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

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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.

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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 $\mu$l 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 $\mu$l 1M TE buffer, 3 $\mu$l 10% SDS (Merck KGaA), 100 $\mu$l 10 mg/ml lysozyme enzyme (Merck KGaA) and 5 $\mu$l 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 $\mu$l 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 $\mu$l, containing 10 $\mu$M forward and reverse primer pairs, 10 $\mu$M 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 $\mu$g) 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.

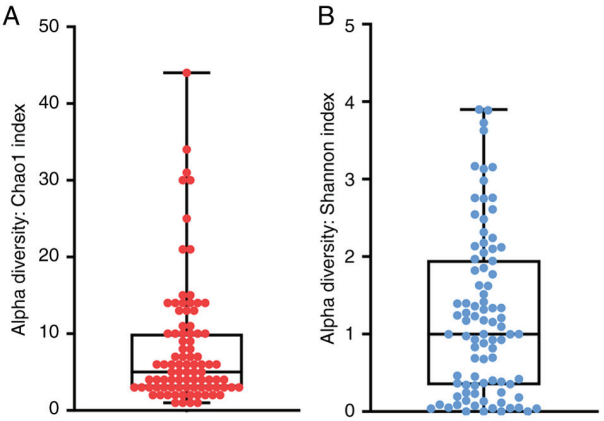


Figure 1. The alpha diversity of bacteria in peritoneal dialysis effluent from 89 patients with chronic kidney disease. (A) Chao1 and (B) Shannon indexes are shown as scatterplots.

