* (5) demonstrates that infections occur more frequently in patients on HD (62.6%) compared with those on PD (37.4%). Furthermore, 21.5% of patients on HD (54/251 patients) developed bloodstream infections, a number of which involved antibiotic-resistant organisms including methicillin-resistant *Staphylococcus aureus* and multidrug-resistant Gram-negative bacteria such as Escherichia coli and *Klebsiella pneumoniae* (6). Comprising diverse microbial communities, biofilms can also form on catheter surfaces, making HD catheters a potential source of contamination and infection among patients on HD (7). Therefore, characterizing the bacterial composition of these biofilms may enable prompt, targeted antimicrobial therapies, with the potential to improve patient outcomes and reduce mortality rates in patients on HD (8,9).

Multiple approaches, including conventional and molecular methods, are used to identify microorganisms in hospitals. In clinical laboratory settings, conventional techniques include selective media culture, blood culture and biochemical tests. However, these traditional methods are associated with notable limitations, including time-intensive processing of bacterial cultures on media or blood cultures, the potential bias of selective media towards specific microorganisms, and issues that arise in cultivating certain microorganisms that are usually difficult to grow under laboratory conditions, or are novel pathogens (10). These limitations highlight the need for a more efficient and accurate method of identifying microorganisms in biofilms. The present study aimed to provide such a method by using molecular techniques.

Molecular techniques are required for the rapid identification of pathogens with high sensitivity and specificity, as exemplified by polymerase chain reaction (PCR)-based methods. Relying on PCR as a method of amplification, the metagenomic approach is currently implemented for genomic analysis, and this provides high-throughput data, with examples including shotgun sequencing and amplicon sequencing (11). Shotgun sequencing provides extensive insights into the entire genome, allowing researchers to investigate complex genomic regions and discover novel genes (12). It is well-suited for *de novo* sequencing projects due to its ability to identify pathogens at both the genus and the species levels, and it is even able to predict antimicrobial resistance and virulence (13). However, the high cost is a limitation of this method, especially when extensive coverage is needed (14,15). Amplicon sequencing targets specific genes or genomic regions, which results in a high sensitivity for detecting sequence variations, and enables a species-level resolution at a lower cost compared with whole-genome approaches (16,17). This method is widely applied in microbial community profiling through the amplification of marker genes such as 16S ribosomal DNA (rDNA) being used for the classification and identification of prokaryotes. Due to its conserved nature and broad utility, 16S rDNA is still a cornerstone of metagenomic amplicon sequencing (18).

Oxford Nanopore Technologies is a third-generation sequencing method that sequences individual molecules in real-time (19). The technology offers anticipated read lengths that surpass those achieved by second-generation methods, potentially reaching 30-150 kbp, while also being more cost-effective, and having improved turnaround times compared with other sequencing methods such as Illumina and Ion Torrent (20,21). These advantages offer promising possibilities for this technology, enabling researchers to identify the bacterial composition within the biofilm of HD catheters with increased accuracy and efficiency. It may potentially revolutionize how researchers understand and treat infections in patients undergoing HD. Furthermore, it may lead to breakthroughs in pathogen identification, with further innovations in CKD and microbiology likely to be made possible. Therefore, the present study aimed to identify and analyze the bacterial composition of HD catheters obtained from patients with CKD using 16S rDNA amplicon sequencing.

Patients and methods

Participants. A total of 140 residual HD catheter samples [70 from each of the sampled sites; from the patient to the dialysis machine (site A) and from the dialysis machine to the patient (site V)] were originally collected between April 2025 and June 2025 from patients at King Chulalongkorn Memorial Hospital (Bangkok, Thailand) during a previously approved study conducted under the approval of the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (COA approval no. 0302/2025; IRB approval no. 0292/67; Bangkok, Thailand). These samples (stored at -20˚C) were accessed for the purpose of the present study in July 2025. The present study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (COA approval no. 0834/2025; IRB approval no. 0282/68; Bangkok, Thailand). Written informed consent was obtained from all patients before enrollment in the previously approved study. The consent covered participation in the study and the collection and storage of residual HD catheter samples and associated clinical data for future research purposes.

The present study included patients aged ≥18 years with ESKD undergoing HD who experienced catheter malfunction, catheter-related bloodstream infection (CRBSI) or non-CRBSI. Eligible samples were obtained from either prevalent or incident cases who had undergone HD for >1 month, and required catheter removal based on clinical indications including catheter malfunction (persistently low blood flow), CRBSI associated with hemodynamic instability or severe sepsis/septic shock, persistent fever or bacteraemia despite 48-72 h of appropriate antimicrobial therapy, tunnel infection, or isolation of a virulent organism, and non-CRBSI indications, including elective or planned catheter removal for transition to a maturing arteriovenous fistula or graft (AVF/AVG), kidney transplantation, or recovery of kidney

function. Samples were excluded from the present study if any of the following exclusion criteria were met: i) Patients who received combined renal replacement therapy (HD and PD) but no prior history of HD treatment at King Chulalongkorn Memorial Hospital before catheter removal; ii) HD catheters with suspected external contamination, defined as specimens with documented breaches in aseptic handling or suspected contamination occurring after catheter removal during specimen handling or transport; and iii) incomplete catheter removal from both the arterial and venous sites. Prior oral or intravenous antibiotic administration before catheter sampling was not considered an exclusion criterion.

