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Case report

Peritonitis with bacteremia due to *Christensenella hongkongensis* identified via ribosomal RNA sequencing in a Japanese patient with advanced colorectal adenocarcinoma: A case report

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ABSTRACT

Background: Christensenella hongkongensis is an obligately anaerobic, catalase-positive, motile, non-sporulating, gram-positive coccobacillus. Human infections are rare and have not been previously reported in Japan. Herein, we report the first case of perforated peritonitis with *C. hongkongensis* bacteremia in Japan.

Case presentation: A 61-year-old Japanese man with advanced colorectal adenocarcinoma presented with fever and abdominal pain. Abdominal computed tomography revealed a low-density area with thinning of the sigmoid colon wall and air outside the intestinal tract, which was diagnosed as perforated peritonitis. Cultures of the ascitic fluid isolated *Bacteroides fragilis*, *Bacteroides eggerthii*, *Parabacteroides distasonis*, *Enterococcus avium*, and *Candida albicans*. Gram-positive rods were detected in the blood culture on admission after 4 days. The isolate was identified as *C. hongkongensis* via 16S ribosomal RNA (16S rRNA) sequencing. The patient underwent open abdominal washout and drainage via a transverse colon bifurcation colostomy. Intravenous meropenem (3 g/day) was administered for 5 days, followed by intravenous piperacillin-tazobactam (9 g/day) for 6 days, and then levofloxacin (500 mg/day) and metronidazole (1500 mg/day) intravenously for 15 days. Postoperatively, the patient gradually recovered. He was transferred to another palliative care hospital on day 38 after admission for worsening advanced colorectal cancer condition.

Conclusion: Bacteremia caused by *C. hongkongensis* is rare. 16S rRNA sequencing should be considered for the identification of gram-positive anaerobic rods that are difficult to diagnose by conventional methods.

Background

Christensenella hongkongensis is a rare microorganism reclassified from *Catabacter hongkongensis* based on whole-genome analysis in 2021 [1,2]. *C. hongkongensis* is an obligate anaerobe. It is a catalase-positive, motile, and non-sporulating gram-positive coccobacillus [3]. It was first isolated from the blood cultures of four patients in Hong Kong and Canada in 2007 [4]. A total of 17 human cases have been reported in various countries, including Hong Kong, Canada, South Korea, New Zealand, Italy, Sweden, France, and the US; however, no cases have been reported in Japan [3–12].

All reported cases were associated with bacteremia [3–12]. They were linked to intestinal and biliary infections, such as intestinal perforation, acute appendicitis, cholangitis, and cholecystitis, suggesting that the intestinal tract is the source of bacteremia. Patients with advanced malignancies are at high risk of mortality from *C. hongkongensis* bacteremia. In addition to causing human infections, *C. hongkongensis* has been found in different environmental samples worldwide, including urban aerosols, mangrove sediments, rice paddy field soil, and fecal microflora of a captive dugong (*Dugong dugong*) at the Toba Aquarium in Japan [13–16].

Here, we describe the first case of perforation peritonitis associated with bacteremia caused by *C. hongkongensis* in Japan. It was identified using 16S ribosomal RNA (16S rRNA) sequencing in a patient with advanced colorectal cancer.

Case presentation

The patient was a 61-year-old Japanese man with advanced colorectal adenocarcinoma who presented with fever and abdominal pain

Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PIPC/TAZ, piperacillin-tazobactam.

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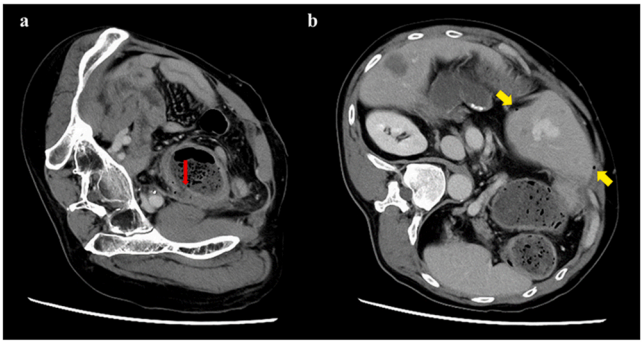

Fig. 1. Abdominal computed tomography scan showing a low-density area with thinning of the sigmoid colon wall (red arrow) and extraluminal air (yellow arrow).

