Bacterial classification based on metagenomic analysis in peritoneal dialysis effluent of patients with chronic kidney disease

SUTHIDA VISEDTHORN[1,2], PAVIT KLOMKLIEW[2], VORTHON SAWASWONG[2,3], PAVARET SIVAPORNNUKUL[2], PRANGWALAI CHANCHAEM[2], THUNVARAT SAEJEW[4], PREEYARAT PAVATUNG[4], TALERNGSAK KANJANABUCH[4-6] and SUNCHAI PAYUNGPORN[2,7]

[1]Medical Biochemistry Program, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand; [2]Center of Excellence in Systems Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand; [3]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand; [4]Center of Excellence in Kidney Metabolic Disorders, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand; [5]Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand; [6]CAPD Excellence Center, King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand; [7]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

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Abstract. End-stage kidney disease (ESKD) is the final stage of chronic kidney disease (CKD), in which long-term damage has been caused to the kidneys to the extent that they are no longer able to filter the blood of waste and extra fluid. Peritoneal dialysis (PD) is one of the treatments that remove waste products from the blood through the peritoneum which can improve the quality of life for patients with ESKD. However, PD-associated peritonitis is an important complication that contributes to the mortality of patients, and the detection of bacterial pathogens is associated with a high culture-negative rate. The present study aimed to apply a metagenomic approach for the bacterial identification in the PD effluent (PDE) of patients with CKD based on 16S ribosomal DNA sequencing. As a result of this investigation, five major bacteria species, namely *Escherichia coli*, *Phyllobacterium myrsinacearum*, *Streptococcus gallolyticus*, *Staphylococcus epidermidis* and *Shewanella algae*, were observed in PDE samples. Taken together, the findings of the present study have suggested that this metagenomic approach could provide a greater potential for bacterial taxonomic identification compared with traditional culture methods, suggesting that this is a practical and culture-independent alternative approach that will offer a novel preventative infectious strategy in patients with CDK.

Correspondence to: Professor Sunchai Payungporn, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Road, Pathum Wan, Bangkok 10330, Thailand
E-mail: sp.medbiochemcu@gmail.com

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Introduction

Chronic kidney disease (CKD) is a long-term condition that causes progressive damage to kidney parenchyma, leading to the deterioration of kidney function and gradually progressing to end-stage kidney disease (ESKD) (1). ESKD is life-threatening without kidney replacement treatment, such as dialysis and kidney transplantation. Peritoneal dialysis (PD) uses the peritoneum in the patient's abdomen as the membrane through which the dialysate passes, and via this process, fluid and dissolved substances are exchanged with the blood. The excess fluid, toxins and other substances that result from kidney failure are also removed through this process (2). PD has the advantage that it can be performed at home, thereby obviating the need for the patient to be admitted to hospital; moreover, it is more cost-effective, is associated with fewer dietary restrictions, and increases both the perception of freedom for patients and patient satisfaction, thereby improving the patient's quality of life (3). In spite of these benefits, however, PD still carries a high risk of peritoneal infection, subcutaneous tunnel infection and catheter exit site infection (4-6). However, even though this dialysis technique has a number of benefits when compared with other available methods, there is an increased risk of peritonitis and microbial contamination of the blood through the catheter which compromises the immune defense system of patients, leading to complications, morbidity and mortality (3,4).

Currently, next-generation sequencing (NGS) provides a promising alternative approach for broad microbial identification in clinical samples (7,8). This approach allows for the unbiased detection of almost all potential microorganisms,

including bacteria, fungi, viruses and parasites. The NGS approach can be used to overcome the limitations of traditional methods, whereby the presence of microorganisms can be detected through the unique classification of DNA/RNA sequences (7). This approach has been successfully applied in the clinical diagnosis of infectious diseases such as severe acute respiratory syndrome coronavirus (9), nosocomial infection detection (10), *Clostridium difficile*-associated disease (11), as well as in response to outbreaks of disease and the discovery of novel pathogens (7,12). Particularly purulent fluids are often indicative of an infectious etiology, and application of the NGS approach has the potential to decrease the assay sensitivity and shorten time detection (13). The majority of metagenomic studies have used Illumina sequencing platforms, with sequencing turnaround times exceeding 16 h and overall process turnaround times of 48-72 h. By contrast, the company Oxford Nanopore Technologies (ONT) offers a third-generation sequencing technology that has several advantages compared with second-generation technologies in terms of longer reads and real-time sequence analysis capabilities, allowing the detection of potential microorganisms within hours of sequencing, and requiring shorter turnaround times of <6 h (13,14).

However, the precise nature of the microbial contamination that may influence the occurrence of infection remains unclear, and this represents a gap in our knowledge that urgently needs to be filled. Few studies have used NGS techniques to explore the microbial associated with PD-related peritonitis through short-reads sequencing platform; however, the taxonomic resolution is limited at the genus level (15,16). Therefore, the present study aimed to develop the rapid diagnosis pipeline for bacterial species classification in the PD effluent (PDE) of patients with ESKD during an early phase of infection based on 16S ribosomal DNA (rDNA) long-reads amplicon sequencing. Furthermore, the accuracy of the technique was comparable with the gold standard culture-dependent method. Thus, the present study was conducted to utilize the advantages of ONT in discovery and characterization of the PD-related microbial infection along with a comparison with the culture-dependent approach.

