

# Peritoneal dialysis-related peritonitis due to *Halomonas hamiltonii*

## A first case report

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### Abstract

**Introduction:** *Halomonas hamiltonii* is a Gram-negative, halophilic, motile, and nonspore-forming rod bacterium. Although most *Halomonas* sp. are commonly found in saline environments, it has rarely been implicated as a cause of human infection. Herein, the authors present a case report of continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis attributed to *H hamiltonii*.

**Case presentation:** An 82-year-old male patient who had been receiving CAPD therapy presented to an emergency department with complaints of abdominal pain and cloudy dialysate that had persisted for 2 days. The peritoneal dialysate was compatible with CAPD peritonitis, with white blood cell count of peritoneal effluent of 810/mm<sup>3</sup> and neutrophils predominated (60%). Two days after culture on blood agar medium, nonhemolytic pink mucoid colonies showed, with cells showing Gram-negative, nonspore-forming rods with a few longer and larger bacilli than usual were found. We also performed biochemical tests and found negative responses in K/K on the triple sugar iron test and H<sub>2</sub>S and equivocal (very weak) response in the motility test, but positive responses to catalase, oxidase, and urease tests. The partial sequence of the 16S rRNA gene of a bacterium detected by peritoneal fluid culture was utilized for a Basic Local Alignment Search Tool search, which revealed that the organism was *H hamiltonii*. Intraperitoneal antibiotics were administered for 21 days, and the patient was discharged without clinical problems.

**Conclusion:** We present here the first case report of CAPD-related peritonitis caused by *H hamiltonii*, which was identified using molecular biological techniques. Although guidelines do not exist for the treatment of infections caused by this organism, conventional treatment for Gram-negative organisms could be effective.

**Abbreviations:** ALT = alanine aminotransferase, AST = aminotransferase, BLAST = The Basic Local Alignment Search Tool, CAPD = continuous ambulatory peritoneal dialysis, DDBJ = DNA Data Bank of Japan, EMBL = European Molecular Biology Laboratory, ESRD = end-stage renal disease, GNB = Gram-negative bacteria, HD = hemodialysis, I = Intermediate, IP = intra-peritoneal, ISPD = International Society for Peritoneal Dialysis, PD = peritoneal dialysis, R = resistant, S = susceptible, TSI test = triple sugar iron test, WBC = white blood cell.

**Keywords:** first case report, *Halomonas hamiltonii*, PD peritonitis, peritoneal dialysis, peritonitis

Editor: Sanket Patel.

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The authors have no funding and conflicts of interest to disclose.

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Medicine (2016) 95:47(e5424)

Received: 18 April 2016 / Received in final form: 26 October 2016 / Accepted: 28 October 2016

<http://dx.doi.org/10.1097/MD.0000000000005424>

## 1. Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is an important treatment option for end-stage renal disease (ESRD). Although the rate of peritonitis has declined in recent years because of improvements in the CAPD technique, peritonitis remains a major complication that causes significant morbidity and mortality in ESRD patients on CAPD.<sup>[1,2]</sup>

Halophilic bacteria inhabit a wide range of environments and play important roles in the microbial diversities of saline environments.<sup>[3–7]</sup> *Halomonas* strains are halophiles usually found in highly saline environments, such as the Dead Sea and the frigid waters of Antarctica.<sup>[3–7]</sup> The organisms also exhibit great versatility in terms of their abilities to successfully grow in environments with widely varying temperatures and pH values.

Some renal care centers provide hemodialysis patients with written information stating that hemodialyzers can be contaminated in the bicarbonate used to prepare dialysis fluid, and that bacteria may persist despite cleaning and flushing procedures because machines can become contaminated by biofilms and bicarbonate inflow.<sup>[9]</sup> However, there have been no previous reports of *Halomonas* infection in ESRD patients on CAPD.

Here, we describe the first case report of CAPD-related peritonitis due to *H hamiltonii*.