*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

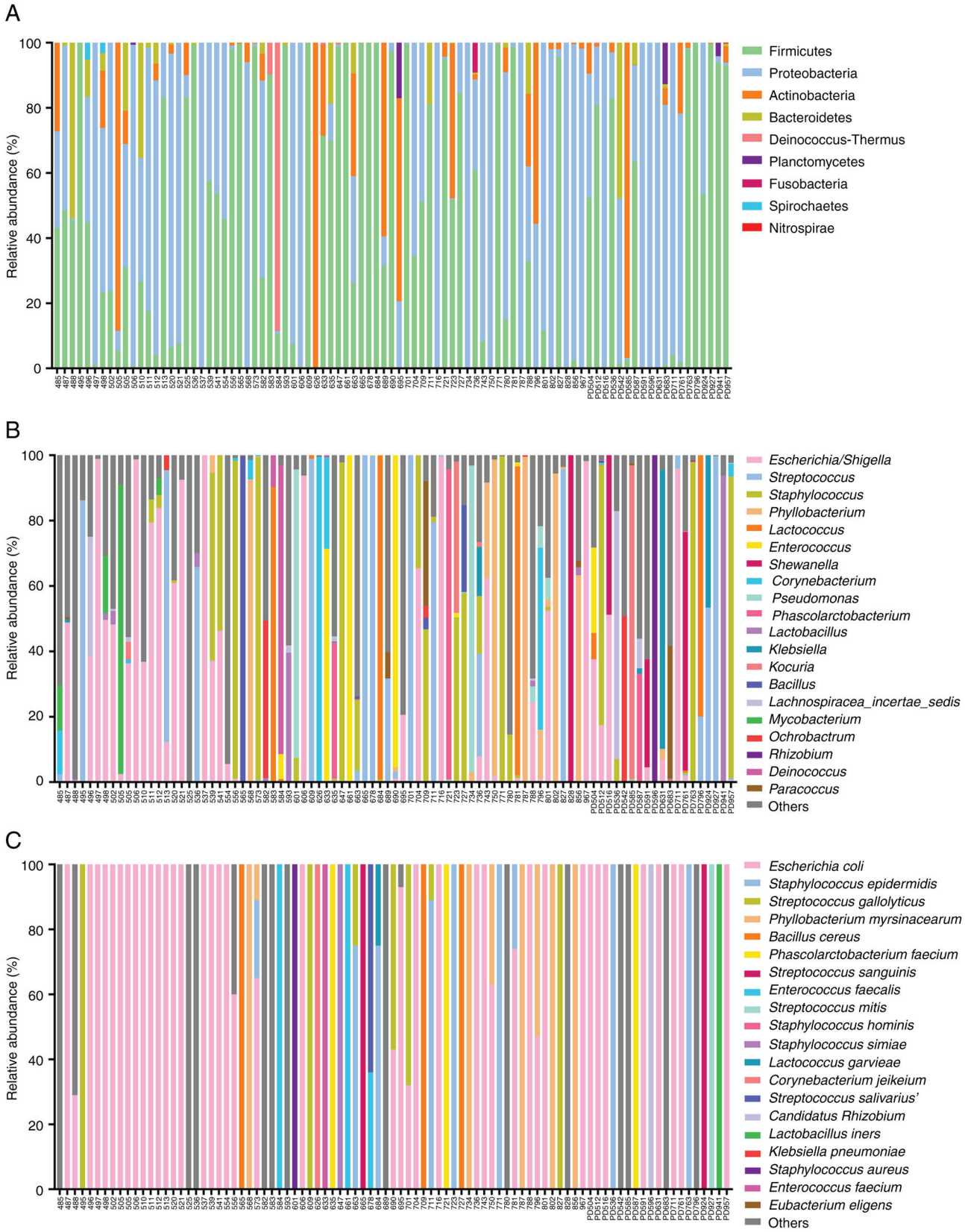


Figure 2. The relative abundance (%) of bacteria at the (A) phylum, (B) top 20 genera and (C) top 20 species levels. The colored bar charts represent the different bacterial taxonomy. The taxa below the top 20 were classified as 'Others'.

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

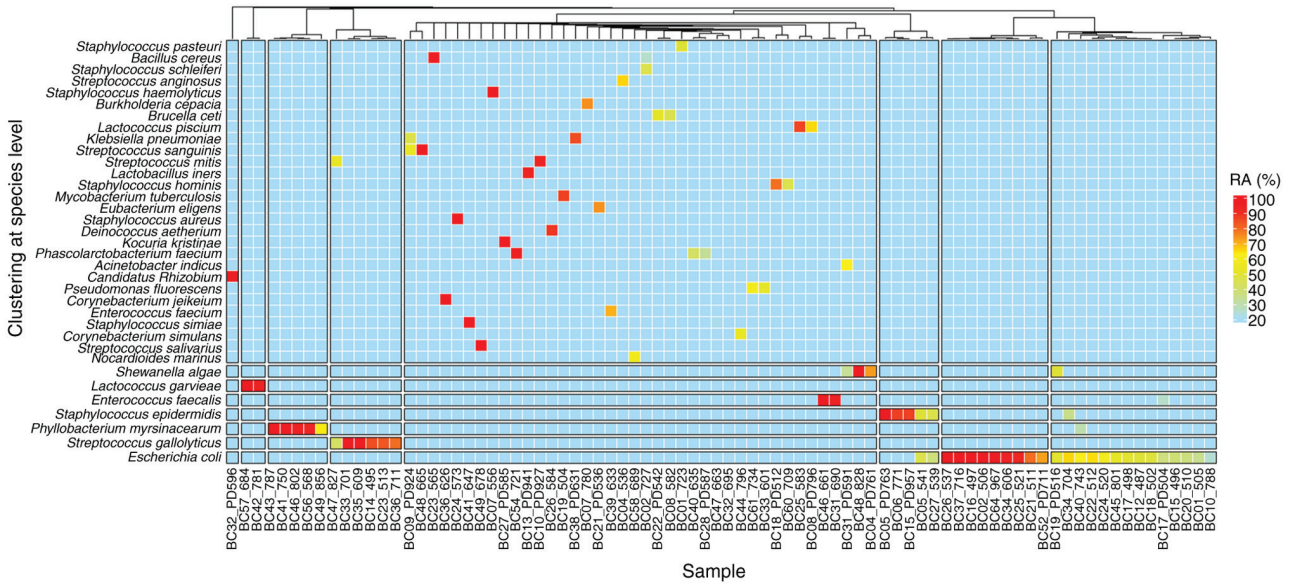


Figure 3. Heatmap analysis, visualizing the hierarchical clustering of the bacterial community in each sample based on the analysis of the 35 most abundant species. The color represents the relative abundance (%) for bacterial species. RA, relative abundance.

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.

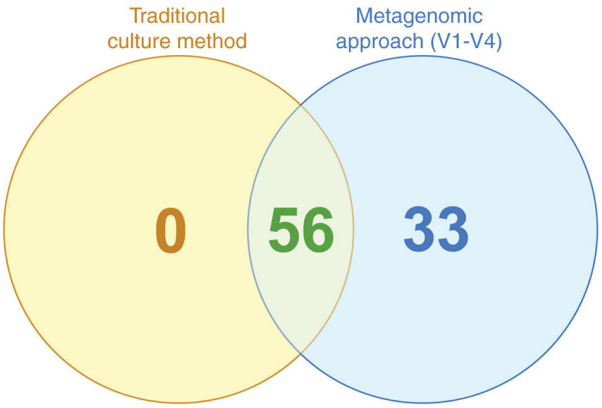


Figure 4. A Venn diagram illustrating the number of peritoneal dialysis effluent samples that can be classified for bacterial species, based on the metagenomic approach (V1-V4) and a traditional culture method. The numbers in the overlapping circles revealed the positive results applicable to both methods.