Patients and methods

Participants. The present study utilized 104 PDE samples obtained from patients (62 men and 42 women; age, 57.88±14.43 years) at the Thailand-Peritoneal Dialysis Outcomes and Practice Patterns Study (PDOPPS) facility of 22 hospitals in Thailand between January 2019 and December 2021. The PDOPPS was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (approval no. 1544/2020; IRB no. 499/58; Bangkok, Thailand). The present study was performed with remains of previously obtained samples from the PDOPPS, which was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (COA no. 0754/2022; IRB no. 0253/65; Bangkok, Thailand). Informed consent was obtained from all included patients.

All participants were >18 years of age, and were diagnosed with kidney failure, having undergone continuous ambulatory peritoneal dialysis or automated peritoneal dialysis for at least 1 month. Peritonitis was diagnosed according to the presence of two out of three of the following inclusion criteria: i) PDE from the first episode of infection was observed to be cloudy, ii) peritonitis caused by the infection had been reported and iii) the patient had a white blood cell count of >100 cells and/or the percentage of polymorphonuclear neutrophils was >50%. By contrast, patients <18 years of age, and those undergoing hemodialysis, acute peritoneal dialysis or combination renal replacement therapy were excluded from participation in the present study.

Sample processing and DNA extraction. Briefly, the collected samples (50 ml) were subjected to centrifugation at 25˚C for 15 min in a microcentrifuge at 18,500 x g, with subsequent removal of the supernatant. The resultant pellet was suspended in 400 μl sorbitol buffer containing 50 U Lyticase enzyme (Merck KGaA), and then incubated at 30˚C for 60 min to break the cells. The cell suspension was then centrifuged at 18,500 x g at 4˚C for 5 min, followed by discarding of the supernatant. Subsequently, 567 μl 1M TE buffer, 3 μl 10% SDS (Merck KGaA), 100 μl 10 mg/ml lysozyme enzyme (Merck KGaA) and 5 μl 20 mg/ml Proteinase K (Worthington Biochemical Corporation) were added to the solution. The resultant mixture was incubated at 65˚C for 90 min to digest the proteins and to inhibit the RNases that were present. Finally, the samples were subjected to centrifugation in a microcentrifuge at 18,500 x g at 4˚C for 5 min, and the resultant pellets were collected and resuspended in 200 μl sterile water. Total genomic DNA (gDNA) extraction was performed using the magLEAD® 12gC system (Precision System Science Co., Ltd.), following the manufacturer's protocol. The quantity and quality of the DNA were assessed using a 260/280 spectrophotometry ratio as a measure of DNA purity (1.70-1.85) and 1.5% agarose gel electrophoresis with RedSafe™ Nucleic Acid Staining Solution (cat. no. 21141; Intron Biotechnology, Inc.), respectively, and the extracted DNA was stored at 20˚C until further use.

Bacterial culture. Three 50 ml PDE bags were centrifuged (3,500 x g) at 25˚C for 15 min, and the supernatants were discarded. Of the remaining solution, 5 ml was mixed into the pellet and injected into BACTEC™ Plus Aerobic/F and BACTEC Plus Anaerobic/F vials (Becton, Dickinson and Company). The mixture was subsequently spread onto various agar plates, including blood agar, Oxoid® MacConkey agar and chocolate agar (Thermo Fisher Scientific, Inc.), and thiosulfate citrate bile salt sucrose agar (Biomedia Thailand Co., Ltd.), as required. The plates were then incubated at 37˚C for 5-7 days to facilitate bacterial culture. Identification of bacterial pathogens was subsequently performed via Gram staining, utilizing the VITEK® MS system (BIOMÉRIEUX).

16S rDNA gene amplification. PCR amplification of partial 16S rDNA (the V1-V4 region) was selected as the method to classify bacterial communities at the species level. The primers included specific target primer sequences (shown underlined) and nanopore adaptor tails, as follows: 16S_27 forward, 5' -TTTCTGTTGGTGCTGATATTGCAGRGTTYGATYMTGGCTCAG-3'; and 16S_806 reverse, 5'-ACTTGCCTGTCGCTCTATCTTCGGACTACHVGGGTWTCTAAT-3'. The representative PCR products (~800 bp) obtained from the amplification of the 16S rDNA gene using 16S_27F/16S_806R primers are shown