Sample size calculation. In order to calculate the sample size required, the following formula was used: $n=[2(((Z_{(1-(\alpha/2))}) + (Z_{(1-\beta)}))^2)]/(\Delta^2)$; where n represents the sample size required per group; $Z_{(1-(\alpha/2))}$ represents the Z score of 1.96, which corresponded to the α=0.05 significance level; $Z_{(1-\beta)}$ represents the Z score of 0.84, which corresponded to an 80% statistical power; and Δ represents the effect size (given that the two groups have the same number of samples) (22). For Δ, the conventional value of 0.5 was used in the calculation, since this value represents the standard effect size (23). When substituting the values into the formula, n=62.72. Using the sample size calculation of 62.72, an additional error rate of 10% was included to account for potential sample loss or sequencing failure, resulting in a minimum required sample size of 68.99, which was rounded up to 69 samples per site. Catheter samples from both sites (A and V) were collected once per patient.

Sample processing and DNA extraction. Catheter samples were collected in NAPseq™ nucleic acid preservation buffer (BioEntist Co., Ltd.), and stored at -20˚C until further processing. For DNA extraction, biofilm suspensions from the catheter samples were lysed using a TissueLyser LT (Qiagen GmbH) at 50 Hz for 3 min. DNA was subsequently extracted using a ZymoBIOMICS™ DNA Miniprep kit (Zymo Research Corp.), following the manufacturer's protocol. The concentration and purity of the extracted DNA were determined using a NanoPhotometer C40 (Implen GmbH) based on the absorbance at 260/280 nm.

16S rDNA amplification. The V1-V4 regions of the bacterial 16S rDNA gene were amplified in two PCR cycles using the primers 16S 27 forward, 5'-TTTCTGTTGGTGCTGATATTG CAGRGTTYGATYMTGGCTCAG-3' and 16S 806 reverse, 5'-ACTTGCCTGTCGCTCTATCTTCGGACTACHVGGGTW TCTAAT-3'. Using a total volume of 20 μl, the PCR mixture included 0.5 μl of each forward and reverse primer at a concentration of 10 μM, 10 μl 2X UltraHiFi Mix (with dye), 4 μl PCR Enhancer, 3 μl water treated with diethylpyrocarbonate and 20 ng DNA template. The first PCR reaction included an initial denaturation step at 94˚C for 2 min, followed by 25 cycles of denaturation at 98˚C for 10 sec, annealing at 60˚C for 10 sec, extension at 68˚C for 45 sec and a final extension at 68˚C for 1 min. Subsequently, amplicons from the first PCR cycle were barcoded using the PCR Barcoding Expansion 1-96 kit (cat. no. EXP-PBC096; Oxford Nanopore Technologies) and 25 cycles of the aforementioned conditions used for the first PCR reaction. Barcoded products were separated using 1% agarose gel electrophoresis with RedSafe™ Nucleic Acid Staining Solution, and then purified using a QIAquick® PCR Purification kit (cat. no. 28704; Qiagen GmbH), following the manufacturer's protocol.

Library preparation and sequencing. Following agarose gel verification and purification, library concentrations were quantified using the Quant-iT™ dsDNA High Sensitivity Assay kit (cat. no. Q32851; Thermo Fisher Scientific, Inc.) with the Invitrogen™ Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Inc.). DNA libraries were then pooled in equimolar ratios to a final concentration of 48 ng/μl (~90 nM, based on an amplicon size of ~800 bp) for further multiplexing. The pooled libraries were enriched for 16S amplicons using 0.5X Agencourt AMPure XP beads (Beckman Coulter, Inc.), followed by end repair and adaptor ligation using the Ligation Sequencing Kit V14 (cat. no. SQK-LSK114; Oxford Nanopore Technologies Ltd.). The resulting libraries were then loaded on to an R10.4.1 flow cell (cat. no. FLO-MIN114; Oxford Nanopore Technologies Ltd.), and long-read sequencing of the 16S rDNA gene (V1-V4 region) was performed on the MinION™ Mk1C platform (Oxford Nanopore Technologies Ltd.).

Statistical analysis. FASTQ files were generated from FAST5 data using Guppy basecaller software (version 6.0.7; Oxford Nanopore Technologies plc) (24) with the super-accuracy model and a minimum acceptable quality score of Q>15. Read quality was assessed using MinIONQC (a tool for visualization and quality control of nanopore data) (25), and adaptor trimming and demultiplexing were carried out using Porechop (version 0.2.4; https://github.com/rrwick/Porechop). Filtered reads were clustered, polished and taxonomically classified using NanoCLUST (an analysis pipeline for Uniform Manifold Approximation and Projection-based classification of amplicon-based full-length 16S rDNA nanopore reads) (26) using full-length 16S rDNA sequences from the Ribosomal Database Project as the reference (27). The resulting files were converted into QIIME format using the QIIME2 (version 2021.2) toolkit (28).

Rarefaction curves were subsequently generated to assess sequencing depth and coverage. α-diversity (Chao1 and Shannon indexes) and β-diversity [permutational multivariate analysis of variance (PERMANOVA)] metrics were calculated to evaluate intra- and inter-site microbial community differences, respectively. Mann Whitney U test was used to compare the data between the two catheter sites (sites A and V). Relative abundances at the phylum, genus and species levels were also determined, and visualized as the top 20 genera and species. The linear discriminant analysis effect size (LEfSe) was determined using P<0.05 and a logarithmic linear discriminant analysis (log LDA) score ≥3.0. α-diversity, β-diversity, relative abundance profiles and the LEfSe were determined using MicrobiomeAnalyst (version 2.0) (29). Spearman correlation analysis was carried out to assess associations between clinical variables and bacterial species abundance. The correlation matrix was visualized as a heatmap using the pheatmap package in R (https://cran.r-project.org/package=pheatmap). P<0.05 was considered to indicate a statistically significant difference.

Table I. A summary of the clinical characteristics of the participants included in the present study (n=70).