## 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.

## References

1. Akchurin OM: Chronic kidney disease and dietary measures to improve outcomes. Pediatr Clin North Am 66: 247-267, 2019.
2. NIDDK: Peritoneal Dialysis. Journal 1950.
3. Zimmerman AM: Peritoneal dialysis: Increasing global utilization as an option for renal replacement therapy. J Glob Health 9: 020316, 2019.
4. Akoh JA: Peritoneal dialysis associated infections: An update on diagnosis and management. World J Nephrol 1: 106-122, 2012.
5. Brook NR, White SA, Waller JR and Nicholson ML: The surgical management of peritoneal dialysis catheters. Ann R Coll Surg Engl 86: 190-195, 2004.
6. Amato D, de Jesus Ventura M, Miranda G, Leaños B, Alcántara G, Hurtado ME and Paniagua R: Staphylococcal peritonitis in continuous ambulatory peritoneal dialysis: Colonization with identical strains at exit site, nose, and hands. Am J Kidney Dis 37: 43-48, 2001.
7. Deng X, Achari A, Federman S, Yu G, Somasekar S, Bártolo I, Yagi S, Mbala-Kingebeni P, Kapetshi J, Ahuka-Mundeke S, *et al*: Metagenomic sequencing with spiked primer enrichment for viral diagnostics and genomic surveillance. Nat Microbiol 5: 443-454, 2020.
8. Simner PJ, Miller S and Carroll KC: Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. Clin Infect Dis 66: 778-788, 2018.
9. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Peñaranda S, Bankamp B, Maher K, Chen MH, *et al*: Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300: 1394-1399, 2003.
10. Greninger AL and Zerr DM: NGSocomial infections: High-Resolution views of hospital-acquired infections through genomic epidemiology. J Pediatric Infect Dis Soc 10 (Supplement 4): S88-S95, 2021.
11. Chiu CY and Miller SA: Clinical metagenomics. Nat Rev Genet 20: 341-355, 2019.
12. Chiu CY: Viral pathogen discovery. Curr Opin Microbiol 16: 468-478, 2013.
13. Gu W, Deng X, Lee M, Sucu YD, Arevalo S, Stryke D, Federman S, Gopez A, Reyes K, Zorn K, *et al*: Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids. Nat Med 27: 115-124, 2021.
14. Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, Stryke D, Bouquet J, Somasekar S, Linnen JM, *et al*: Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis. Genome Med 7: 99, 2015.
15. Nie S, Zhang Q, Chen R, Lin L, Li Z, Sun Y, Huang J, Feng Z, Cao X, Ye K, *et al*: Rapid detection of pathogens of peritoneal dialysis-related peritonitis, especially in patients who have taken antibiotics, using metagenomic next-generation sequencing: A pilot study. Ren Fail 45: 2284229, 2023.
16. Ye P, Xie C, Wu C, Yu C, Chen Y, Liang Z, Chen Y, Chen Q and Kong Y: The application of metagenomic next-generation sequencing for detection of pathogens from dialysis effluent in peritoneal dialysis-associated peritonitis. Perit Dial Int 42: 585-590, 2022.
17. Wick RR, Judd LM and Holt KE: Performance of neural network basecalling tools for Oxford Nanopore sequencing. Genome Biol 20: 129, 2019.
18. Lanfear R, Schalamun M, Kainer D, Wang W and Schwessinger B: MinIONQC: Fast and simple quality control for MinION sequencing data. Bioinformatics 35: 523-525, 2019.
19. Rodriguez-Perez H, Ciuffreda L and Flores C: NanoCLUST: A species-level analysis of 16S rRNA nanopore sequencing data. Bioinformatics 37: 1600-1601, 2021.
20. Cole JR, Chai B, Marsh TL, Farris RJ, Wang Q, Kulam SA, Chandra S, McGarrell DM, Schmidt TM, Garrity GM, *et al*: The ribosomal database project (RDP-II): Previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Res 31: 442-443, 2003.
21. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, *et al*: Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37: 852-857, 2019.
22. Gu Z, Eils R and Schlesner M: Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32: 2847-2849, 2016.
23. Bradley CW, Flavell H, Raybould L, McCoy H, Dempster L, Holden E and Garvey MI: Reducing Escherichia coli bacteraemia associated with catheter-associated urinary tract infections in the secondary care setting. J Hosp Infect 98: 236-237, 2018.
24. Eggimann P, Sax H and Pittet D: Catheter-related infections. Microbes Infect 6: 1033-1042, 2004.
25. Fu Y, Chen Q, Xiong M, Zhao J, Shen S, Chen L, Pan Y, Li Z and Li Y: Clinical performance of nanopore targeted sequencing for diagnosing infectious diseases. Microbiol Spectr 10: e0027022, 2022.
26. Midha MK, Wu M and Chiu KP: Long-read sequencing in deciphering human genetics to a greater depth. Hum Genet 138: 1201-1215, 2019.
27. Matsuo Y, Komiya S, Yasumizu Y, Yasuoka Y, Mizushima K, Takagi T, Kryukov K, Fukuda A, Morimoto Y, Naito Y, *et al*: Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. BMC Microbiol 21: 35, 2021.