(day 0). He had undergone laparoscopic lower anterior resection for newly diagnosed colorectal adenocarcinoma 5 years prior. He currently has stage IV colorectal adenocarcinoma with lung and liver metastases and is receiving chemotherapy and radiation therapy for postoperative recurrence. His last chemotherapy session included bevacizumab plus infusional 5-fluorouracil/leucovorin plus oxaliplatin (3 months before admission). A month before, he had received a total of 21 Gy of radiation therapy for left buttock pain associated with lymph node metastasis. On the day before admission, the patient developed abdominal pain.

On examination, his body temperature was 38.8 °C; heart rate was 117 bpm, blood pressure was 159/96 mmHg, respiratory rate was 20 bpm, and oxygen saturation was 93 % on room air. Tenderness with palpation was observed in the left lower abdomen. The other physical examination findings were unremarkable. Laboratory investigations revealed a white blood cell count of 11,420/mL (neutrophil count: 11,020/mL), normocytic anemia (hemoglobin level: 12.2 g/dL), C-reactive protein level of 31.32 mg/dL (normal < 0.30 mg/dL), aspartate transaminase level of 22 U/L (normal 13–30 U/L), alanine aminotransferase level of 21 U/L (normal 10–42 U/L), gamma-glutamyltransferase level of 136 U/L (normal 13–64 U/L), alkaline phosphatase level of 242 U/L (normal 38–113 U/L), total bilirubin level of 1.6 (normal 0.4–1.5 mg/dL), blood urea nitrogen of 21.8 mg/dL (normal 8.0–20.0 mg/dL), and creatinine level of 0.96 (normal 0.65–1.07 mg/dL). Abdominal computed tomography (CT) revealed a low-density area with thinning of the sigmoid colon wall and air outside the intestinal tract (Fig. 1).

Two sets of blood cultures were obtained from peripheral blood samples collected in BacT/ALERT FA PLUS culture bottles using the BacT/ALERT 3D system (bioMérieux Japan Co. Ltd., Tokyo, Japan). Intravenous meropenem was administered at a dose of 3 g/day as empirical antimicrobial therapy for perforation peritonitis. Open abdominal washout with drainage and transverse colon bifurcation colostomy were performed (day 0). Intraoperative findings indicated severe contamination around the sigmoid colon, although the perforation

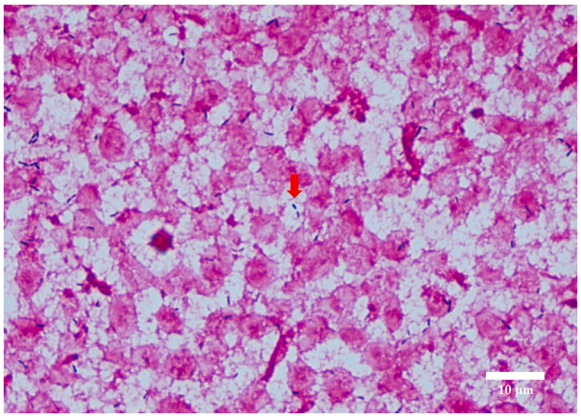

Fig. 2. Blood culture smear showing gram-positive rods (red arrow). Gram staining, 1000 ×.

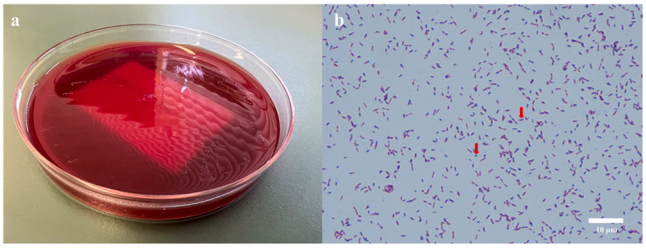

Fig. 3. **(a)** Non-hemolytic pinpoint colonies after 96 h on Brucella HK agar plate. **(b)** Smear of the colonies showing gram-variable coccobacillus (red arrow). Gram staining, 1000 ×.

