—Short Communication—

Micrococcus aloeverae - A Rare Cause of Peritoneal Dialysis-Related Peritonitis Confirmed by 16S rRNA Gene Sequencing

Su Hyun Song[1], Hong Sang Choi[1], Seong Kwon Ma[1], Soo Wan Kim[1], Jong-Hee Shin[2] and Eun Hui Bae[1]

[1]Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea
[2]Department of Laboratory Medicine, Chonnam National University Medical School, Gwangju, Korea

The number of patients receiving peritoneal dialysis has increased worldwide. Herein, we report the first case to our knowledge of continuous ambulatory peritoneal dialysis (CAPD) peritonitis caused by *Micrococcus aloeverae*, which was initially reported to be caused by *Micrococcus luteus* in the dialysate culture report but later identified by 16S ribosomal ribonucleic acid (rRNA) gene sequencing as *M. aloeverae*. A 59-year-old woman visited the emergency room due to abdominal pain. She was hospitalized with CAPD peritonitis. The patient initially responded to empirical antibiotic treatment comprising intraperitoneal cefazolin (15 mg/kg/day) and ceftazidime (1 g/day); however, the leukocyte count of dialysate effluent increased again. *M. luteus* was isolated four times from peritoneal dialysate cultures. We treated the patient with intraperitoneal administration of vancomycin (2 g loading, followed by 1 g every 7 days) but needed to switch from CAPD to temporary hemodialysis. We analyzed the 16S rRNA sequence to confirm the exact causative organism, and the results revealed that the organism was *M. aloeverae*. Because *M. aloeverae* and *M. luteus* have sequence similarity, 16S rRNA sequencing is a useful method to distingush them. (J Nippon Med Sch 2019; 86: 55–57)

Key words: *Micrococcus*, peritonitis, 16S rRNA

Introduction

Micrococcus spp. are gram-positive, aerobic, non-endospore-forming cocci. The organisms can be found in many places such as human skin, water, dust, and soil. *Micrococcus* is generally considered a harmless bacterium, but there have been rare cases of *Micrococcus* infection in people with compromised immune systems[1]. The case of continuous ambulatory peritoneal dialysis (CAPD) peritonitis herein was first attributed to *Micrococcus luteus* based on peritoneal dialysis cultures but later confirmed to be due to *Micrococcus aloeverae* using 16S ribosomal ribonucleic acid (rRNA) sequencing.

Case Report

A 59-year-old woman visited the emergency department complaining of abdominal pain and cloudy dialysate. She had been taking medicine for hypertension and type 2 diabetes mellitus for over 20 years. She had undergone CAPD for end-stage renal disease (ESRD) for four years without any previous episodes of peritonitis. Her blood pressure was 130/80 mmHg, heart rate was 80 beats/min, respiratory rate was 20 breaths/min, and body temperature was 36.0℃ at the time of admission. The white blood cell (WBC) count of dialysate effluent was 1,210 cells/mm^3 with a neutrophil count of 980 cells/mm^3 (81% of the total WBC). The initial laboratory tests showed a WBC count of 4,400 cells/mm^3 (normal range: 4,800–10,800 cells/mm^3). The level of C-reactive protein, blood urea nitrogen, and creatinine was 2.2 (normal range: 0–0.3 mg/dL), 20.5 (normal range: 8–16.5 mg/dL), and 3.5 mg/dL (normal range: 0.5–1.3 mg/dL), respectively. The patient was initially treated empirically with intraperitoneal (IP) cefazolin (15 mg/kg/day) and ceftazidime (1 g/day) for the first 3 days, and the WBC count of dialysate effluent decreased to 637 cells/mm^3. However, the WBC count of dialysate effluent increased to 1,267 cells/mm^3

Correspondence to Eun Hui Bae, MD, PhD, Department of Internal Medicine, Chonnam National University Medical School, 42 Jebongro, Gwangju 61469, Korea
E-mail: baedak76@gmail.com
Journal Website (http://www2.nms.ac.jp/jnms/)

on the fourth day. Furthermore, on the fourth day of hospitalization, we received the peritoneal fluid culture result from an aerobic incubation on a blood chocolate agar plate (**Fig. 1**), which indicated that *M. luteus* was present in the peritoneal dialysate (VITEK®2, bioMérieux, France) collected on the day of admission. Therefore, we began IP administration of vancomycin (2 g loading, followed by 1 g every 7 days). After the second IP vancomycin treatment, the WBC count of dialysate effluent decreased to 174 cells/mm^3. Nonetheless, the WBC count of dialysate effluent increased again to 1,692 cells/mm^3 before the third course of IP vancomycin. After the third course of IP vancomycin, the WBC count of dialysate effluent decreased to 259 cells/mm^3, followed by another increase to 792 cells/mm^3. We incubated the peritoneal dialysate repeatedly before vancomycin administration, and *M. luteus* was isolated from four of the cultures. Based on M45-A2 standard guidelines (clinical and laboratory standard institute, PA, USA), *in vitro* susceptibility testing for selected antibiotics (erythromycin, penicillin, and vancomycin) was performed using the E-test method. The cultured strain showed resistance to erythromycin and susceptibility to vancomycin (**Fig. 2**). However, after the third administration of vancomycin, the patient complained of abdominal pain again. We decided to remove the peritoneal catheter and switched her to hemodialysis via a permanent jugular catheter. After removal of the peritoneal dialysis catheter, she felt better, and the serum C-reactive protein level returned to within the normal range, therefore, we terminated the antibiotic therapy. The removed CAPD catheter was incubated, but no bacterial growth was observed. We performed CAPD catheter re-insertion after a month and restarted peritoneal dialysis without any complication.