28. D'Andreano S, Cusco A and Francino O: Rapid and real-time identification of fungi up to species level with long amplicon nanopore sequencing from clinical samples. Biol Methods Protoc 6: bpaa026, 2020.
29. Monger XC, Saucier L, Gilbert AA and Vincent AT: Stabilization of swine faecal samples influences taxonomic and functional results in microbiome analyses. MethodsX 9: 101716, 2022.
30. Menke S, Gillingham MA, Wilhelm K and Sommer S: Home-Made cost effective preservation buffer is a better alternative to commercial preservation methods for microbiome research. Front Microbiol 8: 102, 2017.
31. Jeong J, Yun K, Mun S, Chung WH, Choi SY, Nam YD, Lim MY, Hong CP, Park C, Ahn YJ and Han K: The effect of taxonomic classification by full-length 16S rRNA sequencing with a synthetic long-read technology. Sci Rep 11: 1727, 2021.
32. Shi Y, Wang G, Lau HC and Yu J: Metagenomic sequencing for microbial DNA in human samples: Emerging technological advances. Int J Mol Sci 23: 2181, 2022.
33. Simoes-Silva L, Araujo R, Pestana M, Soares-Silva I and Sampaio-Maia B: Peritoneal microbiome in end-stage renal disease patients and the impact of peritoneal dialysis therapy. Microorganisms 8: 173, 2020.
34. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, Ni Z, Nguyen TH and Andersen GL: Chronic kidney disease alters intestinal microbial flora. Kidney Int 83: 308-315, 2013.
35. Dzekova-Vidimliski P, Nikolov IG, Gjorgjievski N, Selim G, Trajceska L, Stojanoska A, Rambabova-Bushljetik I, Simeonov R and Stojkovski L: Peritoneal dialysis-related peritonitis: Rate, clinical outcomes and patient survival. Pril (Makedon Akad Nauk Umet Odd Med Nauki) 42: 47-55, 2021.
36. Feng X, Yang X, Yi C, Guo Q, Mao H, Jiang Z, Li Z, Chen D, Cui Y and Yu X: Escherichia coli Peritonitis in peritoneal dialysis: The prevalence, antibiotic resistance and clinical outcomes in a South China dialysis center. Perit Dial Int 34: 308-316, 2014.
37. Chao CT, Lee SY, Yang WS, Chen HW, Fang CC, Yen CJ, Chiang CK, Hung KY and Huang JW: Viridans streptococci in peritoneal dialysis peritonitis: Clinical courses and long-term outcomes. Perit Dial Int 35: 333-341, 2015.
38. Salzer WL: Peritoneal dialysis-related peritonitis: Challenges and solutions. Int J Nephrol Renovasc Dis 11: 173-186, 2018.
39. Gadola L, Poggi C, Dominguez P, Poggio MV, Lungo E and Cardozo C: Risk factors and prevention of peritoneal dialysis-related peritonitis. Perit Dial Int 39: 119-125, 2019.
40. Yan Y, Chai X, Chen Y and Zhang X: The fulminating course of infection caused by shewanella algae: A case report. Infect Drug Resist 15: 1645-1650, 2022.
41. Hughes G, Pallard C, Chavdab S and Davida MD: The first case report of human infection with Phyllobacterium myrsinacearum causing spondylodiscitis. Clin Infec Pract 7-8: 100029, 2020.