Table 1
Antibiotic susceptibility of the isolated microorganisms in the ascitic fluid culture.

	Bacteroides fragilis		*Bacteroides eggerthii*		*Parabacteroides distasonis*		*Enterococcus avium*	
	MIC (μg/mL)	Susceptibility	MIC (μg/mL)	Susceptibility	MIC (μg/mL)	Susceptibility	MIC (μg/mL)	Susceptibility
Penicillin G	≥ 2	R	0.5	S	≥ 2	R	2	S
Ampicillin	≥ 2	R	0.25	S	≥ 2	R	≤ 2	S
Ampicillin-sulbactam	≥ 32	R	≤ 2	S	≥ 128	R	ND	
Amoxicillin-clavulanate	≥ 16	R	≤ 1	S	≥ 64	R	ND	
Piperacillin-tazobactam	64	I	≤ 2	S	4	S	ND	
Ceftriaxone	≥ 64	R	4	S	≥ 64	R	ND	
Cefmetazole	≥ 64	R	4	S	32	I	ND	
Imipenem	≥ 16	R	≤ 0.25	S	0.5	S	ND	
Meropenem	≥ 16	R	≤ 0.25	S	0.5	S	ND	
Erythromycin							≤ 0.25	S
Minocycline							8	I
Teicoplanin							≤ 0.5	S
Vancomycin	ND		ND		ND		≤ 0.5	S
Clindamycin	≥ 8	R	≥ 8	R	≥ 8	R	ND	
Moxifloxacin	2	S	≥ 8	R	≥ 8	R	ND	
Levofloxacin	ND		ND		ND		1	S
Metronidazole	≤ 2	S	≤ 2	S	≤ 2	S	ND	

Abbreviations S, susceptible; I, intermediate; R, resistant; ND, not determined; MIC, minimum inhibitory concentration *Candida albicans* was not tested for susceptibility.

Table 2
Summary of published cases of *Christensenella hongkongensis* infection.