Characteristic	Number of participants, N (%)
Biological sex	
Male	41 (58.57)
Female	29 (41.43)
CRBSI status	
CRBSI	27 (38.57)
Non-CRBSI	43 (61.43)
Prior infection	
Yes	11 (15.71)
No	59 (84.29)
Presenting symptoms	
Fever	27 (38.57)
Chills	15 (21.43)
Edema	6 (8.57)
Dyspnea	10 (14.28)
Nausea and vomiting	8 (11.43)
Patient outcome	
Continue HD	33 (47.14)
Resume PD	10 (14.28)
Stop HD	10 (14.28)
Renal recovery	2 (2.86)
Kidney transplant	2 (2.86)
Mortality	13 (18.57)

CRBSI, catheter-related bloodstream infection; HD, hemodialysis; PD, peritoneal dialysis.

Results

Demographic data. A total of 140 HD catheter samples were collected (70 from site A and 70 from site V). Data from the participants whose catheters were removed were subsequently obtained, anonymized and summarized, and is presented in Table I. Among the 70 eligible participants (41 males and 29 females), 27 were diagnosed with CRBSI, and 11 had a documented history of prior infections. Regarding patient outcomes, 33 participants continued HD as the form of renal replacement therapy, whereas 13 patients died, 10 patients transitioned to PD and the remaining patients discontinued HD, had a full renal recovery, or underwent kidney transplantation.

Bacterial diversity among HD catheter samples. DNA from all catheter samples was sequenced using Oxford Nanopore Technology, resulting in 1,545,377 raw reads (477,138 from site A and 1,068,239 from site V). The average classified read count per sample was 5,559±3,413 at site A and 13,040±5,528 at site V. Using the Chao1 and Shannon indices, α-diversity was determined (Fig. 1). Both mean and median values were found to be higher at site V compared with site A, implying greater microbial diversity and a lower proportion of samples with limited diversity. Although the distribution at site V appeared less variable, with fewer outliers, the results consistently suggested higher diversity compared with site A. Subsequent β-diversity analysis further supported these findings (Fig. 2). Although a substantial overlap between the two sites indicated shared bacterial taxa, significant differences were observed (PERMANOVA, P=0.025). Moreover, catheter samples from site V demonstrated partial separation along axis 1, with a more scattered distribution, suggesting the presence of bacterial taxa unique to this site.

Relative abundance of bacteria in HD catheter samples. Relative abundances of bacteria from site A and site V were visualized at the phylum, genus and species levels using stacked bar charts (Fig. 3). At the phylum level, Proteobacteria, Actinobacteria and Firmicutes accounted for the majority of organisms found at both sites. At the genus level, *Lactobacillus*, *Gardnerella*, *Escherichia*, *Shigella*, *Streptococcus* and *Staphylococcus* were the most prevalent genera. The 10 most frequently identified bacterial species included *Lactobacillus iners*, *Gardnerella vaginalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Ralstonia pickettii*, *Corynebacterium striatum*, *Stenotrophomonas maltophilia*, *Lactobacillus mucosae* and *Methylosarcina lacus*. Comparative analysis revealed site-specific differences, with several known pathogens (*Pseudomonas aeruginosa*, *S. maltophilia* and *Klebsiella pneumoniae*) being notably enriched at site V. Given their pathogenic potential and association with catheter-associated bloodstream infections, sepsis and mortality, these findings further highlighted the importance of strict hygiene in patient care, healthcare personnel practices and dialysis machine maintenance.

LEfSe of the bacterial profiles. Based on the LEfSe presented in Fig. 4, analysis of bacterial profiles between catheter sites [P<0.05; log LDA score ≥3.0] revealed site-specific taxa. *Faecalibacterium prausnitzii* and *Sporosarcina globispora* were primarily found at site A, whereas *Aeromonas caviae*, *Parabacteroides distasonis*, *Herbaspirillum huttiense*, *K. pneumoniae*, *L. mucosae*, *Phyllobacterium myrsinacearum* and *Methylosarcina lacus* were primarily observed at site V, showing distinct microbial composition between catheter sites.

Association between clinical variables and microbial species. Spearman correlation analysis was subsequently carried out to evaluate associations between clinical variables and the top 20 bacterial species (Fig. 5). The catheter site demonstrated predominantly positive correlations with several species. The strongest significant association was observed for *P. myrsinacearum* (r=0.26; P<0.01). Significant positive correlations with P<0.05 were also identified for *R. pickettii*, *Staphylococcus hominis*, *A. baumannii*, *Methylosarcina lacus* and *L. mucosae*. These findings indicated enrichment of these taxa at site V. However, age, as a clinical variable, showed a small number of significant correlations. A significant negative correlation was observed for *Staphylococcus capitis* (r=-0.18; P<0.05) and *Corynebacterium pseudogenitalium* (r=-0.22; P<0.01), suggesting a decreased abundance with increasing age. However, no other species demonstrated statistically