in Fig. S1. The PCR products were amplified using Phusion™ Plus DNA Polymerase (Thermo Fisher Scientific, Inc.) to minimize errors during amplification. The PCR reactions were performed in a total volume of 20 μl, containing 10 μM forward and reverse primer pairs, 10 μM dNTPs, 0.4 U Phusion™ Plus DNA Polymerase and 10 ng DNA template. The thermocycling conditions were as follows: 98˚C for 30 sec, 25 cycles of 98˚C for 10 sec, 60˚C for 10 sec, 72˚C for 50 sec, and a final extension at 72˚C for 5 min. The DNA library of each sample was pooled using the PCR Barcoding Expansion 1-96 kit (cat. no. EXP-PBC096; Oxford Nanopore Technologies). The barcoding step made use of a thermal profile similar to the first PCR, albeit with the barcode primers instead of primer-specific 16S rDNA genes. The barcoding step comprised the following thermocycling conditions: 98˚C for 30 sec, 25 cycles of 98˚C for 10 sec, 60˚C for 10 sec, 72˚C for 50 sec and a final extension at 72˚C for 5 min. The PCR products were subsequently separated using 1% agarose gel electrophoresis with RedSafe™ Nucleic Acid Staining Solution and purified using a QIAquick Gel Extraction Kit (cat. no. 28704; Qiagen GmbH), following the manufacturer's protocol.

Nanopore library preparation and sequencing. The DNA libraries were quantified using Quant-iT™ dsDNA High Sensitivity Assay Kits (cat. no. Q32851) for the Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Inc.). Subsequently, all libraries were pooled (final concentration 1 μg) for multiplexing, and underwent purification using 0.5X Agencourt AMPure XP beads (Beckman Coulter, Inc.). Following purification, the Ligation Sequencing Kit of Oxford Nanopore Technologies (cat. no. SQK-LSK112) was used to repair and ligate the ends of the DNA library. Finally, the pooled DNA library was sequenced (single-end, 800 bp) using a MinION™ Mk1C sequencing device and an R10.4 flow cell (cat. no. FLO-MIN112), both from Oxford Nanopore Technologies.

Data analysis. The raw data or FAST5 data were base-called using Guppy base-caller version 6.0.7 (Oxford Nanopore Technologies) with a super-accuracy model to generate pass reads in FASTQ format with a minimum acceptable quality score of Q>10 (17). Subsequently, MinIONQC was used to assess the quality of reads for nanopore data (18). The FASTQ sequences underwent demultiplexing and adaptor-trimming using Porechop, version 0.2.4. Filtered reads were clustered, polished and taxonomically classified using NanoCLUST (19). The classification was based on the V1-V4 region of 16S rDNA gene sequences from the Ribosomal Database Project database (20). Relative abundance and taxonomic assignment data were converted into the QIIME2 data format, demonstrating bacterial species' richness and evenness based on taxa abundances, and visualized using GraphPad Prism 9.5.0. This analysis utilized a plug-in implemented for QIIME2 software version, 2021.2 (21). The unsupervised clustering was conducted and represented in a heatmap using the ComplexHeatmap R package (22).

Results

Diversity of bacteria in PDE. The bacterial 16S rDNA (V1-V4 variable region) was sequenced using a high-throughput MinION™ platform (Oxford Nanopore Technologies). A total of 1,341,989 raw reads were obtained in the present study, with 15,079±11,214 average reads/sample. The average number of classified reads was 10,656±7,762 reads/sample. A rarefaction analysis was subsequently applied to estimate whether there was sufficient sequence coverage both to classify all samples reliably from the bacterial taxa, and to classify them into operational taxonomic units. The results revealed sufficient sequencing depth for diversity in 89 PDE samples (Fig. S2). The alpha diversity was assessed based on Chao1 and Shannon indexes, as shown in Fig. 1A and B. The Chao1 index (8.15±8.06) indicated the richness of bacteria in each sample, whereas the Shannon index (1.24±1.05) indicated the richness and evenness of bacteria in each sample. This result demonstrated that the samples were highly heterogeneous, which suggests that differences in patient hygiene may account for the variety of bacterial diversity amongst patients.

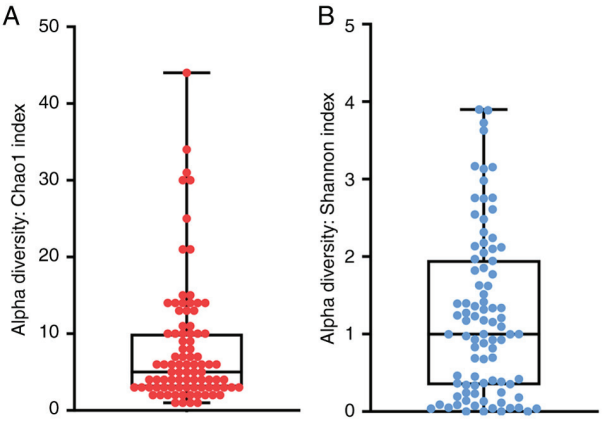

Figure 1. The alpha diversity of bacteria in peritoneal dialysis effluent from 89 patients with chronic kidney disease. (A) Chao1 and (B) Shannon indexes are shown as scatterplots.

Relative bacterial abundance in PDE of patients with ESKD. The relative abundance of bacterial composition in 89 PDE samples was classified. At the phylum level, the dominant bacteria were Firmicutes, Proteobacteria and Actinobacteria (Fig. 2A). The relative abundance of bacteria at the genus level is illustrated in Fig. 2B. The five major bacterial genera were found to be *Escherichia/Shigella*, *Streptococcus*, *Staphylococcus*, *Phyllobacterium* and *Lactococcus*. Several abundant bacterial species were identified in patients receiving PD (Fig. 2C). The results showed that *Escherichia coli* (*E. coli*) was the most abundant bacterial species, followed (in order) by *Phyllobacterium myrsinacearum* (*P. myrsinacearum*), *Streptococcus gallolyticus* (*S. gallolyticus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Shewanella algae* (*S. algae*).