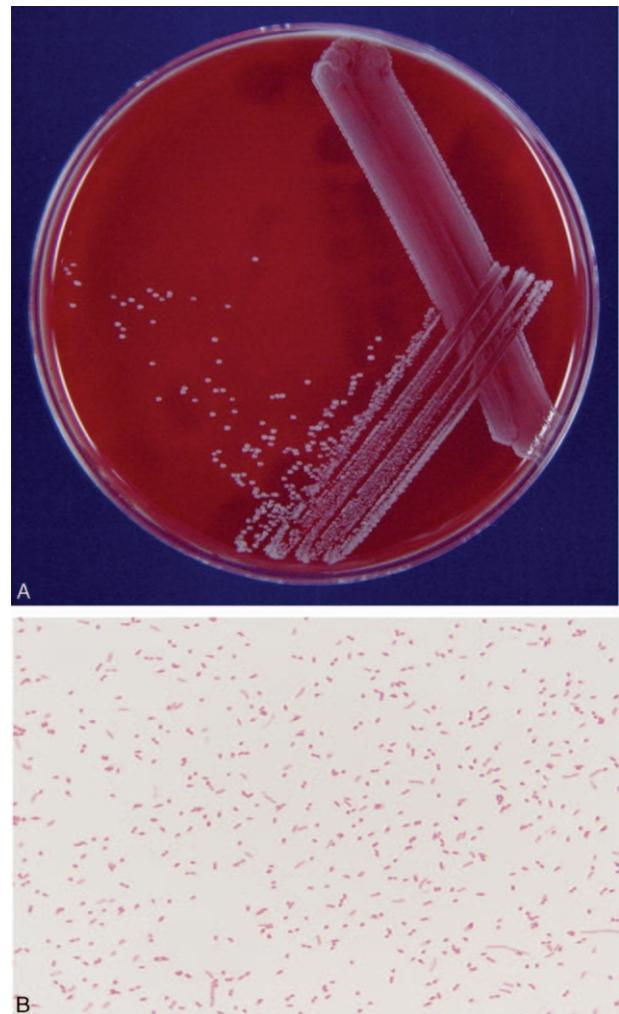
## 2. Case report

An 82-year-old male patient who had been receiving CAPD therapy for ~1 year was admitted because of abdominal pain and turbid peritoneal effluent that persisted for 3 days. The underlying cause of the ESRD was hypertensive nephropathy, and his dialysis treatment consisted of 4 exchanges of 2 L of dialysate daily. The patient had never been on hemodialysis and had been administered peritoneal dialysis after receiving a diagnosis of ESRD. There was no relevant family history, and the patient had no alcohol consumption or smoking history. The patient was a monk who lived in the countryside and used groundwater for toiletry and drinking.

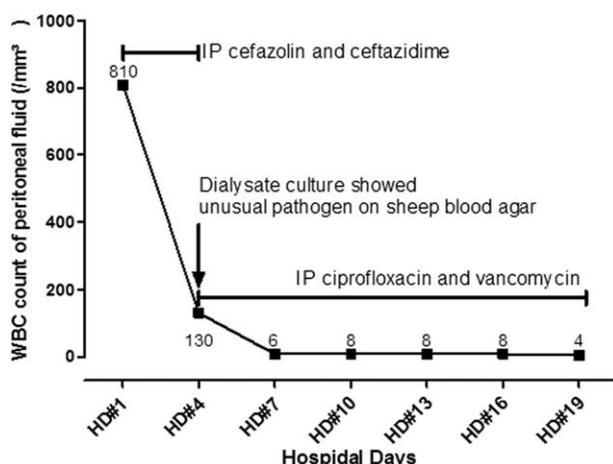
On admission, his blood pressure, heart rate, respiratory rate, and body temperature were 120/80 mm Hg, 72 beats/min, 19/min, and 36.5°C, respectively. Abdominal examination showed diffuse tenderness; however, the CAPD catheter tunnel was normal, and its exit site was clear. Initial laboratory findings were: leukocytes 4250/mm<sup>3</sup>, hemoglobin 9.5 g/dL, platelets 164,000/mm<sup>3</sup>, sodium 136 mEq/L, potassium 2.9 mEq/L, chloride 100 mEq/L, blood urea nitrogen 33 mg/dL, creatinine 4.6 mg/dL, aspartate aminotransferase (AST) 25 IU/L, alanine aminotransferase (ALT) 10 IU/L, total bilirubin 0.3 mg/dL, total protein 5.4 g/dL, and albumin 2.7 g/dL. The peritoneal dialysate showed CAPD peritonitis; the white blood cell (WBC) count of the peritoneal effluent was 810/mm<sup>3</sup> and neutrophils predominated (60%) (Fig. 1).