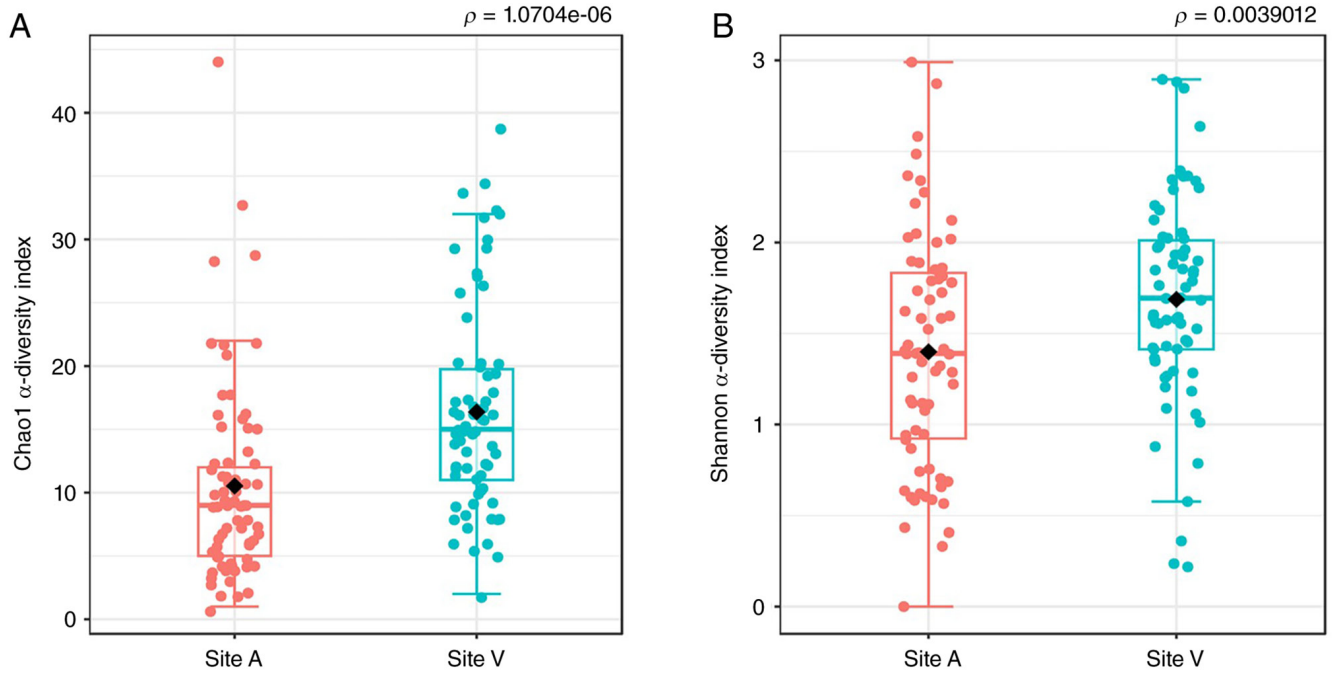

Figure 1. Comparison of bacterial α-diversity between HD catheter sites A and V. The α-diversity of bacterial communities in HD catheter samples was assessed using (A) Chao1 richness and (B) Shannon diversity indices. Box plots illustrate the distribution of diversity values, including median, interquartile range, and overall range, comparing samples from site A (patient-to-dialysis machine) and site V (dialysis machine-to-patient).

