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【 CASE REPORT 】

First Case Report of Peritoneal Dialysis-associated Peritonitis Caused by *Lysinibacillus Sphaericus*

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Abstract:
We herein report a case of peritoneal dialysis-associated peritonitis caused *by Lysinibacillus sphaericus* in a 40s-year-old patient. Treatment was initiated with intermittent intraperitoneal cefazolin and ceftazidime. Later, both peritoneal dialysate and blood cultures detected *L. sphaericus*, so the antibiotic was changed to ampicillin (ABPC). The patient was treated with a combination of intraperitoneal intermittent and intravenous ABPC for 7 days, followed by 14 days of amoxicillin. The patient experienced no adverse events and no recurrence for 30 days. The patient had four dogs, and the infection was deemed likely to have been caused by environmental contamination and inadequate catheter replacement.

Key words: Peritoneal dialysis, Peritonitis, *Lysinibacillus sphaericus*

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Introduction

Lysinibacillus sphaericus is an aerobic, Gram-positive, rod-shaped, spore-forming bacterium that lives primarily in soil. *L. sphaericus* produces toxins, such as binary toxin, Mtx1 toxin, and Mtx2 toxin, which have prominent larvicidal activity and have been used to control malaria and other diseases (1-3). *L. sphaericus* is rarely pathogenic in humans, and while a few cases of bacteremia in immunocompromised individuals have been reported, deaths have been rare (4-8). However, to our knowledge, there have been no reports of peritoneal dialysis-associated peritonitis (PDAP) caused by *L. sphaericus*.

We herein report a case of PDAP attributed *to L. sphaericus.*

Case Presentation

This is the case of a 40-year-old woman with a 3-year history of chronic renal failure from an unknown primary disease who had been on peritoneal dialysis (PD) for 5 months. The woman sought medical attention after developing a fever the night prior to presentation. At a routine visit four days prior to presentation, no drainage or abnormalities had been noticed at the catheter exit site, but she had reported fatigue for the past three days.

The patient's medical history included angina pectoris, Hashimoto's disease, and antiphospholipid syndrome. She was not on any immunosuppressive drugs, such as corticosteroids or calcineurin inhibitors, and she had no history of endoscopy of the upper or lower abdomen or gynecological procedures prior to admission. Her PD schedule was 1,500 mL/18-22 h of glucose-containing peritoneal dialysate (Midpeliq 135®; Terumo, Tokyo, Japan) and 2,000 mL/22-6 h of icodextrin-containing peritoneal dialysate (Nicopeliq®; Terumo). A manual connection system (Click Safe; Terumo) was used for bag replacement. She maintained a urine output of 300-1,000 mL/day. The patient kept four dogs indoors and had regular and intense contact with them. The dogs had access to the room where dialysis equipment was stored and where bag changes were performed.

On arrival at the hospital, the patient had a temperature of 37.6 °C, blood pressure of 117/64 mmHg, pulse rate of 90

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Table. Drug Susceptibility of *Lysinibacillus Sphaericus* (Based on the Criteria for *Bacillus* spp. in CLSI M45-3rd).

Abbreviation	Drug	MIC μg/mL
PCG	Benzylpenicillin	≤0.12
ABPC	Ampicillin	≤0.25
ABPC/SBT	Ampicillin/Sulbactam	≤0.5
AMPC/CVA	Amoxicillin/Clavulanate	≤0.25
PIPC/TAZ	Piperacillin/Tazobactam	1
CEZ	Cefazolin	≤0.5
CTRX	Ceftriaxone	≤1
CAZ	Ceftazidime	16
CFPM	Cefepime	2
IPM/CS	Imipenem/Cilastatin	≤0.25
MEPM	Meropenem	≤0.25
GM	Gentamicin	≤0.5
CAM	Clarithromycin	≤0.5
CLDM	Clindamycin	≤0.25
MINO	Minocycline	≤1
CPFX	Ciprofloxacin	≤0.25
LVFX	Levofloxacin	≤0.5
ST	Sulfamethoxazole/Trimethoprim	40
VCM	Vancomycin	≤0.5
TEIC	Teicoplanin	≤0.5
LZD	Linezolid	0.5

MIC: minimum inhibitory concentration

bpm, white blood cell (WBC) count of 7,600/μL with 93.1% neutrophils, C-reactive protein (CRP) of 7.97 mg/dL, and no abnormal findings on computed tomography (CT). The patient was at her baseline level of consciousness and had no abdominal pain, but her PD drainage was turbid. An analysis of the PD effluent showed an elevated WBC count of 8,197/μL, with 823/μL mononuclear cells and 7,374/μL polymorphonuclear cells; therefore, the patient was admitted as a PDAP patient (day X). No obvious damage to the dialysis tubing was noted at admission. No redness or discharge of pus was noted at the exit site, so wet contamination was suspected. Empiric treatment for peritonitis, namely cefazolin (CEZ) 1 g and ceftazidime (CAZ) 1 g, were then started intraperitoneally at intervals (22-6 h).

