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# Application of 16S rRNA Gene-Targeted Next-Generation Sequencing for Bacterial Pathogen Detection in Continuous Ambulatory Peritoneal Dialysis Peritonitis

**Young Ah Kim[1], Ea Wha Kang[2], Hye Su Moon[4], Daewon Kim[3,4], Dongeun Yong[3,4]***

[1]Department of Laboratory Medicine, National Health Insurance Service Ilsan Hospital, Goyang, Korea

[2]Department of Internal Medicine, National Health Insurance Service Ilsan Hospital, Goyang, Korea

[3]Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea

[4]Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea.

***Corresponding author:** Dongeun Yong, deyong@yuhs.ac



## Abstract

*Background:* 16S rRNA gene-targeted next-generation sequencing (NGS) can detect microorganisms in a comprehensive reference database. To date, NGS has been successfully applied to samples such as urine, blood, and synovial fluid. However, there is no data for continuous ambulatory peritoneal dialysis (CAPD) fluid. The purpose of this study was to evaluate the clinical usefulness of microbiome analysis of CAPD fluids for the diagnosis of CAPD peritonitis. *Methods:* We included 21 patients with high suspicion of CAPD peritonitis. Routine CAPD fluid culture was performed using a pellet of 50 mL CAPD fluid onto the chocolate and blood agar for two days, and thioglycollate broth for one week. 16S rRNA gene-targeted NGS of pellets, stored at -70°C was performed with MiSeq (Illumina, USA). *Results:* Many colonized or pathogenic bacteria were detected from CAPD fluids using NGS and the microbiomes were composed of 1 to 29 genera with a cut-off 1.0. Compared to the culture results, NGS detected the same pathogens in 6 of 18 valid results (three samples failed with low read count). Additionally, using NGS, anaerobes such as *Bacteroides* spp. and *Prevotella* spp. were detected in six patients. In two of five samples in which no bacterial growth was detected, possible pathogens were detected by NGS. *Conclusion:* To our knowledge, this is the first report about the application of 16S rRNA gene-targeted NGS for the diagnosis of CAPD peritonitis. The etiology of culture-negative CAPD peritonitis can be better defined in NGS. Furthermore, it also helped in the detection of anaerobic bacteria.

## Keywords

Continuous ambulatory peritoneal dialysis
Next-generation sequencing
Peritonitis
Rapid diagnosis
16S rRNA gene

# 1. Introduction

Peritoneal dialysis peritonitis is a major adverse event in patients undergoing peritoneal dialysis, and prompt diagnosis and appropriate treatment are crucial for the long-term success of peritoneal dialysis [1]. In microbiological laboratories, routine peritoneal dialysate culture methods such as centrifugation, leukolysis, and automated blood culture systems are used to increase the detection rate of pathogens, but the number of bacteria present in the peritoneal dialysate is often very low, and unculturable or recalcitrant organisms are often difficult to detect by routine culture methods, especially after antimicrobial therapy.

Recently, the application of polymerase chain reaction (PCR) and sequencing targeting 16S rRNA for bacterial detection and internal transcribed spacer (ITS) for fungal detection was introduced, but the sensitivity of PCR (88.9%) compared to culture (82.2%) was reported to be not significantly different [2]. 16S rRNA and ITS sequencing can help identify bacteria that are difficult to identify by conventional methods but have limitations such as the location and size of the amplified sequence and limited discriminatory power in strains with high genetic identity between strains [3].

The advent of next-generation sequencing (NGS) has transformed the field of clinical microbiological testing [4]. NGS can be used for etiological typing of outbreaks of nosocomial infections and detection of genes associated with antimicrobial resistance or virulence and can detect bacteria that are difficult to culture [5]. Previous studies have used urine, blood, and orthopedic specimens [6-8], but no studies have applied NGS to peritoneal dialysis fluid. In this study, we applied NGS to the diagnosis of peritonitis caused by peritoneal dialysis and evaluated its diagnostic usefulness to improve the care of dialysis patients.

# 2. Materials and methods

A total of 21 patients with suspected peritoneal dialysis peritonitis who were referred for peritoneal dialysate culture in April 2018 were studied, and the results of conventional culture and NGS targeting the 16S rRNA gene were compared.