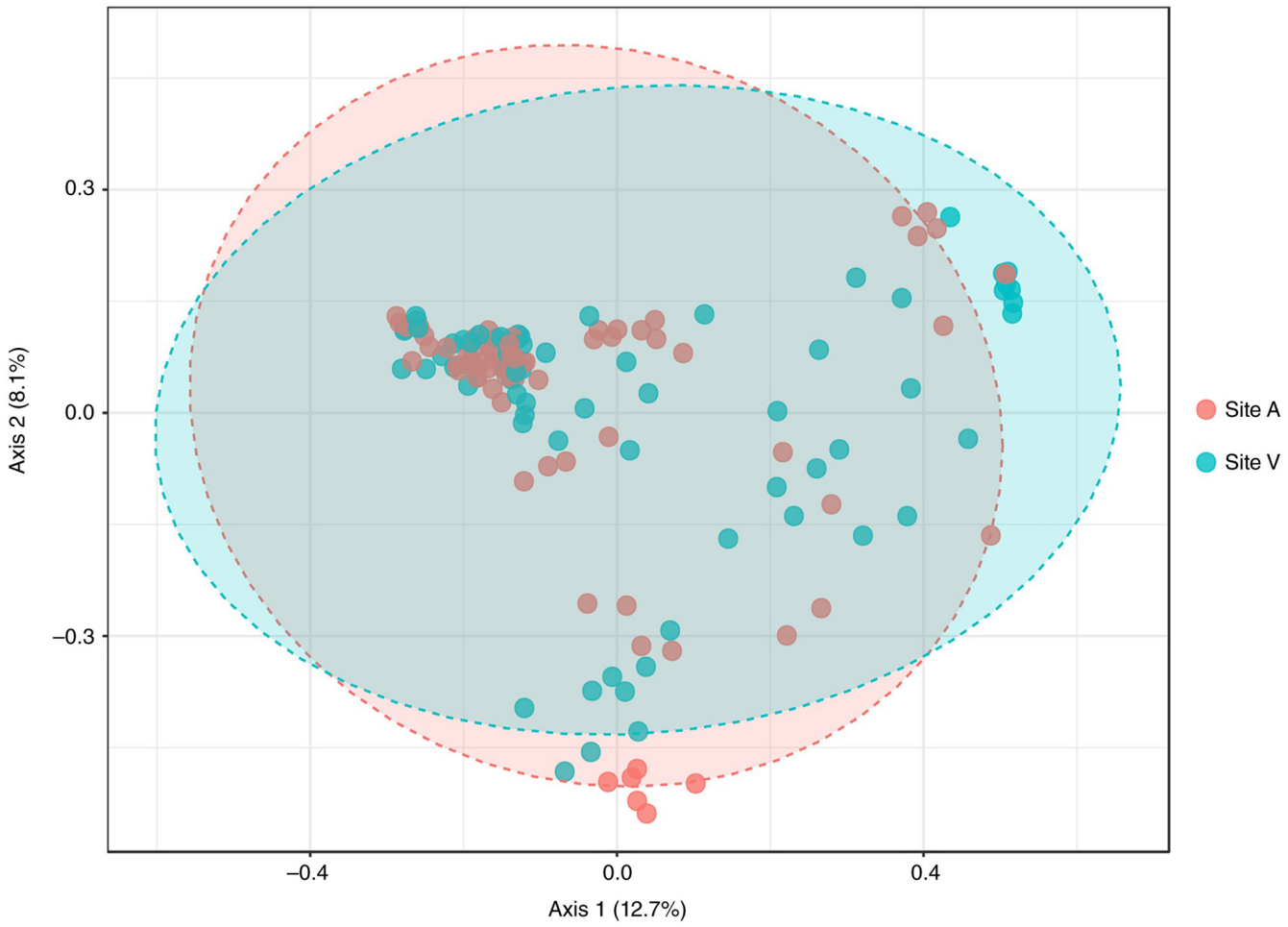

Figure 2. β-diversity analysis of bacterial communities between HD catheter sites A and V using principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity. β-diversity was assessed using ordination plots generated by PCoA based on the Bray-Curtis dissimilarity distance to visualize differences in bacterial community composition between samples from site A (patient-to-dialysis machine) and site V (dialysis machine-to-patient).

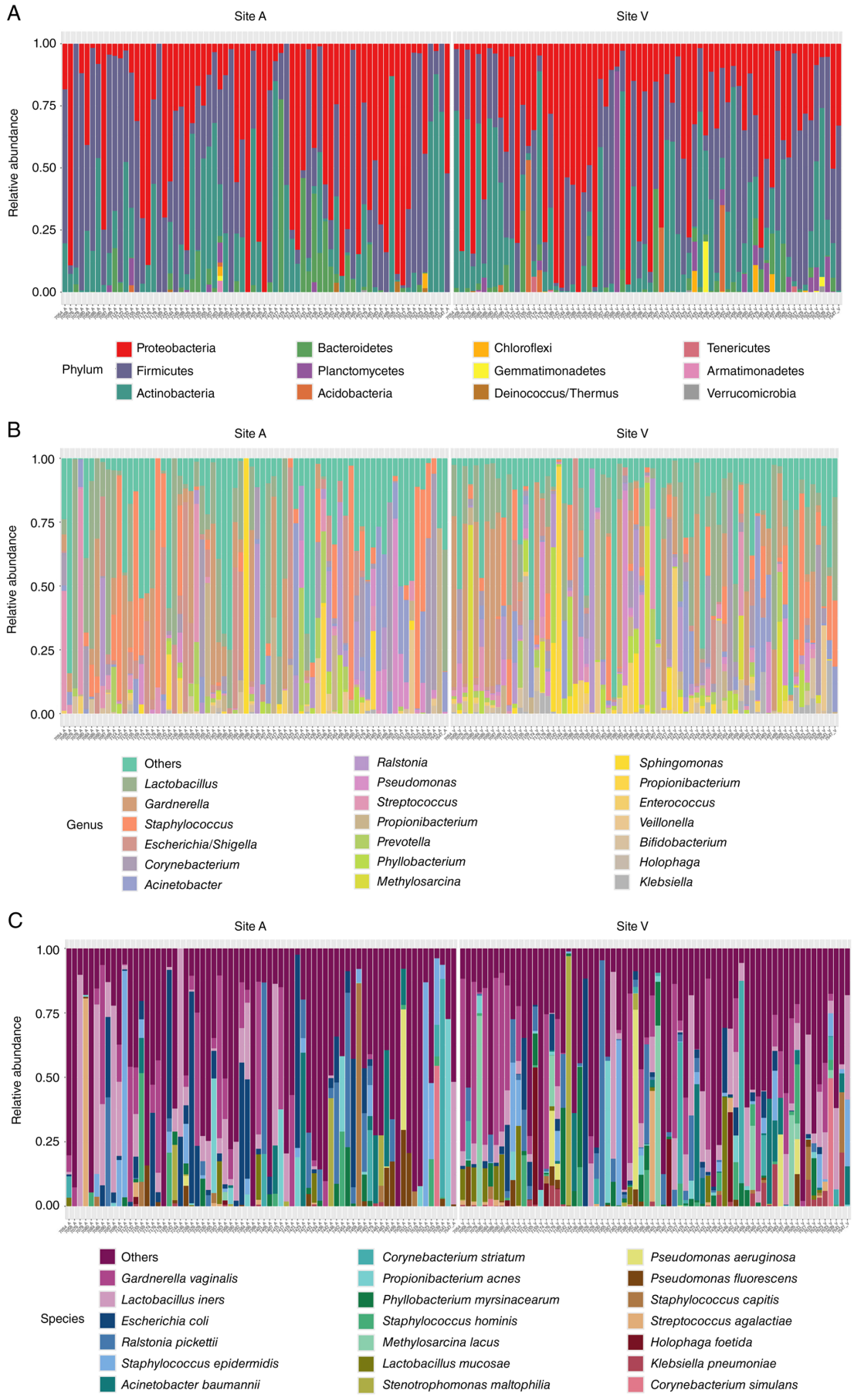

Figure 3. Bacterial relative abundance at the phylum, genus, and species levels shows stacked distributions across samples. Stacked bar charts show (A) all bacterial phyla, (B) the 20 most abundant genera and (C) the 20 most abundant species. Site A, from the patient to the dialysis machine; site V, from the dialysis machine to the patient.