Two peritoneal dialysate samples were inoculated into BacT/ALERT PF Plus culture bottles (bioMérieux, Marcy-l'Étoile, France) and incubated in a BacT/ALERT 3D Blood Culture System (bioMérieux, Marcy-l'Étoile, France). Immediately after peritoneal dialysate was sent for bacterial culture, the patient was empirically started on an antibiotics regimen consisting of cefazolin and ceftazidime intraperitoneally.

On day 4 after admission and 2 days after culture at 37 °C under 5% CO<sub>2</sub>, the peritoneal dialysate culture showed 2-mm gray-white, nonhemolytic colonies on sheep blood agar (Fig. 2A), but did not grow on MacConkey's agar. Cells were Gram-negative, nonspore-forming rods with a few longer and larger bacilli than usual (Fig. 2B).



**Figure 2.** (A) *H hamiltonii* on blood agar medium showing nonhemolytic pink mucoid colonies after incubation for 48 h. (B) Gram stain of *H hamiltonii* showing Gram-negative, nonspore-forming rods with a few longer and larger bacilli than usual (Gram stain ×1000).



**Figure 1.** Time-frame figure of the disease course.

bacilli than usual (Fig. 2B). The isolate was identified as *Methylobacterium* sp. based on a positive reaction to urease (GN 0000000200000000) as determined by a commercial identification kit (Vitek II system, bioMérieux, Marcy-l'Étoile, France).

The WBC count of the peritoneal fluid was 130/mm<sup>3</sup> with neutrophils predominant (61%) on day 4 after admission. On day 6 after admission, empirical antibiotic treatment was stopped, after which intraperitoneal (IP) ciprofloxacin and vancomycin were started, even though the patient was improving because the authors believed that caution should be taken in considering the possibility of cephalosporin-resistant organisms until the causative organism is identified. On day 18 after admission, the clinical condition of the patient had entirely recovered, and the peritoneal WBC count was 8/mm<sup>3</sup>. Intraperitoneal treatment was continued for 21 days, and the patient was discharged without any problems (Fig. 1).

The Vitek 2 (BioMérieux Inc.) automated identification system cannot subdivide *Methylobacterium* sp; therefore, we requested a molecular biological evaluation from Macrogen (Macrogen Inc., Korea) based on DNA isolated from a cultured colony at day 6

Primers	amplification	sequencing
27F AgA aTT TgA TCM TGG CTC Ag	●	
1492R TAC ggY TAC CTT gTT ACg ACT T	●	
518F CCA gCA gCC gCg gTA ATA Cg		●
800R TAC CAg ggT ATC TAA TCC		●

**Figure 3.** Primers used for amplification and sequencing during 16S rRNA analysis.

after admission. The 16S rRNA gene was sequenced using universal primers (Fig. 3) and then compared with sequences retrieved from the GenBank, European Molecular Biology

Laboratory (EMBL), and DNA Data Bank of Japan (DDBJ) databases. The isolate showed the highest 16S rRNA gene sequence similarity (of 99%) to *H hamiltonii* W1025<sup>T</sup> (GenBank accession number AM941396). The partial sequence of the 16S rRNA gene of a bacterium detected by peritoneal fluid culture was utilized for a Basic Local Alignment Search Tool (BLAST) search, which revealed that the organism was *H hamiltonii*. Accordingly, the causative organism was concluded as *H hamiltonii*. These results were received after the patient had been discharged.