Author, year, Ref.	Age, sex	State	Underlying condition	TTP (days)	Diagnostic method	Strain	Susceptibility (MIC, μg/mL) [S or R]				Treatment	Outcome
							VAN	PEN	CEF	MET		
Lau et al. (2007) [4]	48, M	Hong Kong	Small bowel obstruction with secondary sepsis	3	16S rRNA sequencing	HKU16	2 [S]	0.75 [S]	> 32 [R]	< 0.016 [S]	Cefuroxime + Metronidazole	Recovered
Lau et al. (2007) [4]	39, M	Hong Kong	Acute appendicitis with perforation	3	16S rRNA sequencing	HKU17	2 [S]	0.5 [S]	> 32 [R]	< 0.016 [S]	Laparoscopic appendectomy, Cefuroxime + Metronidazole	Recovered
Lau et al. (2007) [4]	74, M	Canada	Plasmacytoma with biliary obstruction, Exchange of biliary stent	ND	16S rRNA sequencing	CA1	2 [S]	4 [R]	> 32 [R]	< 0.016 [S]	Ciprofloxacin	Recovered
Lau et al. (2007) [4]	66, F	Canada	Sepsis, Metastatic cancer of the lung	5	16S rRNA sequencing	CA2	2 [S]	4 [R]	> 32 [R]	< 0.016 [S]	Cefuroxime + Ciprofloxacin	Died
Elsendoorn et al. (2011) [5]	52, M	France	Intestinal perforation peritonitis with Pneumoperitonitis	3	16S rRNA sequencing	ND	< 0.016 [S]	2 [R]	> 32 [R]	< 0.016 [S]	Amoxicillin-clavulanate + gentamicin	Died
Lau et al. (2012) [3]	91, F	Hong Kong	Liver abscess, suspected gastrointestinal malignancy	3	16S rRNA sequencing	HKU 16	0.75–1.0 [S]	< 0.016–0.032 [S]	> 32 [R]	< 0.016 [S]	Ticarcillin-clavulanate + Gentamicin	Died
Lau et al. (2012) [3]	21, M	Hong Kong	Acute gangrenous perforated Appendicitis	3	16S rRNA sequencing	HKU 16	0.75–1.0 [S]	< 0.016–0.032 [S]	> 32 [R]	< 0.016 [S]	Laparoscopy with appendectomy, Cefuroxime + Metronidazole	Recovered
Lau et al. (2012) [3]	81, F	Hong Kong	Sepsis, Metastatic colorectal cancer	3	16S rRNA sequencing	HKU 16	0.75–1.0 [S]	< 0.016–0.032 [S]	> 32 [R]	< 0.016 [S]	Amoxicillin-clavulanic acid, Piperacillin-Tazobactam	Died
Lau et al. (2012) [3]	76, M	Hong Kong	Acute calculous cholecystitis	3	16S rRNA sequencing	HKU 16	0.75–1.0 [S]	< 0.016–0.032 [S]	> 32 [R]	< 0.016 [S]	Cefuroxime + Metronidazole	Recovered
Lau et al. (2012) [3]	81, F	Hong Kong	Infected tumor, carcinoma of ascending colon with liver metastasis	3	16S rRNA sequencing	HKU 16	0.75–1.0 [S]	< 0.016–0.032 [S]	> 32 [R]	< 0.016 [S]	Cefuroxime + Metronidazole	Died
Smith et al. (2012) [6]	47, M	New Zealand	Acute appendicitis with perforation	4	16S rRNA sequencing	HKU 16	2 [S]	4 [R]	ND	< 0.016 [S]	Laparoscopy and pelvis wash out, Cefuroxime + Metronidazole, Amoxicillin-clavulanic acid	Recovered
Torri et al. (2016) [7]	55, M	Italy	Septic shock, Road accident with multiple pelvic fractures and splenic Hematoma	4	16S rRNA sequencing	HKU 16	0.75 [S]	ND	ND	< 0.016 [S]	Ceftazidime + Gentamicin, vancomycin + meropenem	Recovered
Kaden et al. (2017) [8]	83, M	Sweden	Isolated fever	3	16S rRNA sequencing	ABBA15k	[S] Genotypic Determination	[S] Genotypic Determination	[S] Genotypic Determination	[S] Genotypic Determination	No antibiotic treatment	Recovered
Choi et al., 2016 [9]	77, M	South Korea	Acute cholecystitis	3	16S rRNA sequencing	NA	0.5–0.75 [S]	> 32 [R]	> 32 [R]	- [S]	Endoscopic retrograde biliary drainage, Cefodizime + metronidazole	Recovered
Cabrol et al. (2021) [10]	80, F	France	Febrile diarrhea on sigmoid adenocarcinoma	2–3	16S rRNA sequencing	NA	< 2 [S]	1 [S]	> 32 [R]	< 4 [S]	Cefazolin + Gentamicin, Piperacillin-Tazobactam + Vancomycin	Died

(*continued on next page*)

Table 2 (*continued*)

Author, year, Ref.	Age, sex	State	Underlying condition	TTP (days)	Diagnostic method	Strain	Susceptibility (MIC, μg/mL) [S or R]				Treatment	Outcome
							VAN	PEN	CEF	MET		
Kamau et al. (2021) [11]	55, M	USA	Colonic perforation	4	Metagenomic sequencing	DSM 18959	ND	1.5 [R]	ND	0.023 [S]	Colostomy takedown + right hemicolectomy + ileostomy creation, Vancomycin + Metronidazole + cefepime, Meropenem + Vancomycin + Caspofungin	Recovered
Mandin et al. (2022) [12]	62, M	France	Septic shock, Non-small cell lung cancer with adrenal metastases without any intraperitoneal involvement	3	16S rRNA sequencing	NA	ND	ND	ND	ND	Amoxicillin-clavulanic acid, Piperacillin-Tazobactam + Amikacin	Died
Present case	61, M	Japan	Colonic perforation with advanced colorectal adenocarcinoma	4	16S rRNA sequencing	HKU 16	ND	ND	ND	ND	Abdominal washout + transverse colon bifurcation colostomy, Meropenem, Piperacillin-Tazobactam, Cefepime + Metronidazole	Recovered