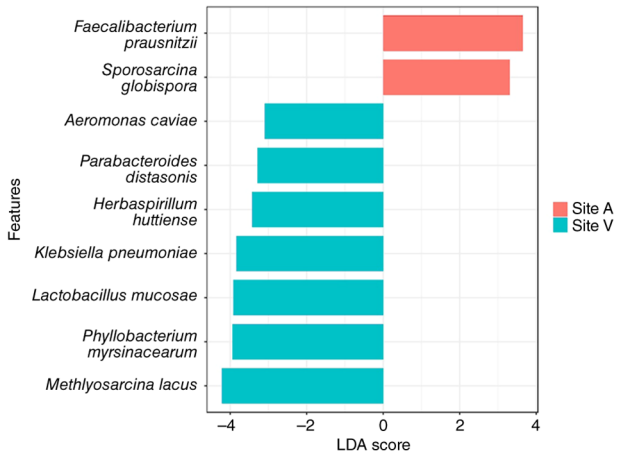

Figure 4. An LDA effect size comparison between site A and V. The analysis revealed a significant separation in bacterial composition between the two catheter sites. Site A, from the patient to the dialysis machine; site V, from the dialysis machine to the patient; LDA, linear discriminant analysis.

significant associations with age; moreover, sex-specific differences were observed for a limited number of species. A strong negative correlation was identified for *S. capitis* (r=-0.29; P<0.001), indicating enrichment in female patients. In addition, *P. aeruginosa* showed a significant negative correlation with the male sex (r=-0.19, P<0.05), also suggesting a higher abundance in female patients. No species demonstrated a statistically significant positive correlation with the male sex. Neither were any statistically significant correlations observed between CRBSI and non-CRBSI for any of the evaluated species (all P>0.05), although weak trends were noted for several taxa.

Discussion

The growing prevalence of antibiotic-resistant bacteria highlights the limitations of conventional diagnostic methods, and the necessity for more accurate strategies in pathogen identification. Molecular approaches serve to provide a rapid and reliable detection of bacterial strains to facilitate the implementation of targeted therapeutic interventions (11). Among these techniques, 16S rDNA amplicon sequencing, a widely utilized tool in metagenomic studies, offers enhanced specificity for the identification of genes or genomic regions of interest, while maintaining cost-effectiveness (16,17). This sequencing technique is used to characterize microbial communities across a variety of sample types, including clinical specimens (such as blood and stool), environmental samples (such as soil, wastewater and freshwater), and host-associated microbiomes, such as those of humans, animals and plants (2).

In the present study, full-length (V1-V9) 16S rDNA amplification of representative catheter samples provided limited information. This may be attributable to factors associated with sample handling and preservation. Multiple freeze-thaw cycles and prolonged storage at -20˚C may have resulted in compromised DNA integrity, reducing the suitability of the material for full-length amplification. Consequently, partial 16S rDNA amplification targeting the V1-V4 region was selected in subsequent analyses to ensure sufficient DNA for further amplification. Another potential limitation was the choice of lysis buffer used during DNA extraction. Although mammalian cells are readily disrupted due to their relatively fragile plasma membranes, bacterial and fungal cells are encapsulated within structurally resilient cell walls. Mild lysis conditions can selectively lyse mammalian cells, while sparing microbial populations. Nevertheless, certain microorganisms may remain vulnerable under these conditions, leading to a suboptimal DNA quantity. Such variability in DNA yield may negatively influence the efficiency of downstream library preparation and sequencing.

A previous study demonstrates the applicability of 16S rDNA amplicon sequencing in characterizing bacterial and fungal communities in peritoneal dialysis fluid samples (30). However, to the best of our knowledge, the present study is the first to use such an approach to identify the bacterial composition within HD catheter samples. Differences in bacterial communities between the catheter insertion site (site A) and the vascular lumen (site V) were evident at each of the phylum, genus and species levels.

The predominant phyla identified across the samples were Proteobacteria, Actinobacteria and Firmicutes, findings which are consistent with those of another study (31). At the genus level, *Lactobacillus*, *Gardnerella*, *Escherichia*, *Shigella*, *Streptococcus* and *Staphylococcus* emerged as the most abundant genera. Moreover, species-level analysis revealed that *L. iners*, *G. vaginalis*, *E. coli*, *S. epidermidis*, *A. baumannii*, *R. pickettii*, *C. striatum*, *S. maltophilia*, *L. mucosae* and *Methylosarcina lacus* were among the 10 most prevalent species. Furthermore, several of these organisms are recognized either as established pathogens or as opportunistic species with clinical relevance, suggesting the potential relevance of these findings to catheter-associated infections.

The 10 most frequently identified bacterial species in the present study may be broadly categorized into three groups, namely environmental organisms, potential pathogens and beneficial commensals. *A. baumannii*, a species commonly associated with hospital-acquired infections, was detected in samples from both sites, despite its usual environmental reservoirs in soil and water (32). Similarly, *S. maltophilia*, typically regarded as an environmental organism, has emerged as a clinically relevant opportunistic pathogen, especially in immunocompromised patients (33). Finally, *R. pickettii*, although generally considered a contaminant, has also occasionally been identified as a rare opportunistic pathogen (34).