Heatmap analysis was used to visualize the hierarchical clustering of bacterial diversity, and thereby reveal the top 35 most abundant bacterial species. All subjects were divided into eight clusters according to the microbial community patterns in the samples (Fig. 3). The dominant bacterial community included *Candidatus Rhizobium* (cluster 1), *Lactococcus garvieae* (cluster 2), *P. myrsinacearum* (cluster 3), *S. gallolyticus* (cluster 4) and *S. epidermidis* (cluster 6). *E. coli* dominated in clusters 7 and 8, which were distinguished

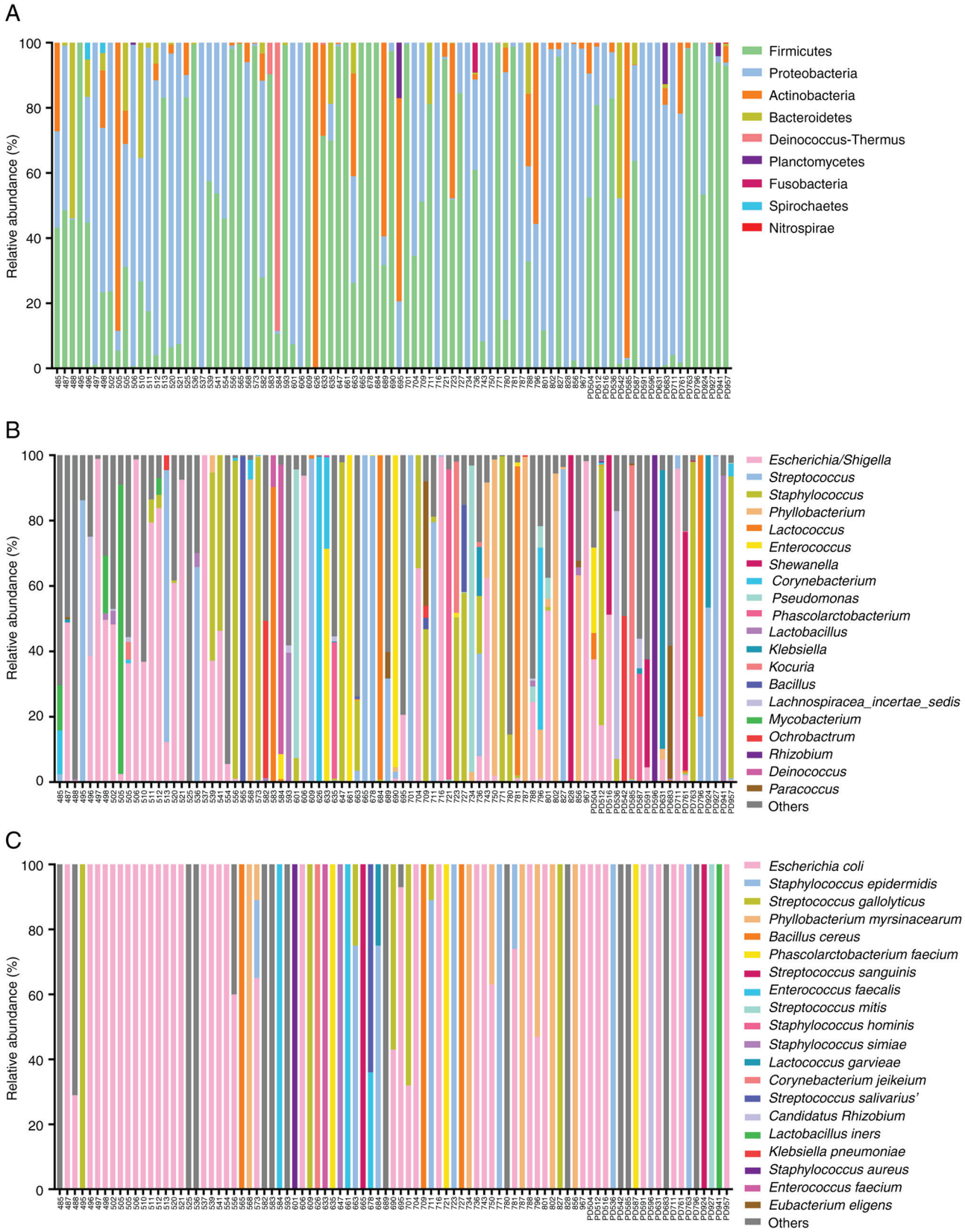

Figure 2. The relative abundance (%) of bacteria at the (A) phylum, (B) top 20 genera and (C) top 20 species levels. The colored bar charts represent the different bacterial taxonomy. The taxa below the top 20 were classified as 'Others'.