Abbreviations: M, male; F, female; MIC, minimum inhibitory concentration; CEF, cefotaxime; R, resistant to agents based on CLSI clinical breakpoints for anaerobes; S, susceptible to agents based on CLSI clinical breakpoints for anaerobes; TTP, time to positive results; VAN, vancomycin; PEN, penicillin; MET, metronidazole; ND, no data.

site could not be identified. Drain tubes were placed under the left and right diaphragms, in the rectovesical space, and around the sigmoid colon.

An ascitic fluid culture was obtained intraoperatively. The fluid revealed numerous white blood cells, gram-negative rods, gram-positive rods, and gram-positive cocci. The ascitic fluid was cultured on a Sheep Blood Agar plate (Nissui Pharmaceuticals Co., Ltd., Tokyo, Japan), a chocolate agar EX II plate (Nissui Pharmaceutical Co., Ltd.), a MacConkey II agar plate (Becton Dickinson Co., Ltd., Tokyo, Japan), a Brucella HK (RS) agar plate (Kyokuto Pharmaceutical Co., Tokyo, Japan), a CHROMagar MRSA plate (Kanto Chemical Co, Inc., Tokyo, Japan), and a CHROMagar Candida plate (Becton Dickinson, Co, Inc., Tokyo, Japan). The culture specimens were identified using VITEK2 ver. 9.02 with the VITEK 2 ANC ID card and VITEK 2 GP ID card (bioMérieux Japan Co., Ltd.). *Bacteroides fragilis*, *Bacteroides eggerthii*, *Parabacteroides distasonis*, and *Enterococcus avium* were identified with probabilities of 97 %, 98 %, 97 %, and 99 %, respectively. *Candida albicans* was identified based on the colony color on the CHROMagar Candida plate. Antimicrobial susceptibility tests were performed according to the methodology recommended by the Clinical and Laboratory Standards Institute document M100-S32 (2022) [17], and the minimum inhibitory concentration of antimicrobial agents was determined by broth microdilution (Table 1).

Gram-positive rods were detected in one of two sets of anaerobic bottles of blood culture upon admission and after 88 h and 38 min (day 3) (Fig. 2). Positive bottles were sub-cultured on sheep blood, MacConkey II, and Brucella HK (RS) agar plates. After 96 h of anaerobic incubation at 35 °C, non-hemolytic pinpoint colonies of gram-positive coccobacilli with variable staining were observed on the Brucella HK agar plates (Figs. 3**a**, 3**b**). Attempts to identify the bacteria using VITEK2 and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with VITEK MS ver. 4.7.1 (bioMérieux Japan Co., Ltd.) were unsuccessful. Identification was achieved using 16S rRNA sequencing. After performing a Basic Local Alignment Search Tool (BLAST) search of the 16S rRNA sequence of the isolated strain, 100 % homology (1429/1429 bp) with the standard *Christensenella hongkongensis* strain HKU 16 (GenBank Accession No.: NR 115269.1) was obtained.

On day 5, erythema appeared on the patient's buttocks and chest, and meropenem was changed to intravenous piperacillin-tazobactam (PIPC/TAZ) at a dose of 9 g/day because of a suspected drug eruption. Based on the ascitic fluid culture results, PIPC/TAZ was changed to intravenous levofloxacin (500 mg/day) and metronidazole (1500 mg/day) on day 11. Postoperatively, the fever gradually subsided, and the patient recovered. The left subdiaphragmatic drainage tube was removed on day 18, followed by the drainage tube around the sigmoid colon on day 24 and the Douglas fossa drain on day 28. However, the right diaphragmatic drain remained in place, which continued to drain purulent fluid. Levofloxacin and metronidazole were administered for 15 days. Patient was transferred to another palliative care hospital on day 38 due to deterioration of activities of daily living associated with worsening advanced colorectal cancer condition.