Fig. 1 Colony morphology of *Micrococcus spp.* in blood chocolate agar which was cultured on the day of admission.

We analyzed the 16S rRNA sequence to confirm the causative organism. The genomic DNA was extracted from the cultured microorganisms, and polymerase chain reaction was performed with 16S rRNA. The 16S rRNA was amplified using universal primers (forward, 5′-AGT TTGATCCTGGCTCAG-3′; reverse, 5′-GTATTGCCGCGG CTGCTG-3′) and sequenced. *M. aloeverae* strain AE-6 was verified with 605/607 (99.7%) similarity (GenBank acces-

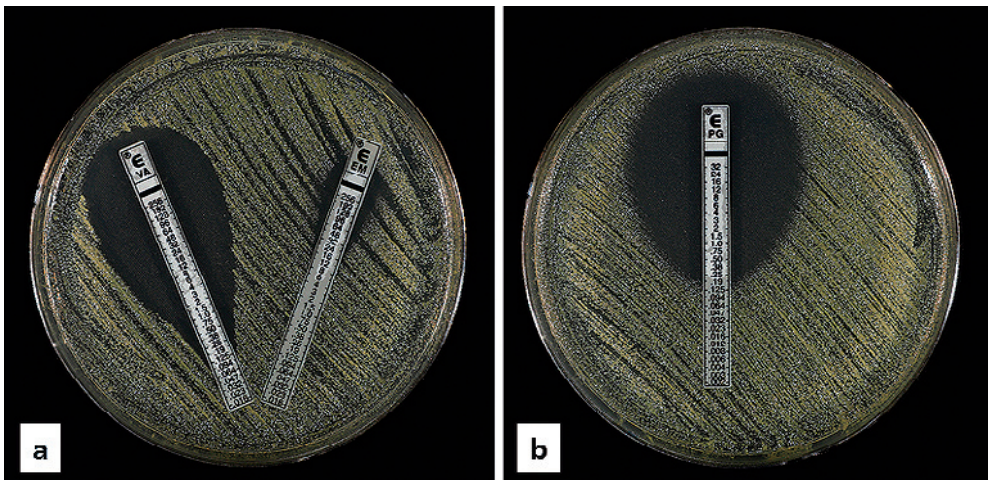

Fig. 2 E-test for vancomycin and erythromycin (a), and penicillin (b). The results show resistance to erythromycin (MIC 32 μg/mL), sensitivity to vancomycin (MIC 0.19 μg/mL) and could not be determined for penicillin (MIC 0.25 μg/mL). MIC, minimal inhibitory concentration; VA, vancomycin; EM, erythromycin; PC, penicillin.

sion number: NR134088.1).

Discussion

Recently, the number of patients receiving peritoneal dialysis has increased worldwide[2]. Therefore, it is important to prevent and diagnose the complications of peritoneal dialysis. Peritonitis is a major complication of peritoneal dialysis and is associated with mortality in patients receiving peritoneal dialysis[3]. The typical spectrum of organisms causing peritonitis includes: gram-positive organisms (67%), gram-negative organisms (28%), fungi (2.5%), and anaerobic organisms (2.5%)[4].

The case discussed herein involved CAPD peritonitis resulting in removal of the CAPD catheter. In addition, the causative organism was initially reported as *M. luteus* in the dialysate culture report but later identified as *M. aloeverae* by 16S rRNA sequencing. *M. aloeverae* is known to have sequence similarity with *M. luteus* and *Micrococcus yunnanensis*[5]. Thus, it is difficult to distinguish these three organisms. *Micrococcus* spp. are susceptible to a wide range of antibiotics. However, the resistance of the pathogens to penicillin and oxacillin has been reported[6], but most *Micrococcus* spp. are susceptible to vancomycin[7]. At least two weeks of IP vancomycin is recommended to treat *Micrococcus* peritonitis[8]. Nevertheless, vancomycin-resistant *Micrococcus* has been reported recently[9]. The present case also showed the initial response of the patient to vancomycin, but the dialysate WBC count increased again after the third IP administration of vancomycin, leading to the removal of the CAPD catheter.

16S rRNA sequencing is known to be a useful method for rapid and accurate identification of pathogens causing peritonitis[10]. Because *Micrococcus* is considered a harmless organism, there was a possibility of other causative organisms. Thus, we performed 16S rRNA gene sequencing to identify the causative organism, which was finally confirmed as *M. aloeverae*. To the best of our knowledge, this is a unique case of CAPD peritonitis caused by *M. aloeverae*. Although rare, we suggest that *Micrococcus* spp. should be included in the differential diagnosis of peritoneal dialysis-related peritonitis, and their resistance to antibiotics should not be ignored. In addition, 16S rRNA gene sequencing is a useful method to confirm the causative organism.

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Conflict of Interest: None declared.

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