by relative abundances of >70 and <70%, respectively. The other microbial community patterns were classified in cluster 5. This result showed that the overwhelming presence of *E. coli* in clusters 7 and 8, with obvious differences in relative abundance, could imply a differential impact on the health of the patient, potentially associated with varying infection risks

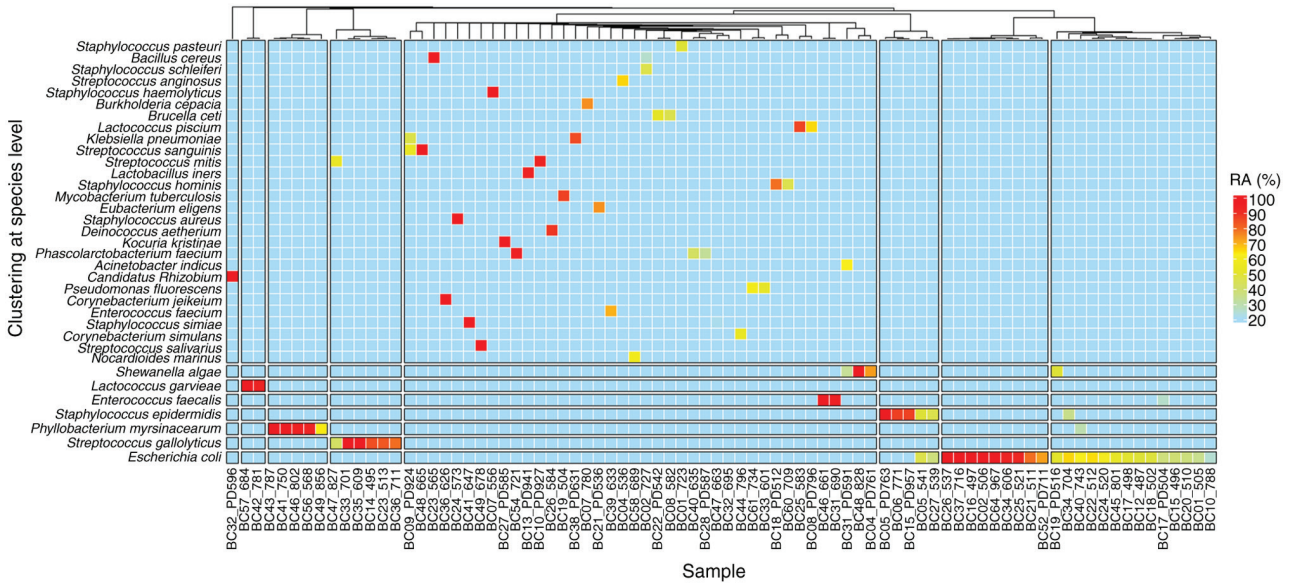

Figure 3. Heatmap analysis, visualizing the hierarchical clustering of the bacterial community in each sample based on the analysis of the 35 most abundant species. The color represents the relative abundance (%) for bacterial species. RA, relative abundance.

or outcomes. This distinction between the high-abundance (>70%) and moderate-abundance (<70%) groups of *E. coli* may point to different colonization or infection dynamics, suggesting that cluster 7 could be at a higher risk of infection-related complications compared with cluster 8 (23). The classification of other microbial community patterns into cluster 5 might represent a mixed or transitional flora, possibly including organisms that are less common or less dominant but could still play a role in the catheter ecosystem, either as commensals or opportunistic pathogens (24). This diversity within cluster 5 might also indicate a fluctuating microbial environment influenced by external factors such as antibiotic use, patient hygiene, or the procedure of catheter insertion and maintenance.

Comparison between metagenomic approach and traditional culture for bacterial identification. Among the 89 PDE samples, bacterial species were identified from only 56 samples (62.92%) based on the traditional culture method, whereas all samples (100%) could be classified through the metagenomic approaches (Fig. 4). In a comparison between metagenomic and traditional culture methods for bacterial classification in the 56 samples, concordant results from both techniques were observed in 42/56 samples (75%). Briefly, the dominant bacterial species were *E. coli* (eight cases), *S. epidermidis* (six cases), *K. pneumoniae* (three cases), *S. aureus* (three cases), *E. faecalis* (two cases), *P. aeruginosa* (two cases), *S. mitis* (two cases), and 16 other bacterial species (one case each), as summarized in Table I. On the other hand, regarding the remaining 14/56 samples (25%), different results were demonstrated between the metagenomic approaches and traditional culture methods, as shown in Table II. Interestingly, the metagenomic approaches could be applied for bacterial classification in 33/89 samples (37.08%), which were negative as far as the traditional culture method was concerned. The metagenomic results are summarized in Table III.