Needles obtained by centrifugation of 50 mL of peritoneal dialysate were streaked onto the chocolate and blood agar media and incubated for 48 hours in a 37°C, 5% $CO_2$ incubator, while the remaining needles were inoculated on thioglycollate and incubated for up to 1 week. If bacterial growth was observed, pure culture colonies were used for identification by MALDI Biotyper® Systems (BrukerDaltonik, Bremen, Germany). NGS analysis was performed using peritoneal dialysate needles stored at -70°C. DNA was extracted using the Fast DNA Spin Kit for Soil DNA Extraction (MP Biomedicals™, Fountain Parkway, CA, USA), and V3 and V4 of the 16S rRNA gene was amplified using MiSeq (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The detailed protocol can be found at https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf. Metagenomic analysis was performed at the genus level using EzBioCloud 16S-based Microbiome Taxonomic Profiling (ChunLab Inc., Seoul, Korea). Species identification by NGS was performed at the genus level. For precision control, negative and positive controls were included in each run, and the data of all samples and the data of gDNA Mock V34 (1707_C) and MiSeq QC (1903_C) positive controls performed in each run were analyzed together by principal coordinate analysis to ensure that the variation between runs was within the acceptable range (**Figure 1**). To determine whether the occurrence of peritoneal dialysis peritonitis was associated with the diversity of the microbiome during 16S rRNA-targeted NGS, α-diversity was analyzed. Electronic medical records were reviewed to identify clinical characteristics of the patients, including the presence of true peritoneal infection, final antimicrobial treatment for peritonitis, clear-up on day 10 of treatment, peritoneal dialysis catheter removal, and relapse with the same strain within 4 weeks of treatment.