### 3. Discussion

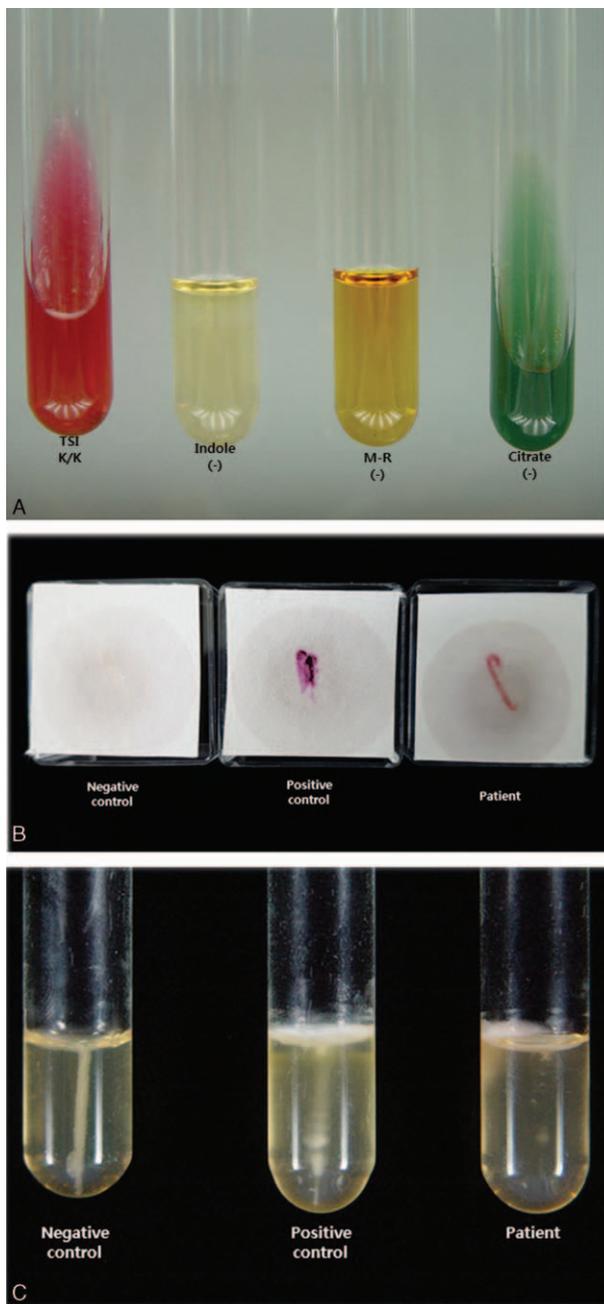
To date, the gold standard for identifying pathogens in peritoneal dialysate is culture, but CAPD-related peritonitis is usually treated empirically. So culture-negative peritonitis often exists