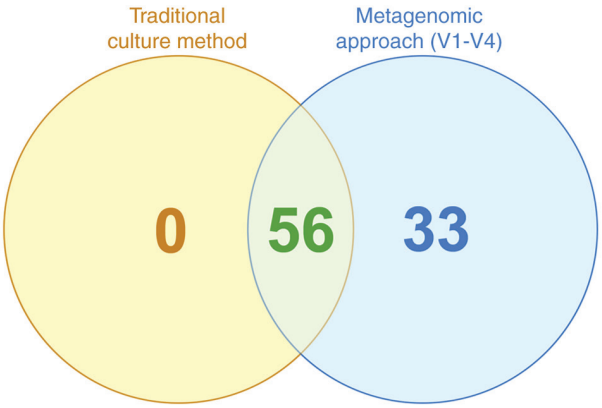

Figure 4. A Venn diagram illustrating the number of peritoneal dialysis effluent samples that can be classified for bacterial species, based on the metagenomic approach (V1-V4) and a traditional culture method. The numbers in the overlapping circles revealed the positive results applicable to both methods.

Discussion

Amplicon sequencing using the Oxford Nanopore Technologies platform is a powerful strategy for microbial identification, and has been popularly employed for microbiome analysis in diverse patient clinical samples (25). This sequencing platform offers a culture-free method that both provides a cost-effective technique and is associated with a number of essential benefits regarding long-read data (26). The amplification and sequencing of the full-length 16S rDNA gene (~1,500 bp) can allow bacterial identification up to the species level with high accuracy and sensitivity (27,28). However, a favorable-quality DNA sample is initially required to amplify the full-length gene for long-read sequencing; therefore, one limitation of this approach is the difficulty of achieving full-length gene amplification in low-quality DNA samples.

Table I. Summary of concordant bacterial species obtained from both methods.

Bacterial species	Samples ID	Number of cases (%)
Acinetobacter indicus	PD591	1/42 (2.38)
Burkholderia cepacia	780	1/42 (2.38)
Candidatus Rhizobium	PD596	1/42 (2.38)
Citrobacter freundii	485	1/42 (2.38)
Corynebacterium simulans	796	1/42 (2.38)
Corynebacterium striatum	568	1/42 (2.38)
Corynebacterium striatum	633	1/42 (2.38)
Enterococcus faecium		
Escherichia coli	505, 487, 497, 510, 511, 716, 750, PD711	8/42 (19.05)
Enterobacter cloacae	520	1/42 (2.38)
Enterococcus faecalis	690, 661	2/42 (4.76)
Klebsiella pneumoniae	736, PD924, PD631	3/42 (7.15)
Lactococcus garvieae	684	1/42 (2.38)
Pseudomonas aeruginosa	734, 601	2/42 (4.76)
Shewanella algae	828	1/42 (2.38)
Staphylococcus aureus	573, 647, 663	3/42 (7.15)
Staphylococcus epidermidis	541, 539, 711, 771, PD763, PD957	6/42 (14.29)
Staphylococcus haemolyticus	556	1/42 (2.38)
Staphylococcus hominis	709	1/42 (2.38)
Staphylococcus pasteuri	723	1/42 (2.38)
Staphylococcus schleiferi	727	1/42 (2.38)
Streptococcus anginosus	536	1/42 (2.38)
Streptococcus gallolyticus	495	1/42 (2.38)
Streptococcus mitis	827, PD927	2/42 (4.76)

In the present study, the DNA in PDE samples was degraded for several reasons, including being collected without nucleic acid preservation (NAP), RNA/DNA Stabilization Buffer, multiple freeze-thaw events, and being kept at -20˚C for a long period of time (29). Degraded samples may have insufficient quantities of DNA to amplify the full-length 16S rDNA gene. To solve this problem, the sample should be preserved in NAP buffer (30), and avoiding multiple freeze-thaw steps would be more appropriate for full-length amplicon sequencing. Normally, V3-V4 hypervariable region of 16S rDNA gene (~500 bp) is widely used for bacterial identification studies which limits the taxonomic diversity, specified only at the genus level (31). For V1-V4 (~800 bp), target sequence was longer than V3-V4 hypervariable. Therefore, the partial 16S rDNA gene (V1-V4 region) should be applied for identifying the bacterial species in samples without the preservation buffer and low abundance of bacterial DNA. Another factor that may contribute towards DNA degradation would be the lysis buffer used in the DNA extraction process. A cell wall is found in the majority of different species of bacteria, and this is substantially more rigid than the plasma membrane of mammalian cells. A mild lysis buffer can therefore be used to ensure that the plasma membrane is selectively lysed with no resultant damage to the microorganisms. However, certain microorganisms are more likely to be destroyed by using a selective lysis buffer, which leads to an undesirably low DNA quantity for library preparation and sequencing (32).

In the present study, the Chao1 and Shannon diversity indexes in PDE were relatively lower than those found in the previous study that investigated the microbial diversity in peritoneal tissue samples (33). This result suggested that the bacterial composition in the PDE sample might be diluted compared with bacterial community in the peritoneal tissue sample. However, the PDE sample collection is non-invasive and more convenient process. The present study demonstrated that the traditional bacterial culture method provided positive results in only 56 of the 104 samples (53.8%), whereas the 16S rDNA metagenomic approach was able to identify up to 89 samples (85.6%). Moreover, the current study showed that the same results were obtained comparing between the traditional culture method and 16S rDNA sequencing in 42/56 samples (75%). Notably, the bacterial species were classified for the 33 samples (31.73%) that lacked traditional culture results through the use of 16S rDNA amplicon sequencing. Considered altogether, the results obtained from the present study were comparable with those of a recent study (16), wherein shotgun metagenomic analysis was performed to identify pathogens in PDE samples based on the BGISEQ platforms, as summarized in Table IV.