The next day (day X+1), Gram-positive rods (GPRs) were detected in two sets of blood cultures and a peritoneal dialysate culture. No other bacterial species were detected in blood or peritoneal dialysate cultures. The bacterium was identified as *L. sphaericus*. On the following day (day X+3), due to the proven susceptibility of *L. sphaericus* (Table), intraperitoneal administration of CEZ and CAZ was discontinued and replaced with intravenous (1 g/day) and intraperitoneal (4 g/day) ampicillin (ABPC). Intravenous ABPC was administered at 10:00, and intraperitoneal ABPC was mixed with icodextrin-containing peritoneal dialysis fluid (Nicopeliq®; Terumo) and administered from 22-6 h. Blood tests revealed a WBC count of 5,900/μL with 70.9% neutrophils, and a PD drainage test showed improvement with a leukocyte count of 69/μL (34/μL mononuclear cells and 35/μL polynuclear cells).

To prevent future recurrence, the hospital nurses instructed the patient to not allow the dogs in rooms where dialysis equipment was being stored or where bag changes were performed and to wash her hands rigorously during bag changes and before and after caring for her pets. ABPC treatment was continued for a total of 7 days. The patient showed improvement where her results showed a CRP measurement of 1.19 mg/dL, WBC count of 8,100/μL with 72.8% neutrophils, and PD drainage WBC count of 48/μL (24/μL mononuclear cells and 24/μL polynuclear cells). The antibiotic treatment was switched to amoxicillin (500 mg/day) on Day X+11, and the patient was discharged. Antimicrobial chemotherapy was administered intraperitoneally for 3 days, combined intraperitoneally and intravenously for 7 days, and orally for 14 days, for a total of 24 days. The patient had no relapse for 30 days.

Blood culture was performed using an automated blood culture device (BACTEX FX®; Becton Dickinson, Franklin Lakes, New Jersey, USA). Two aerobic lesion bottles were positive after 12 h of incubation. GPRs were detected in a Gram-stained specimen (Figure). Isolated cultures were grown on sheep blood agar medium (Nissui Separated Plate CA Sheep Blood Agar/Chocolate Agar EX II with vancomycin®; Nissui Pharmaceutical, Tokyo, Japan) under aerobic conditions at 35 °C for 6 h. The sample was identified as *L. sphaericus* (Score value: 2.23) by Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (MALDI Biotyper®; Bruker Japan, Kanagawa, Japan) using the cell smear method with formic acid addition. The same bacteria were detected in PD drainage cultures. Gram staining of colonies from blood and PD effluent cultures showed spores (Figure). Using another mass spectrometry test (Vitek® MS; bioMérieux, Tokyo, Japan) and a 16SrRNA gene analysis: 100% (GenBank: CP 019980.1), the organism was identified as *L. sphaericus*. Drug susceptibility testing was performed using a dry plate (Dry Plate Eiken 192®; Eiken Chemical Co., Ltd., Tokyo Japan) and measured with a microbial susceptibility analyzer (DPS192iX®; Eiken Chemical Co., Ltd.) based on the criteria for *Bacillus spp*. of the Clinical and Laboratory Standards Institute (CLSI) M45-3rd.

Discussion

L. sphaericus was originally classified as *Bacillus sphaericus* but was reclassified as *L. sphaericus* in 2007 (9). *Lysinibacillus* spp. are rarely pathogenic and often considered contaminants when detected in laboratory specimens. There have been reports of bacteremia due to *L. sphaericus* in immunocompromised patients associated with intravenous heroin products, splenectomy (4), malignancy, and bone marrow transplantation, but no deaths have been reported in these cases (5).