**Table 1**  
**Antimicrobial susceptibility tests (AST) for *H hamiltonii*.**

Antibiotics	Reference <sup>[1]</sup>	Study sample using Mueller–Hinton Agar			
		N224	N225	P600	P601
Ampicillin	S	S	I	S	
Carbenicillin	S	S			
Cefotaxime	S	S	S		
Cefoxitin	S	S	S		
Penicillin	S				R
Tetracycline	S	S		S	S
Erythromycin	R			R	R
Trimethoprim/sulfametho	R	R	S		S
Vancomycin	R			R	I
Cephalothin		R			
Ceftriaxone		S			
Ceftazidime		S	S	S	
Ciprofloxacin		S	S	S	S
Gentamicin		S	S	S	S
Clindamycin				R	R
Amoxicillin/avulanic ac		S	S		
Imipenem		S	S	S	
Amikacin		S	S	S	
Aztreonam		S	S	S	
Oxacillin					R
Teicoplanin				R	R
Ampicillin/sulbactam		S		S	
Piperacillin/tazobactam		S	S		
Levofloxacin				S	
Norfloxacin				S	
Quinupristin/dalfopristin				R	R
Linezolid				R	R
Tigecycline			S	S	S
Nitrofurantoin	S			S	S
Habekacin					S
Telithromycin					R
Fusidic acid					R
Mupirocin					S
Rifampicin	S				S
Cefazolin			S		
Cefepime			S	S	
Aztreonam				S	
Ticarcillin/clavulanic acid				S	
Piperacillin				R	
Minocycline				S	
Colistin				S	

Antimicrobial susceptibility tests (AST) were performed in our study specimen using the N224, N225, P600, and P601 cards (bioMérieux) to check for enterobacteriaceae, nonfermentative Gram-negative bacteria (GNB), enterococci, and staphylococci, respectively.

AST=antimicrobial susceptibility tests, GNB=Gram-negative bacteria, I=intermediate, R=resistant, S=susceptible.



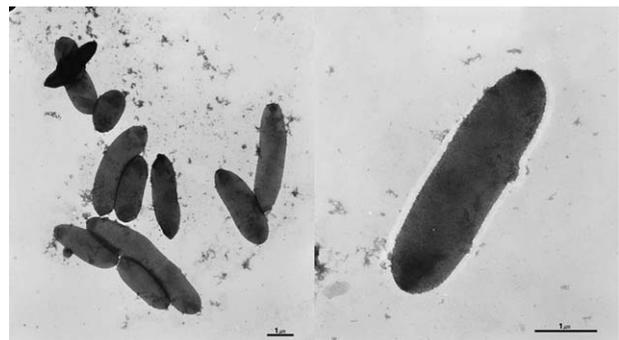
**Figure 4.** Biochemical tests (A), oxidase test (B), and motility test (C) for strain characterization.

and remains a clinical issue with renal care centers. To increase susceptibility to bacterial culture, we use blood culture bottles instead of conventional media. In particular, the pediatric blood culture bottles used at our center enable bacterial cultures to be performed on samples as small as 5 mL. And pediatric blood culture bottle can absorb antibiotics, so bacteria that do not grow well in conventional media grow at high rate. Gram-positive bacteria usually grow in blood agar and Gram-negative bacteria in MacConkey agar. In our case, the Gram-negative bacterium grew in blood agar and not definite in MacConkey agar. Because the bacterium did not grow on this medium, we requested a molecular biological evaluation to enable definitive organism

identification, and subsequently, we identified the organism as *H hamiltonii*.

*H hamiltonii* is an aerobic, halophilic, Gram-negative, non-spore-forming rod bacterium, which contains Q-9 as the predominant ubiquinone and C18:1  $\omega$ 7 c and C16:0 as the major fatty acids.<sup>[9]</sup> In this study, cultured colonies are circular, smooth, cream-colored, and translucent. Good growth is observed on sheep blood agar. The family *Halomonadaceae* was first described in 1988 by Franzmann et al,<sup>[8]</sup> and although most *Halomonas* sp. are commonly found in saline or high-salt environments, it has rarely been implicated as a causative organism in human infections. Moreover, a few cases of infections attributed to hemodialysis, hemodialysis machines, and environmental sources in renal care centers have been reported.<sup>[9,10,11]</sup>