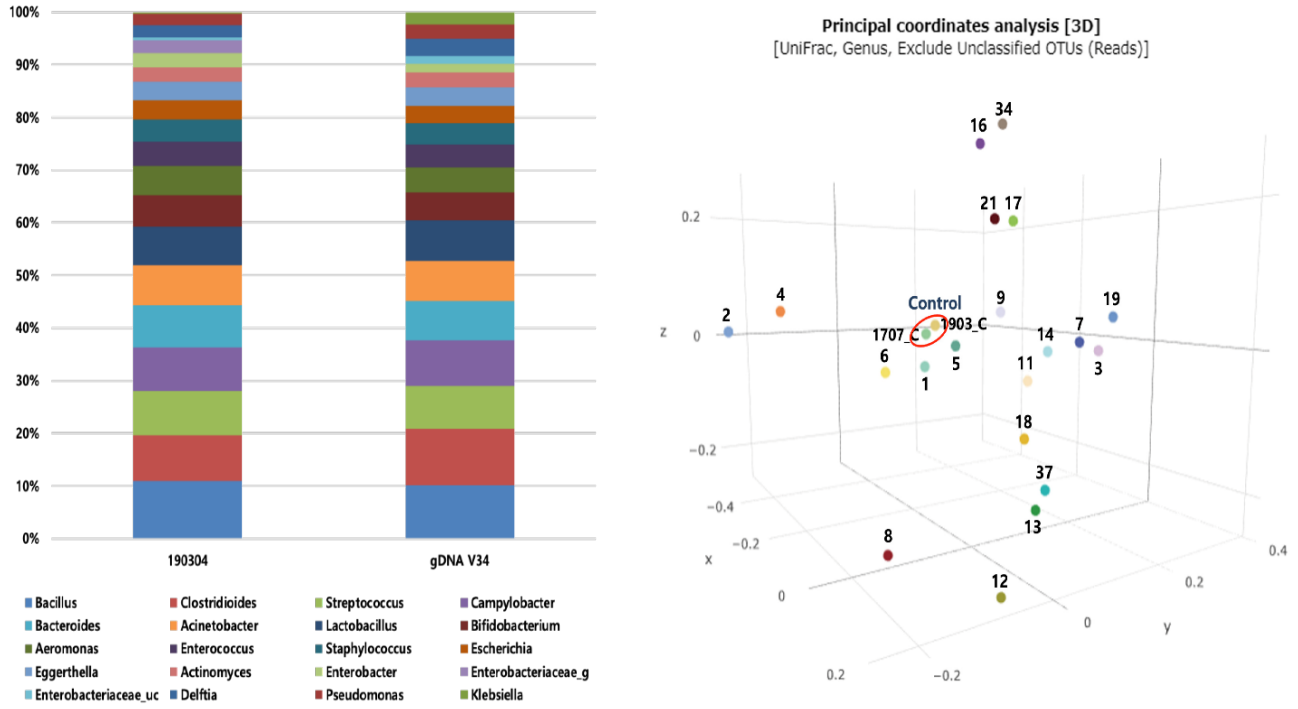


**Figure 1.** (A) Composition of gDNA Mock V34 (1707_C) and MiSeq QC (1903_C); (B) The principal coordinates analysis with CAPD fluid samples with QC controls in each run. Abbreviation: CAPD, continuous ambulatory peritoneal dialysis; QC, quality control; OTU, operational taxonomic unit

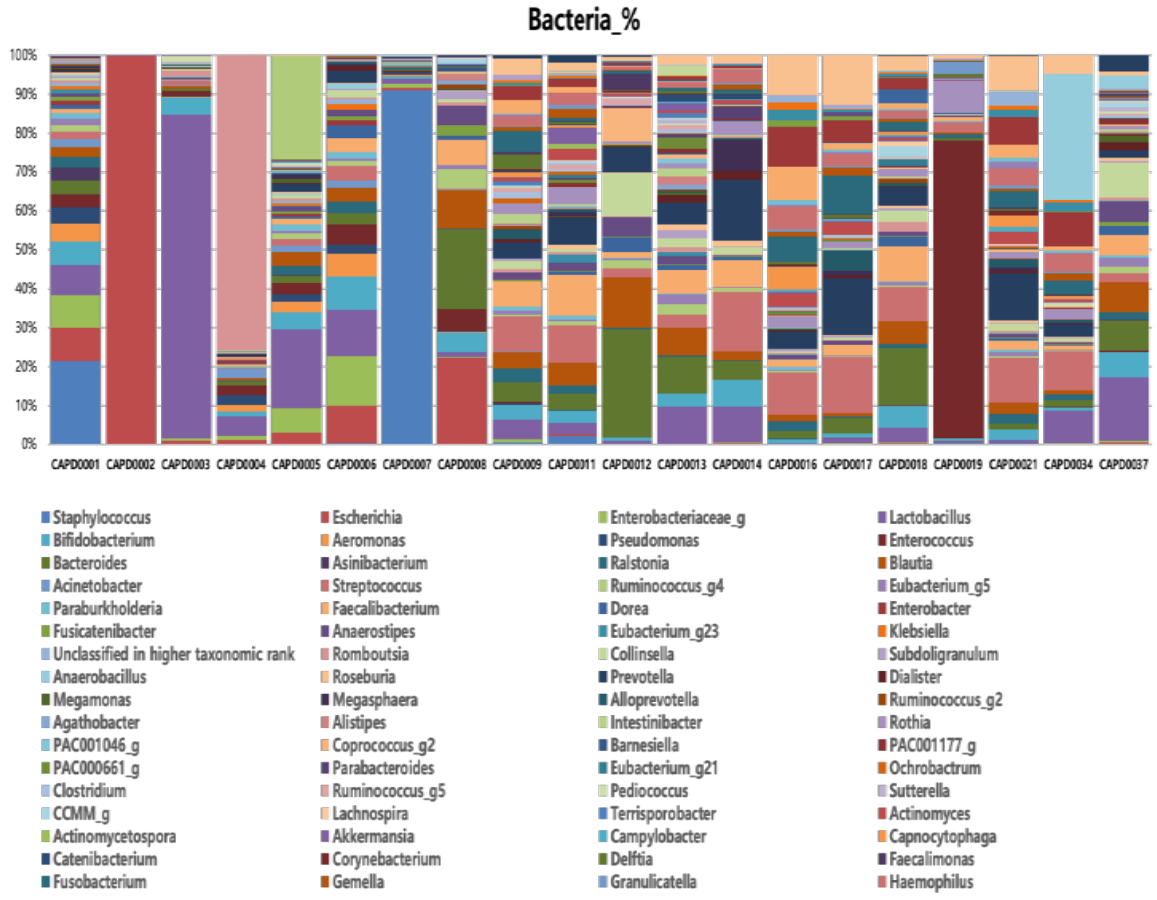


**Figure 2.** The microbiome of CAPD fluid with a 1% cut-off of relative abundance. *CAPD 10, 15, and 20 couldn't be analyzed due to low read count. Abbreviation: CAPD, continuous ambulatory peritoneal dialysis.