Among the identified enteric organisms, *E. coli* represents a species with both commensal and pathogenic strains, the latter capable of causing intestinal and extraintestinal infections, including urinary tract infections and septicemia (35). *G. vaginalis*, detected in samples from both sites, is a urogenital pathogen, and the principal organism associated with bacterial vaginosis (36). Regarding skin-associated organisms, *S. epidermidis*, an important component of the skin microbiota and a contributor to skin homeostasis, is implicated in CRBSIs based on its capacity in biofilm formation (37). Additionally, *C. striatum*, a skin commensal, is reported as an emerging opportunistic pathogen causing severe bloodstream infections (38). The genus *Lactobacillus* demonstrates contrasting roles. *L. iners*, often described as a vaginal symbiont, is associated with vaginal dysbiosis and an increased susceptibility

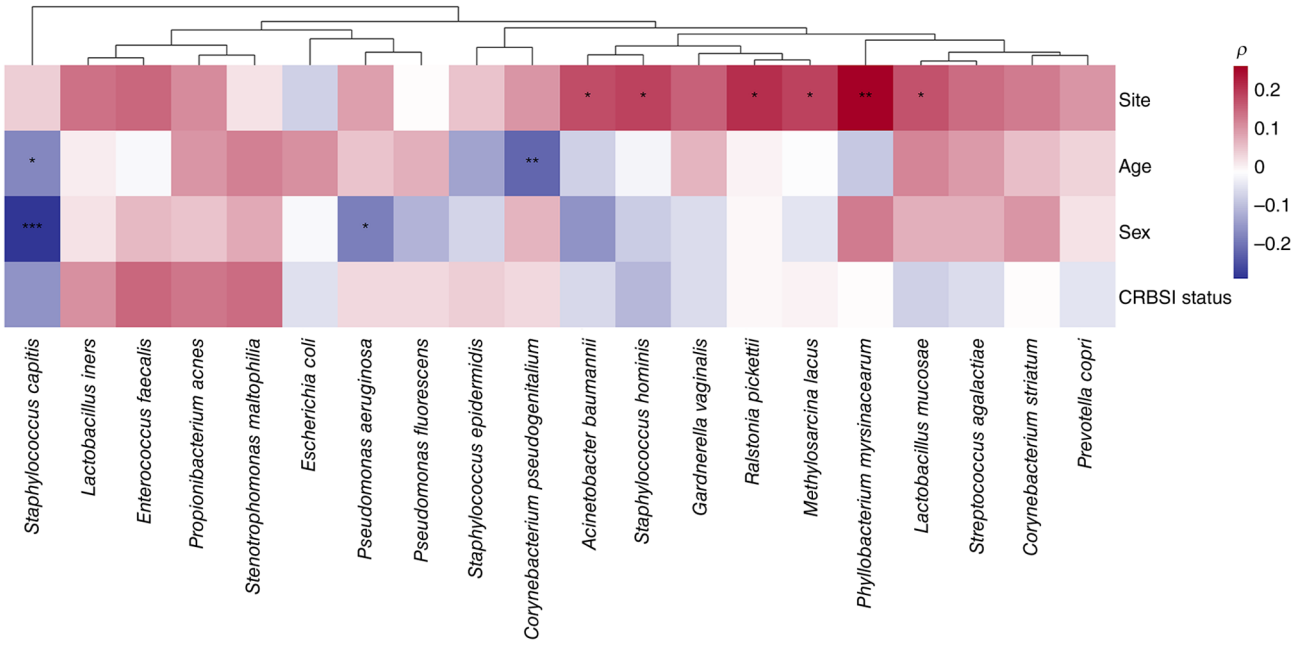

Figure 5. Correlations between clinical variables and the top 20 bacterial species. A heatmap analysis of the Spearman's rank correlation coefficients between clinical variables (catheter site, age, sex and CRBSI status) and the relative abundance of bacterial species. Catheter site, sex and CRBSI status were coded as follows: The patient to the dialysis machine site, being biologically female and having a CRBSI were ranked as 0; the dialysis machine to the patient site, being biologically male and having a non-CRBSI were ranked as 1. The red and blue colors represent positive and negative correlations, respectively. Correlation coefficients are shown within each cell. *P<0.05, **P<0.01 and ***P<0.001. Hierarchical clustering was carried out on bacterial species according to their correlation profiles. CRBSI, catheter-related bloodstream infection.

to sexually transmitted infections, whereas *L. mucosae* is considered beneficial, and has been proposed for probiotic applications (39). Finally, *Methylosarcina lacus*, a methanotrophic organism capable of survival in methane-rich environments, is classified as an environmental species with limited clinical significance.

When comparing the bacterial profiles between the two catheter sites, it was identified from the relative abundances that host-associated bacterial species, including *L. iners*, *G. vaginalis*, *E. coli* and *S. epidermidis* were the most common at site A. Although these species were also present in site V, additional environmental species were also found to be increasingly abundant at the location. Such species included *R. pickettii* and *P. myrsinacearum*, which both act as human pathogens, and may also serve as indicators of water contamination (40-42). According to the relative abundance and LEfSe analyses, there was a significantly increased abundance of *G. vaginalis*, as well as the persistent presence of environmental species at site V. These findings indicated the possible introduction of such species via the water system that was used in the procedure. In addition, several of the detected bacterial species were likely to have translocated from other body sites, including the skin, the intestines and the female reproductive tract. Examples of such organisms included *G. vaginalis*, *E. coli*, *S. epidermidis* and *C. striatum*. This also implicated their possible introduction during HD, catheterization and catheter removal. Given that dialysis machines are expected to be cleaned and disinfected, these findings highlighted the potential need to re-evaluate the cleaning and disinfection protocols used in dialysis centers, as well as the quality control of dialysate and water used in the dialysis system. Given the presence of known human pathogens (such as *A. baumannii* and *K. pneumoniae*) among the samples, an emphasis on enhancement of the cleaning and disinfection protocols of the dialysis equipment, along with improving personal hygiene among healthcare personnel and patients with CKD, may be essential in preventing such infections.