Little is known about the clinical implications of peritoneal dialysis-associated peritonitis (PD peritonitis) caused by *Halomonas* species. Antibiotic susceptibility to *H hamiltonii* has been revealed in previous studies. Specifically, Lee et al described the characteristics of *Halomonas*, especially in ESRD patients with maintenance hemodialysis.<sup>[9,10]</sup> They had previously reported the treatment of *Halomonas*-infected hemodialysis patients. However, to the best of our knowledge, there is no *Halomonas*-specific treatment protocol in PD peritonitis. In hemodialysis patients, conventional antibiotics coverage for Gram-negative rod was successful.<sup>[9]</sup> Moreover, the results of antimicrobial susceptibility tests (ASTs) have been reported.<sup>[9]</sup> In the present study, we also conducted ASTs of our study specimen using the N224, N225, P600, and P601 cards (bioMérieux) for enterobacteriaceae, nonfermentative Gram-negative bacteria (GNB), enterococci, and staphylococci, respectively (Table 1). The results of our study were consistent with those of previous studies.<sup>[9,10]</sup> In this study, we additionally attempted to conduct microscopic examination of *Halomonas* in peritoneal dialysis fluid. Good growth was observed on blood agar medium, and the biochemical tests for strain characterization revealed negative responses in K/K on the triple sugar iron (TSI) test and H<sub>2</sub>S, a weak positive equivocal response in the motility test, and positive responses to the catalase, oxidase, and urease tests (Fig. 4). These strains of *Halomonas* species differ from each in the previous study in terms of having flagella. In our specimen, the motility test is not conclusive. Thus, we performed electron microscopic observation of flagellation in our specimen. We failed to demonstrate that the culture organisms are motile with flagella (Fig. 5). In our specimen, we concluded that this *Halomonas* species has no flagellae and no motility.



**Figure 5.** Electron microscopic observation of cultured *H hamiltonii*.

The patient described in this report was discharged after 21 days of intraperitoneal antibiotic treatment and was later readmitted at our renal care center with CAPD-related peritonitis on October 3rd, 2014. Culture and genetic analyses on this second occasion identified *Pseudomonas pseudoalcaligenes* as the pathogenic organism. Thus, we concluded that on this second occasion the peritonitis was caused by a different bacterium and that *H hamiltonii* was causative at first admission.

Patients with maintaining PD have reduced immunity and can therefore be infected with bacteria that do not cause problems for healthy individuals, although infections by *Halomonas* are rare. Because peritoneal dialysis patients are susceptible to recurrent bacterial infections, accurate identification of the causative organism is essential, and molecular biological methods can be helpful in this context.

It is often not clear why an causal organism cannot be cultured, and the underlying microbiological characterization is poorly understood.<sup>[12]</sup> To increase susceptibility to bacterial culture, we used the blood culture bottle-based technique with large volume of effluent as recommended by ISPD 2016 peritonitis guideline in this study. However, in our case, the Gram-negative bacterium seemed to grow in blood agar and appeared not to in MacConkey's agar. As a result, molecular biological techniques can be useful for this group of patients. Several studies have shown that the use of molecular biological techniques such as quantitative PCR for bacterial DNA, rRNA amplification by PCR, and so on, can detect and identify bacterial pathogens, thereby increasing diagnosis of CAPD-related peritonitis.<sup>[13,14]</sup> Moreover, molecular biological analysis could help diagnose culture-negative peritonitis.<sup>[15]</sup> The patient in the present case was a monk who lived deep in the mountains. Therefore, we collected water from the wells and groundwater that the monk used, and had done culture study. However, we failed to disclose the causative organism, contrary to our expectation. Our institute was founded by the Jogye Order of Korean Buddhism in the rural area. This case might be interesting report of CAPD-related peritonitis due to *H hamiltonii* whose profession is Buddhist priest, practicing asceticism.

In summary, we provide here the first case report of CAPD-related peritonitis caused by *H hamiltonii*, which was identified using molecular biological techniques. Although guidelines do not exist for the treatment of infections caused by this organism, conventional treatment for Gram-negative organisms could be effective.

## Acknowledgments

This study is a retrospective, noninterventional study, and written informed consent was waived. Approval from institutional review board was not necessary, because of case report with fewer 3 patients not requiring additional resource of patient designed to contribute to generalizable medical knowledge under Health Insurance Portability and Accountability Act (HIPAA).

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