## 3. Results

A variety of bacteria were detected by 16S rRNA-targeted NGS in a total of 15 peritoneal dialysate samples, and when analyzed with a relative abundance cut-off of 1%, the microbiome in peritoneal dialysate contained 1–29 different genera (**Figure 2**). Three samples (CAPD10, CAPD15, CAPD20) could not be analyzed due to low read counts, and three samples (CAPD09, CAPD11, CAPD13) had no dominant bacteria with relative abundance exceeding 10% (**Table 1**).

Six of the 16 culture-positive specimens (CAPD01, CAPD02, CAPD04, CAPD07, CAPD08, CAPD19) were detected by NGS as strains of the same genus as those isolated from routine CAPD cultures (**Table 1**). Of the five culture-negative samples, NGS detected bacteria in three samples: *Lactobacillus* spp. (CAPD03, CAPD06), *Enterobacteriaceae* (CAPD06), *Streptococcus* spp. (CAPD14), and *Prevotella* spp. (CAPD14). Anaerobes were additionally detected by NGS in five of the culture-positive samples: *Bacteroides* spp. (CAPD08, CAPD12, CAPD18), *Blautia* spp. (CAPD12), *Prevotella* spp. (CAPD17, CAPD21), and *Veillonella* spp. (CAPD17) (**Table 1**).

Sixteen of the 21 patients in the study were judged by clinicians to have true peritonitis, with one case (CAPD06) having culture-negative peritonitis and detection of *Lactobacillus* spp. and *Enterobacteriaceae* by NGS. The patients with true peritonitis were treated with appropriate antimicrobials, and five of them had their catheters removed. In six cases (CAPD02, CAPD04, CAPD10, CAPD11, CAPD18, and CAPD21) that recurred within 2 months, peritoneal dialysate cultures performed at the time of recurrence re-detected the same organisms as those isolated in the initial culture (**Table 1**). One patient (CAPD03) received antibiotics for what the clinician believed to be a nonperitoneal infection but was given piperacillin-tazobactam from the date of specimen collection and discontinued after the culture was negative.

To determine whether the occurrence of peritoneal dialysis peritonitis was associated with the diversity of the microbiome on 16S rRNA-targeted NGS, we examined the association with α-diversity of the 19 samples available for analysis. Various α-diversity indices such as Shannon ($P$ = 0.7668), Simpson ($P$ = 0.3988), OTUs ($P$ = 0.9632), ACE ($P$ = 0.6131), and Chao1 ($P$ = 0.8899) were compared between true peritonitis and clinically unsuspected infection, and the $P$-values performed by Wilcoxon signed-rank test were all > 0.05, indicating no difference between the two groups.

## 4. Discussion

At least two of three conditions are commonly used as diagnostic criteria for peritonitis: a suitable clinical presentation (abdominal pain), observation of more than 50% polymorphonuclear cells in the peritoneal dialysis fluid and leukocytes >100 μ/L, and a positive microbiological culture [9]. When cultures are performed to detect the etiological agent of peritonitis, it has been reported that approximately 13% of cases are culture-negative, 11% are caused by two or more bacterial species (polymicrobial), and when a single bacterium is isolated, gram-positive bacteria account for the majority (53.4%) [10]. Clinically suspected peritonitis with a negative culture is problematic because it is difficult to determine the appropriate antibiotic, and is often caused by atypical organisms such as mycobacteria and fungi [11].

Microbiological cultures provide essential data for the selection of appropriate antimicrobials, but it is difficult to obtain satisfactory data due to the long time required for culturing, recent antimicrobial therapy, very low bacterial numbers, and poorly growing organisms. Until now, NGS has not been suitable for use in routine microbiological testing due to its high cost and difficulty, but with the development of more user-friendly instruments, lower testing costs, and the availability of easy-to-use commercialized bioinformatics tools, the use of NGS in routine microbiological testing practices for purposes such as identifying the pathogenic agents of infectious diseases