Intraperitoneal administration of antimicrobials is generally recommended in the treatment of PDAP, unless there is systemic sepsis; however, the optimal route of administration

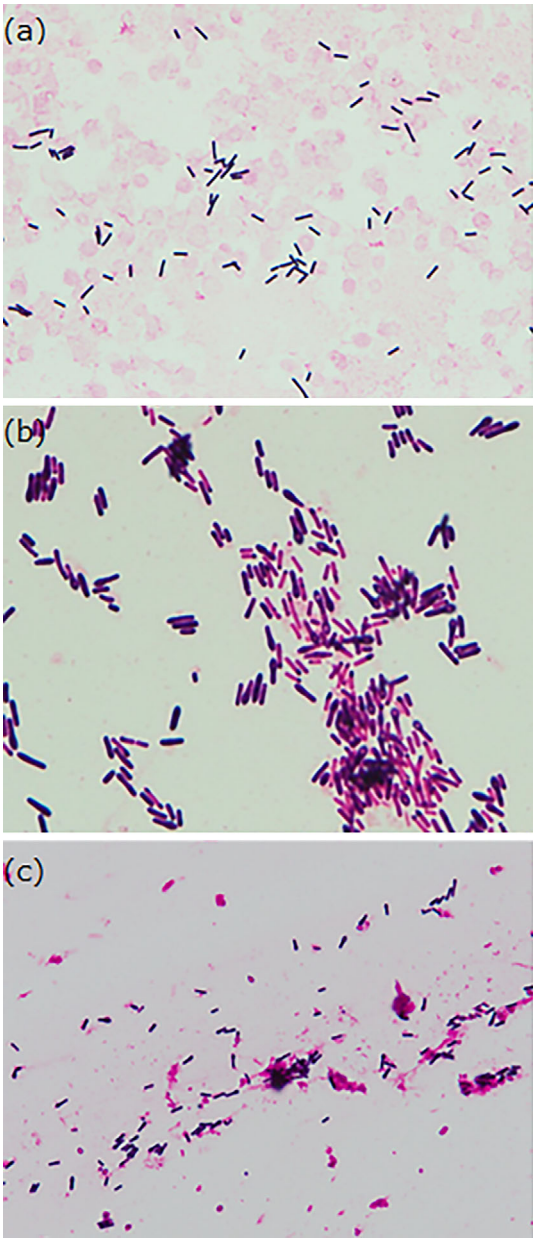


Figure. Gram stain findings for each culture test. (a) blood culture subculture ×1000. (b) blood culture colony ×1000. (c) peritoneal dialysate culture colony ×1000.

in the presence of concurrent bacteremia is unknown. ABPC is recommended at 4 g/day when intraperitoneal intermittent administration is chosen (10), which corresponds to a 60% transfer rate into the systemic circulation (11). This rate would have been higher than 60% in this patient, since the it increases during peritonitis (12, 13).

Since our patient developed bacteremia, it was necessary to maintain high blood levels of ABPC. The ISPD guideline recommends a 25% increase in antimicrobial dosage when urine output is >100 mL/day (10). The patient had a residual urine output of approximately 300-1,000 mL/day. Because of the need to increase the dose of antimicrobial agents and the expected decrease in the rate of transfer to the systemic circulation during intraperitoneal administration with improvement of peritonitis, we decided to use intravenous ABPC in combination with intraperitoneal administration.

In general, PDAP often improves within 48 h of treatment (10), and the number of leukocytes in PD effluent on the third day of treatment has been reported to correlate with the treatment response (14). The recommended duration of PDAP treatment depends on the bacterial species but is generally two to three weeks (10). Although our patient's blood cultures were positive, and subsequent negative blood cultures were not confirmed, she was discharged early from the hospital because the WBC count in the PD effluent decreased early during treatment, subjective symptoms were minimal, and CT showed no disseminated lesions. Therefore, after three days of intraperitoneal antibiotics and seven days of combined intraperitoneal and intravenous therapy, the patient was switched to oral medication and discharged.

Although removal of the PD catheter outlet is recommended for refractory peritonitis, fungal peritonitis, or recurrent peritonitis, the PD catheter was not removed in this case because it was the patient's first episode, and she showed prompt improvement following treatment.

There were no triggers, such as farming or gardening activities, that could have allowed exposure to *L. sphaericus*. However, the patient had four dogs, and the pets were kept indoors and had access to the dialysis environment, which could have contributed to the environmental contamination. In addition, there was inadequate sanitation during a bag change procedure check conducted by a nurse during the patient's hospitalization. As such, it is highly likely that the deterioration of the sanitary environment due to the dogs' movements in and out of the room and inadequate hygiene during the bag exchange procedure contributed to the infection in this case. However, as a limitation of this case, no oral secretion culture test was performed on the breeding dogs.