Spearman correlation analysis was also carried out in the present study, which revealed that the catheter site had the strongest influence on species-level microbial distribution, whereas age and sex demonstrated limited, taxa-specific associations. Furthermore, several species, including *A. baumannii*, *S. hominis* and *R. pickettii*, were positively associated with site V. These organisms are commonly implicated in opportunistic infections and biofilm formation, suggesting that the venous segment of the catheter may provide a microenvironment conducive to colonization by potentially pathogenic taxa (41,43). The enrichment of these species at the venous site may reflect differences in blood flow dynamics, nutrient availability or host-device interface characteristics that facilitate microbial persistence (44-46). The sex-specific associations were modest in comparison; however, the populations of *S. capitis* and *P. aeruginosa* were significantly enriched in female patients compared with male patients. Both species are known to be skin- or environment-associated organisms that have documented roles in device-associated colonization (47-49). Taken together, these findings suggested that host biological differences, including hormonal influences or variations in skin microbiota composition, may affect catheter-associated microbial communities (47,50). The age-associated correlations were also limited and primarily negative, indicating a potential decline in certain commensal or skin-associated taxa with increasing age (51). These observations may reflect age-dependent alterations in skin barrier integrity, immune

function or microbiome composition (52). In addition, CRBSI status was not significantly correlated with the abundance of the top 20 species. This suggested that overt bloodstream infection may not be caused by dominant taxa abundance, but rather by complex ecological interactions, virulence factors or biofilm dynamics (53). Overall, the findings generated from the Spearman correlation analysis highlighted the catheter site as the principal determinant of microbial structuring at the species level, whereas host demographic factors appeared to exert more subtle modulatory effects (54). Understanding these spatial and host-associated microbial patterns may aid in the identification of high-risk colonization profiles, and in informing targeted infection prevention strategies in patients on HD (55,56).

In the clinical context, among all 70 participants whose HD catheters were used in the study, 27 cases were confirmed as cases of CRBSI. A total of 36 patients received antibiotic treatment, including 9 patients with negative culture results, but who demonstrated relevant symptoms (for example, fever, nausea/vomiting and diarrhea). The management of CRBSI in patients on HD relies on the prompt administration of antibiotics. Based on the clinical data, a diverse correlation was identified between specific antibiotic regimens and clinical outcomes, with fourth-generation cephalosporins emerging as a main contributor for positive outcomes, frequently associated with patients being able to continue with HD. Vancomycin was often used in combination with carbapenems or cephalosporins to support the maintenance of HD. Meropenem was also used in several patients, allowing them to either continue HD or to resume PD. An observation that concerned the therapeutic limitations when treating multidrug-resistant (MDR) pathogens was that patients with fatal outcomes often received the most intensive antibiotic regimens, including combinations of colistin, fosfomycin and meropenem, or sequences involving piperacillin-tazobactam and vancomycin. These clinical data may highlight the virulence of identified bacterial species, including carbapenem-resistant *P. aeruginosa*, carbapenem-resistant *A. baumannii* and MDR *K. pneumoniae*. Whereas antibiotics such as ceftazidime and vancomycin are instrumental in maintaining the treatment for numerous patients on HD, their efficacy is limited by the presence of MDR gram-negative bacteria. The relative abundance of these pathogens in site V samples highlights the potential need for a rapid pathogen identification to transition from empiric to precision therapy earlier in the clinical course, which may potentially reduce the high mortality rates associated with these resistant organisms.

However, the present study focused on characterizing the taxonomic composition of catheter-associated microbiota, and antibiotic-associated outcomes were not evaluated. Future investigation is required in future studies, which constitutes one of the limitations of the present study. Furthermore, studies on the differences in the fungal, as well as the viral, microbiome should also be carried out. Additionally, the DNA that was sequenced from the biofilm may have resulted from both current and past infections, highlighting that culture results may need to be used as references, and that further validation is required. Furthermore, since data regarding the catheter insertion location and relevant previous infections were not obtained, these results could not be used to determine what the direct causative effects were. Further studies, such as investigations incorporating shotgun metagenomic sequencing, may provide comprehensive functional profiling, including the detection of antibiotic resistance genes, virulence determinants, biofilm-associated bacterial pathways and the symptoms or clinical outcome of the patients. Such an approach would provide additional in-depth insights into the clinical significance of catheter microbiomes and their potential association with CRBSI.

In conclusion, the present study demonstrated differences in the bacterial composition present at two distinct HD catheter sites. Through the application of 16S rDNA amplicon sequencing, the identification and characterization of bacterial profiles were carried out in patients with CKD who were undergoing HD. This molecular approach offers potential clinical utility by enabling the precise and prompt identification of pathogens, which may facilitate the delivery of optimal therapeutic interventions. Therefore, its integration into clinical practice may improve patient outcomes, potentially by reducing the morbidity and mortality associated with CRBSI in this vulnerable population.

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Availability of data and materials

The data generated in the present study may be found in the NCBI database under the BioProject accession number PRJNA1346282 and Sequence Read Archive accession numbers SRR35811925-SRR35812066 or at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1346282.

Authors' contributions

SP and TK designed the study. BS and SV performed the experiments, interpreted the results, and contributed to drafting and revising the manuscript. SV contributed to data analysis. WW contributed to the acquisition and preparation

of clinical samples and the collection of patient data. TS and PP contributed to the handling and transport of clinical samples and the collection and compilation of patient data. SP and TK supervised the study, critically revised the manuscript and confirm the authenticity of all raw data. All authors critically reviewed the manuscript, approved the final version for publication, and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The present study was performed in line with the Declaration of Helsinki (2013), and the protocol of the present study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (approval no. 0834/2025; IRB no. 0282/68; Bangkok, Thailand). Informed consent was obtained from all of the included patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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