**Table 1.** The comparison of the two methods (culture versus NGS) for the diagnosis of CAPD peritonitis pathogens

| No. | Culture | NGS (10% relative abundance) | Read count | Infection | Antibiotics* | Catheter removal | Clear up on day 10 | Relapse within 2 months |
|---|---|---|---|---|---|---|---|---|
| CAPD01 | *Staphylococcus epidermis* | *Staphylococcus* spp. (20.4%) | 14,841 | Yes | Cefazolin, Tobramycin, **Vancomycin** | Yes | Yes | No |
| CAPD02 | *Escherichia coli* | *Escherichia* spp. (99.8%) | 96,528 | Yes | Rifaximin, **Meropenem** | No | Yes | Yes |
| CAPD03 | No growth | *Lactobacillus* spp. (82.1%) | 86,053 | No | Piperacillin-tazobactam | - | - | - |
| CAPD04 | *Serratia marcescens* | *Serratia* spp. (74.6) | 59,609 | Yes | Vancomycin, **Ceftazidime** | No | No | Yes |
| CAPD05 | *Micrococcus* spp. | *Kytococcus* spp. (25.2%) *Lactobacillus* spp. (19.2%) | 13,260 | Yes | Cefazolin, Ceftazidime | No | Yes | No |
| CAPD06 | No growth | *Enterobacteriaceae* (11.9%) *Lactobacillus* spp. (11.0%) | 83,717 | Yes | Meropenem, Rifaximin | No | No | No |
| CAPD07 | *Staphylococcus epidermidis* | *Staphylococcus* spp. (90.9%) | 44,704 | Yes | **Cefazolin, Ceftazidime** | No | Yes | No |
| CAPD08 | *Escherichia coli* | *Escherichia* spp. (21.4%) *Bacteroides* spp. (19.9%) | 76,720 | Yes | Cefazolin, **Ceftazidime** | No | Yes | No |
| CAPD09 | No growth | No dominant organism | 3,350 | No | No | - | - | - |
| CAPD10 | *Enterococcus faecalis* | Low read count | - | Yes | **Cefazolin, Ceftazidime** | No | No | Yes |
| CAPD11 | *Staphylococcus epidermidis* | No dominant organism | 71,751 | Yes | Cefazolin, Ceftazidime | No | No | Yes |
| CAPD12 | *Staphylococcus haemolyticus* | *Bacteroides* spp. (26.4%) *Blautia* spp. (12.3%) *Collinsella* spp. (10.5%) | 74,491 | Yes | Cefazolin, Ceftazidime | No | No | No |
| CAPD13 | No growth | No dominant organism | 58,772 | No | No | - | - | - |
| CAPD14 | No growth | *Prevotella* spp. (15.2%) *Streptococcus* spp. (14.6%) | 92,363 | No | No | - | - | - |
| CAPD15 | *Streptococcus anginosus* | Low read count | - | Yes | **Cefazolin, Ceftazidime** | No | No | No |
| CAPD16 | *Micrococcus* spp. | *Streptococcus* spp. (10.0%) | 47,182 | Yes | Vancomycin | Yes | No | No |
| CAPD17 | *Bacillus* spp. | *Prevotella* spp. (13.3%) *Streptococcus* spp. (13.2%) *Veillonella* spp. (11.6%) | 25,296 | No | No | - | - | - |
| CAPD18 | *Burkholderia cepacian* | *Bacteroides* spp. (13.6%) | 4,174 | Yes | Cefazolin, **Ceftazidime** | Yes | No | Yes |
| CAPD19 | *Candida albicans, Enterococcus avium* | *Enterococcus* spp. (74.6%) | 85,431 | Yes | **Piperacillin-tazobactam** | Yes | No | No |
| CAPD20 | *Citrobacter freundii* | Low read count | - | Yes | **Ceftazidime** | Yes | No | No |
| CAPD21 | *Staphylococcus epidermidis* | *Prevotella* spp. (11.5%) | 2,800 | Yes | Cefazolin, Ceftazidime | No | Yes | Yes |

*Antibiotics for CAPD peritonitis treatment (a bold form is an effective agent); -, not determined. Abbreviation: NGS, next-generation sequencing; CAPD, continuous ambulatory peritoneal dialysis

is no longer a distant prospect [12].