Discussion and conclusions

Here, we report a case of perforated peritonitis with bacteremia caused by *Christensenella hongkongensis*. This infection is rare, and this is the first reported case in Japan. Notably, *Catabacter hongkongensis* was reclassified as *C. hongkongensis* in 2021 [1], which is the first report of *C. hongkongensis*. Although *C. hongkongensis* could not be identified using VITEK2 and MALDI-TOF MS, it was eventually identified using 16S rRNA sequencing.

Only 17 cases of *C. hongkongensis* infection have been reported in humans (Table 2) [3–12]. There have been no reports of human infection or colonization by *C. hongkongensis* in Japan, except for this report. There have been seven cases in Hong Kong, three in France, two in

Canada, and only one each in Korea, Sweden, Italy, New Zealand, and the United States [3–12]. The few reports suggest that *C. hongkongensis* may be underestimated because of the difficulty in identification using routine diagnostic tests [5,7,9,11,12]. Although MALDI-TOF MS has proven to be an accurate and useful technique for rapid bacterial identification, one of its limitations is the limited number of reference datasets available for infrequently isolated microorganisms from clinical specimens. In most previously reported cases, including ours, *C. hongkongensis* was identified using 16S rRNA sequencing.

Among the reported cases of *C. hongkongensis*, 8 out of 18 (44 %) patients were diagnosed with cancer [3–12]. The overall mortality rate was as high as 7 out of 18 (39 %), while 6 out of 8 (75 %) of the patients with cancer died. The patients who died were older and had gastrointestinal perforations. Notably, there is a report from Sweden of a patient who recovered from *C. hongkongensis* bacteremia without antibiotic treatment [8]. The high mortality rate may be due to severe comorbidities rather than the high virulence of *C. hongkongensis*.

Most reported cases of bacteremia caused by *C. hongkongensis* are associated with the gastrointestinal and biliary tracts. From a microbiological standpoint, the patient's ascitic fluid showed commensal flora of the human gastrointestinal tract, including *Bacteroides fragilis*, *Bacteroides eggerthii*, *Parabacteroides distasonis*, *Enterococcus avium*, and *Candida albicans*. Gastrointestinal perforation has been postulated to cause *C. hongkongensis* bacteremia. Therefore, *C. hongkongensis* and other non-spore-forming anaerobic gram-positive rods may be part of the human gut flora. *C. hongkongensis* was not isolated from the ascitic fluid, possibly because of its fastidious nature, which requires strict anaerobic conditions for growth.

In this case, antimicrobial susceptibility testing could not be performed because of poor colony growth. However, the patient recovered with surgery, levofloxacin, and metronidazole. Reports on susceptibility to other antimicrobial agents vary; however, susceptibility to vancomycin and metronidazole has been preserved [3–12]. Several treatment options could be considered. As *C. hongkongensis* bacteremia is mostly of gastrointestinal origin, an initial regimen of metronidazole plus an antimicrobial agent effective against *Enterobacterales* may be appropriate.

In conclusion, we reported the first case of perforated peritonitis with bacteremia caused by *C. hongkongensis* in Japan. If gram-positive rods are detected in anaerobic blood culture bottles and are difficult to identify using conventional methods, 16S rRNA sequencing should be considered if the pathogen is considered to be clinically relevant and could require a change of management.

CRediT authorship contribution statement

Naoya Itoh: Conceptualization, Investigation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Nana Akazawa:** Supervision, Formal analysis, Writing – original draft, Writing – review & editing. **Yuichi Ishibana:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Hiromi Murakami:** Formal analysis, Writing – original draft, Writing – review & editing.

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Ethical approval

Not applicable.

Conflicts of Interest

None.

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Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the editor of this journal.

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