In the present study, the 16S rDNA gene sequencing results showed that Firmicutes, Proteobacteria and Actinobacteria were the dominant phyla in patients with ESKD, similar to the findings of a previously published study (34). In line with the findings of the current study, previous studies identified microbiomes in the peritoneal tissue of patients

Table II. Summary of different bacterial species between the metagenomic approach and traditional culture method.

Sample ID	Traditional culture method result	Top three of metagenomic approach result (abundance, %)
496	*Kocuria kristinae*	*Escherichia coli* (38.54%), *Ruminococcus gnavus* (36.54%), *Prevotella copri* (11.45%)
502	*Ochrobactrum anthropic*	*Escherichia coli* (48.22%), *Methylobacterium dankookense* (26.55%), *Clostridium saccharolyticum* (7.73%)
506	*Pseudomonas aeruginosa*	*Escherichia coli* (98.70%), *Butyricicoccus pullicaecorum* (0.68%), *Schlesneria paludicola* (0.63%)
525	*Staphylococcus aureus*	*Catabacter hongkongensis* (28.33%), *Saccharofermentans acetigenes* (21.85%), *Coprococcus comes* (18.24%)
554	*Corynebacterium* sp.	*Zhizhongheella caldifontis* (40.20%), *Sarcina ventriculi* (37.47%), *Achromobacter ruhlandii* (5.99%)
582	*Pseudomonas aeruginosa*	*Brucella ceti* (48.96%), *Oscillibacter valericigenes* (21.84%), *Achromobacter xylosoxidans* (7.65%)
593	*Rhizobium radiobacter*	*Lactobacillus reuteri* (33.81%), *Catabacter hongkongensis* (21.98%), *Cellulosilyticum lentocellum* (17.36%)
665	*Staphylococcus epidermidis*	*Streptococcus sanguinis* (100%)
695	*Mycobacterium tuberculosis*	*Brachybacterium conglomeratum* (35.84%), *Propionibacterium acnes* (26.55%), *Escherichia coli* (20.58%)
781	*Coagulase Negative Staphylococcus*	*Lactococcus garvieae* (93.95%), *Cellulosilyticum lentocellum* (2.21%), *Escherichia coli* (1.24%)
788	*Shewanella putrefaciens*	*Escherichia coli* (24.51%), *Propionibacterium acnes* (22.27%), *Roseburia faecis* (17.22%)
PD512	*Klebsiella pneumonia*	*Staphylococcus hominis* (79.20%), *Escherichia coli* (17.44%), *Veillonella atypica* (0.71%)
PD542	*Mycobacterium tuberculosis*	*Brucella ceti* (50.73%), *Parabacteroides faecis* (47.73), *Roseburia intestinalis* (1.48%)
PD941	*Staphylococcus haemolyticus*	*Lactobacillus iners* (93.77%), *Pirellula staleyi* (4.15%), *Methylobacterium podarium* (1.93%)

with ESKD who harbored a high abundance of Firmicutes and Proteobacteria (33). The bacterial genera *Escherichia*, *Streptococcus* and *Staphylococcus* were also detected in a recent study (16) that used a traditional culture method and shotgun metagenomic analysis. Therefore, the dominance of *E. coli, P. myrsinacearum*, *S. gallolyticus*, *S. epidermidis* and *S. algae* in the PDE samples in the present study may be of clinical importance. Moreover, a different study (35) revealed the causative microorganisms in PDE samples based on traditional culture that found both gram-positive bacteria (namely, *Staphylococcus, Streptococcus* and *Enterococcus*) and gram-negative bacteria (namely, *Escherichia, Klebsiella* and *Pseudomonas*). This finding was consistent in part with those of the present study.

In general, *E. coli* is a frequent gram-negative peritonitis bacterium that can produce the extended-spectrum β-lactamase that is associated with a poorer prognosis (36). Interestingly, PD-associated peritonitis caused by *Streptococcus sp.* has been reported from the entry routes into the peritoneal cavity, including contamination during the exchange or catheter-associated processes and bacterial translocation (37). A number of studies have shown that the most common pathogens are coagulase-negative *Staphylococcus* species, including *S. epidermidis* and *S. aureus*, which commonly colonize human skin and hands and may also lead to peritonitis via exit-site and tunnel infections (38,39). *Shewanella* sp. are hydrogen sulfide-producing, motile gram-negative bacilli. The clinical syndromes that are commonly encountered are skin and soft tissue infections, including peritoneal catheter-associated infections (40). Finally, *P. myrsinacearum* is a gram-negative bacterium that can cause infections in humans. As *P. myrsinacearum* cannot grow on standard media culture, the identification of this bacterium based on a metagenomic approach raised several outstanding questions about whether it is able to cause severe infection in humans (41).