The ISPD guidelines include a note regarding pets, recommending that pets not be allowed in the room where PD is performed. To prevent cases such as this one, it is very important to know in advance whether a patient has pets, and if so, to thoroughly instruct the patient on how to prevent zoonosis.

To our knowledge, this is the first report of PDAP caused by *L. sphaericus*.

The authors state that they have no Conflict of Interest (COI).

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Informed consent

Written informed consent was obtained from the patient for their anonymized information to be published in this article.

Authors' contributions

Teruhisa Kinoshita, Shoko Sahara, and Tomomi Amano:

Writing of Original Draft.

Masashi Ito, Takashi Sakakibara, and Norio Takimoto: Review & Editing

Yukari Osada, and Keisuke Oka: Supervision

Authorship statement

All authors have met the ICMJE authorship criteria and have given final approval for the submission of the final version.

Declarations of interest

None.

Abbreviations: PD (Peritoneal dialysis); PDAP (Peritoneal dialysis-associated peritonitis); CEZ (cefazolin); CAZ (ceftazidime); CLSI (Clinical and Laboratory Standards Institute); CT (computed tomography); GPR (Gram-positive rod); AMPC (amoxicillin); ABPC (ampicillin); WBC (white blood cell); MALDI-TOF MS (Matrix-assisted laser desorption ionization time-of-flight mass spectrometry)

References

1. Baumann P, Clark MA, Baumann L, et al. Bacillus sphaericus as a mosquito pathogen: properties of the organism and its toxins. Microbiol Rev **55**: 425-436, 1991.
2. Berry C. The bacterium, Lysinibacillus sphaericus, as an insect pathogen. J Invertebr Pathol **109**: 1-10, 2012.
3. Riaz MA, Adang MJ, Hua G, et al. Identification of Lysinibacillus sphaericus Binary toxin binding proteins in a malarial mosquito cell line by proteomics: A novel approach towards improving mosquito control. J Proteomics **227**: 103918, 2020.
4. Wenzler E, Kamboj K, Balada-Llasat JM. Severe sepsis secondary to persistent Lysinibacillus sphaericus, Lysinibacillus fusiformis and Paenibacillus amylolyticus bacteremia. Int J Infect Dis **35**: 93-95, 2015.
5. Castagnola E, Fioredda F, Barretta MA, et al. Bacillus sphaericus bacteraemia in children with cancer: case reports and literature review. J Hosp Infect **48**: 142-145, 2001.
6. Garavelli PL. Bacteremia caused by Bacillus sphaericus. Recenti Prog Med **90**: 628-629, 1999.
7. Isaacson P, Jacobs PH, Mackenzie AM, et al. Pseudotumour of the lung caused by infection with Bacillus sphaericus. J Clin Pathol **29**: 806-811, 1976.
8. Allen BT, Wilkinson HA. A case of meningitis and generalized Shwartzman reaction caused by Bacillus sphaericus. Johns Hopkins Med J **125**: 8-13, 1969.
9. Ahmed I, Yokota A, Yamazoe A, et al. Proposal of Lysinibacillus boronitolerans gen. nov. sp. nov., and transfer of Bacillus fusiformis to Lysinibacillus fusiformis comb. nov. and Bacillus sphaericus to Lysinibacillus sphaericus comb. nov. Int J Syst Evol Microbiol **57**: 1117-1125, 2007.
10. Li PK, Chow KM, Cho Y, et al. ISPD peritonitis guideline recommendations: 2022 update on prevention and treatment. Perit Dial Int **42**: 110-153, 2022.
11. Blackwell BG, Leggett JE, Johnson CA, et al. Ampicillin and sulbactam pharmacokinetics and pharmacodynamics in continuous ambulatory peritoneal dialysis (CAPD). Perit Dial Int **10**: 221-226, 1990.
12. Boyce NW, Wood C, Thomson NM, et al. Intraperitoneal (IP) vancomycin therapy for CAPD peritonitis--a prospective, randomized comparison of intermittent v continuous therapy. Am J Kidney Dis **12**: 304-306, 1988.
13. Lam E, Lien YTK, Kraft WK, et al. Vancomycin in peritoneal dialysis: Clinical pharmacology considerations in therapy. Perit Dial Int **40**: 384-393, 2020.
14. Chow KM, Szeto CC, Cheung KKT, et al. Predictive value of dialysate cell counts in peritonitis complicating peritoneal dialysis. Clin J Am Soc Nephrol **1**: 768-773, 2006.

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