In an example of the application of NGS in clinical specimens, 16S–23S rRNA NGS was applied to 60 urine specimens from patients with suspected UTIs, 23 blood culture-positive diseases, and 21 orthopedic specimens such as tissues, body fluids, and joint fluid [6]. The report showed that the NGS method had better discrimination of strains than PCR sequencing of the 16S rRNA gene, rapid and accurate identification of pathogens commonly detected in urine and blood cultures, and more sensitive detection of pathogens in orthopedic specimens [6]. Rapid detection of pathogens, especially in patients with sepsis, is critical, and NGS enabled rapid detection from specimen preparation to reporting within 30 hours [7]. In a report of pathogen detection by NGS in the blood of pediatric immunocompromised patients, the dominant bacteria detected by NGS in the blood of 12 patients with bloodstream infections were consistent with blood culture results in 8 cases, and 2 patients with catheter-related bloodstream infections, the pathogen of sepsis could be detected as early as 7 days before onset [8]. More recently, metagenomic analysis has been introduced, in which NGS is not only performed on 16S rRNA genes but all DNA present in the specimen, including the host, is analyzed by NGS [13]. Metagenomic analysis requires the application of an appropriate algorithm to remove background DNA and has the limitation that it is mainly applicable to sterile samples such as joint cavity fluid, but it has the advantage of obtaining additional information such as virulence factors and antimicrobial resistance in addition to strain information. In this study, NGS detected a wide variety of bacteria in peritoneal dialysis fluid, but some strains were difficult to interpret. If *Lactobacillus* spp. was detected, it was most likely a contaminant, but rare cases of peritoneal dialysis peritonitis caused by *L. casei*, *L. paracasei*, *L. acidophilus*, and *L. rhamnosus* have been reported [14-17], making it difficult to completely exclude *Lactobacillus* spp. as an etiological agent. *Kytococcus* spp., *Blautia* spp., and *Collinsella* spp. have all been reported as etiological agents of peritoneal dialysis peritonitis. *Kytococcus* spp. are gram-positive cocci that are resistant to methicillin and differentiated from the genus Micrococcus [18], and their role is not well understood, but *Blautia* spp. have been reported to be commonly isolated in gut metagenome analyses of normal individuals [19]. *Collinsella* spp. are known to be part of the gut microbiota [20].

Most laboratories do not use appropriate media and conditions for the detection of anaerobes in routine culture, as it is thought that peritoneal dialysis fluid is highly oxygenated and anaerobes do not grow well. However, NGS has been able to detect anaerobic bacteria in many samples, and while the clinical significance of *Prevotella* spp. and *Veillonella* spp. is difficult to determine, *Bacteroides* spp. is highly virulent, and there have been reports of causing peritonitis, making it a possible pathogenic agent [21,22]. In addition, there are reports that automated blood culture methods can detect anaerobic bacteria such as the *Bacteroides fragilis* group in peritoneal dialysis patients that were not detected by conventional culture methods [23].

The implications of the diversity of the microbiome in peritoneal dialysate are still unknown, but it is likely that in true infections, the microbiome in peritoneal dialysate will decrease in diversity due to the dominant proliferation of pathogenic bacteria and a decrease in commensal bacteria. Unfortunately, when comparing the α-diversity of each group in this study, no statistically significant differences were obtained, which may be due to the small number of subjects analyzed.

This study is the first application of NGS for the diagnosis of peritoneal dialysis peritonitis, and compared to conventional culture, 16S rRNA-targeted NGS was able to detect anaerobes and additional bacteria in culture-negative samples.

## Disclosure statement

The authors declare no conflict of interest.

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## References

[1] Salzer WL, 2018, Peritoneal Dialysis-Related Peritonitis: Challenges and Solutions. Int J Nephrol Renovasc Dis, 11: 173–186.

[2] Ahmadi SH, Neela V, Hamat RA, et al., 2013, Rapid Detection and Identification of Pathogens in Patients with Continuous Ambulatory Peritoneal Dialysis (CAPD) Associated Peritonitis by 16S rRNA Gene Sequencing. Trop Biomed, 30: 602–607.

[3] Sung H, Kim MN, 2008, Reliability of Sequence-Based Identification of Microorganism. Infect Chemother, 40: 355–356.

[4] Motro Y, Moran-Gilad J, 2017, Next-Generation Sequencing Applications in Clinical Bacteriology. Biomol Detect Quantif, 14: 1–6.

[5] Rossen JWA, Friedrich AW, Moran-Gilad J, et al., 2018, Practical Issues in Implementing Whole-Genome-Sequencing in Routine Diagnostic Microbiology. Clin Microbiol Infect, 24: 355–360.