In summary, the present study demonstrated the metagenomic analysis based on the partial gene amplification of 16S rDNA with Oxford Nanopore Technologies, which is suitable for PDE samples containing low abundance of DNA. The present metagenomic approach would be useful for monitoring possible bacterial infections in patients with CKD with peritoneal dialysis. Moreover, this method might be attractive and applicable for other specimens with low amount of DNA such as urine, skin and conjunctival specimens. Furthermore, it can be applied to select appropriate medicine to reduce antibiotics resistance prone in long-term.

Table III. Summary of bacterial species through the only metagenomic method.

Sample ID	Top three of the metagenomic approach result (abundance, %)
488	*M. thermophilus* (28.32%), *C. clostridioforme* (22.94%), *P. copri* (12.99%)
498	*E. coli* (49.62%), *M. tuberculosis* (16.36%), *A. chartisolvens* (12.73%)
504	*M. tuberculosis* (86.92%), *C. segnis* (2.80%), *E. coli* (2.49%)
512	*E. coli* (64.79%), *E. fergusonii* (19.04%), *M. tuberculosis* (5.14%)
513	*S. gallolyticus* (83.13%), *E. coli* (12.31%), *B. ceti* (3.57%)
521	*E. coli* (92.49%), *F. magna* (7.51%)
537	*E. coli* (100%)
565	*B. cereus* (99.62%), *P. acnes* (0.30%), *B. weihenstephanensis* (0.08%)
583	*L. piscium* (86.47%), *D. aetherius* (9.74%), *L. raffinolactis* (3.79%)
584	*D. aetherius* (88.51%), *E. faecalis* (7.62%), *C. oryzae* (2.08%)
606	*E. coli* (93.77%), *M. podarium* (4.13%), *M. radiotolerans* (1.40%)
609	*S. gallolyticus* (98.93%), *L. piscium* (1.07%)
626	*C. jeikeium* (99.50%), *M. halophilus* (0.27%), *P. acnes* (0.23%)
635	*P. faecium* (41.69%), *P. buccalis* (14.21%), *O. valericigenes* (8.52%)
678	*S. salivarius* (97.45%), *S. warneri* (2.36%), *E. faecalis* (0.19%)
689	*N. marinus* (59.48%), *S. gordonii* (31.69%), *P. sediminis* (8.11%)
701	*S. gallolyticus* (99.54%), *E coli* (0.46%)
704	*E. coli* (65.45%), *S. epidermidis* (34.55%)
721	*P. faecium* (94.92%), *B. luteolum* (3.95%), *P. stutzeri* (0.75%)
743	*E. coli* (62.44%), *P. myrsinacearum* (29.22%), *C. minuta* (8.34%)
787	*P. myrsinacearum* (99.53%), *O. pituitosum* (0.47%)
796	*L. piscium* (65.22%), *S. parauberis* (20.05%), *L. raffinolactis* (14.73%)
801	*E. coli* (52.48%), *A. commune* (26.08%), *F. saccharivorans* (10.55%)
802	*P. myrsinacearum* (94.42%), *B. vesicularis* (3.59%), *R. mucilaginosa* (1.99%)
856	*P. myrsinacearum* (63.30%), *B. aurantiaca* (27.09%), *B. vesicularis* (4.72%)
967	*E. coli* (98.17%), *P. acnes* (1.83%)
PD504	*E. coli* (37.35%), *E. faecalis* (25.86%), *P. capillosus* (17.96%)
PD516	*E. coli* (51.20%), *S. algae* (48.80%)
PD536	*E. eligens* (74.34%), *M. podarium* (11.61%), *S. epidermidis* (7.05%)
PD585	*K. kristinae* (95.64%), *G. para-adiacens* (1.62%), *S. suis* (0.71%)
PD587	*P. faecium* (32.69%), *E. cloacae* (14.38%), *E. eligens* (8.00%)
PD683	*P. sediminis* (40.62%), *T. brevis* (12.76%), *P. staleyi* (11.20%)
PD761	*S. algae* (72.93%), *C. cellulans* (15.33%), *N. kribbensis* (5.45%)

Table IV. The comparison of positive rate from bacterial culture and metagenomic analysis between the present study and a recent report.

Result	Present study	Ye *et al*, 2022 (16)
Positive rate from culture method	56/104 (53.85%)	18/30 (60%)
Positive rate from metagenomic analysis	89/104 (85.58%)	26/30 (86.67%)
Positive rate from both techniques	56/104 (53.85%)	15/30 (50%)
Negative rate from both techniques	0/104 (0%)	1/30 (3.33%)

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Availability of data and materials

The data generated in the present study may be found in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession numbers SRR26930019 to SRR26930107. The data generated in the present study may be found in the NCBI GenBank under accession number PRJNA1044279 or at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1044279.

Authors' contributions

SP and TK conceptualized the study. SV conducted the experiments, analyzed the data, interpreted the results and prepared the draft version of the manuscript. PK, VS and PS contributed to data analysis. PC, TS and PP contributed to the experiments. SP and TK oversaw, revised the final manuscript and confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in line with the Declaration of Helsinki (2013), and the protocol of this study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (approval no. 0754/2022; IRB no. 0253/65; Bangkok, Thailand). Informed consent was obtained from all included patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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