[6] Sabat AJ, van Zanten E, Akkerboom V, et al., 2017, Targeted Next-Generation Sequencing of the 16S–23S rRNA Region for Culture-Independent Bacterial Identification – Increased Discrimination of Closely Related Species. Sci Rep, 7: 3434.

[7] Grumaz S, Stevens P, Grumaz C, et al., 2016, Next-Generation Sequencing Diagnostics of Bacteremia in Septic Patients. Genome Med, 8: 73.

[8] Horiba K, Kawada JI, Okuno Y, et al., 2018, Comprehensive Detection of Pathogens in Immunocompromised Children with Bloodstream Infections by Next-Generation Sequencing. Sci Rep, 8: 3784.

[9] Li PK, Szeto CC, Piraino B, et al., 2016, ISPD Peritonitis Recommendations: 2016 Update on Prevention and Treatment. Perit Dial Int, 36: 481–508.

[10] Ghali JR, Bannister KM, Brown FG, et al., 2011, Microbiology and Outcomes of Peritonitis in Australian Peritoneal Dialysis Patients. Perit Dial Int, 31: 651–662.

[11] Fahim M, Hawley CM, McDonald SP, et al., 2010, Culture Negative Peritonitis in Peritoneal Dialysis Patients in Australia: Predictors, Treatment, and Outcomes in 435 cases. Am J Kidney Dis, 55: 690–697.

[12] Pak TR, Kasarskis A, 2015, How Next-Generation Sequencing and Multiscale Data Analysis Will Transform Infectious Disease Management. Clin Infect Dis, 61: 1695–1702.

[13] Ivy MI, Thoendel MJ, Jeraldo PR, et al., 2018, Direct Detection and Identification of Prosthetic Joint Infection Pathogens in Synovial Fluid by Metagenomic Shotgun Sequencing. J Clin Microbiol, 56: 402–418.

[14] Martins AR, Branco P, Gaspar A, 2016, *Lactobacillus casei* Peritonitis in Peritoneal Dialysis: A Rare Case and Literature Review. Ther Apher Dial, 20: 90–92.

[15] Sanyal D, Bhandari S, 1992, CAPD Peritonitis Caused by *Lactobacillus rhamnosus*. J Hosp Infect, 22: 325–327.

[16] Neef PA, Polenakovik H, Clarridge JE, et al., 2003, *Lactobacillus paracasei* Continuous Ambulatory Peritoneal Dialysis-Related Peritonitis and Review of the Literature. J Clin Microbiol, 41: 2783–2784.

[17] Schleifer CR, Benz RL, McAlack R, et al., 1989, *Lactobacillus acidophilus* Peritonitis in CAPD. Perit Dial Int, 9: 222–223.

[18] Sims D, Brettin T, Detter JC, et al., 2009, Complete Genome Sequence of *Kytococcus sedentarius* Type Strain (541T). Stand Genomic Sci, 1: 12–20.

[19] Durand GA, Pham T, Ndongo S, et al., 2017, *Blautia massiliensi* ssp. nov., Isolated from a Fresh Human Fecal Sample and Emended Description of the Genus Blautia. Anaerobe, 43: 47–55.

[20] Gomez-Arango LF, Barrett HL, Wilkinson SA, et al., 2018, Low Dietary Fiber Intake Increases *Collinsella* Abundance in the Gut Microbiota of Overweight and Obese Pregnant Women. Gut Microbes, 9: 189–201.

[21] Nachimuthu S, Visconti EB, Pannone JB, et al., 2001, *Bacteroides* Peritonitis Associated with Colon Cancer in a Continuous Ambulatory Peritoneal Dialysis Patient. South Med J, 94: 1021–1022.

[22] Denis M, Tommasi L, Teyssier G, et al., 1986, *Clostridium butyricum* and *Bacteroides distasonis* Peritonitis in a Child Treated by Peritoneal Dialysis. Usefulness of Anaerobic Culture after Filtration. Presse Med, 15: 2168.

[23] Alfa MJ, Degagne P, Olson N, et al., 1997, Improved Detection of Bacterial Growth in Continuous Ambulatory Peritoneal Dialysis Effluent by Use of BacT/Alert FAN Bottles. J Clin Microbiol, 35: 862–866.