

High Mortality Associated with Catabacter hongkongensis Bacteremia

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Catabacter hongkongensis is a recently described catalase-positive, motile, anaerobic, nonsporulating, Gram-positive coccobacillus that was first isolated from blood cultures of four patients from Hong Kong and Canada. Although DNA sequences representing *C. hongkongensis* have been detected in environmental sources, only one additional case of human infection has been reported, in France. We describe five cases of *C. hongkongensis* bacteremia in Hong Kong, two presenting with sepsis, one with acute gangrenous perforated appendicitis, one with acute calculous cholecystitis, and one with infected carcinoma of colon. Three patients, with gastrointestinal malignancy, died during admission. All five isolates were catalase positive, motile, and negative for indole production and nitrate reduction and produced acid from arabinose, glucose, mannose, and xylose. They were unambiguously identified as *C. hongkongensis* by 16S rRNA gene analysis. Of the total of 10 reported cases of *C. hongkongensis* bacteremia in the literature and this study, most patients had underlying diseases, while two cases occurred in healthy young individuals with acute appendicitis. Six patients presented with infections associated with either the gastrointestinal or biliary tract, supporting the gastrointestinal tract as the source of bacteremia. *C. hongkongensis* bacteremia is associated with a poor prognosis, with a high mortality of 50% among reported cases, especially in patients with advanced malignancies. All reported isolates were susceptible to metronidazole. Identification of more *C. hongkongensis* isolates by 16S rRNA gene sequencing will help better define its epidemiology and pathogenesis.

Medically important anaerobic Gram-positive bacilli are a heterogeneous group of bacteria comprising members of the genera *Clostridium, Actinomyces, Bifidobacterium, Eggerthella, Eubacterium, Lactobacillus,* and *Propionibacterium.* As a result of the difficulties in accurate identification by traditional phenotypic methods in clinical microbiology laboratories, the clinical significance and pathogenicity of these bacteria, especially the less commonly encountered species, have been poorly understood. Based on 16S rRNA gene analysis as a new standard for classification of bacteria (20, 21), many of these bacteria have undergone taxonomic revisions with new genera and species having been introduced (9, 10, 12, 18, 24, 26, 29), and rarely encountered species have become better understood in terms of their disease association and epidemiology (4, 5, 15, 19, 22, 25, 28).

Catabacter hongkongensis is a motile, catalase-positive, strictly anaerobic, nonsporulating, Gram-positive coccobacillus that was first described in 2007 after its isolation from blood cultures of four patients (11). Two of the isolates were recovered from two patients in Hong Kong, one with intestinal obstruction and secondary sepsis and the other with acute appendicitis. The other two isolates were recovered from two patients in Canada, one with biliary sepsis after stent removal and the other with metastatic carcinoma of the lung and sepsis syndrome. The four isolates exhibited similar phenotypic characteristics that do not fit into patterns of any known genus and species. Their 16S rRNA genes were identical but exhibited more than a 16% nucleotide difference from those of all previously described bacteria. Phylogenetic analysis showed that they represent a distinct lineage among the anaerobic Gram-positive rods, only peripherally associated with clusters I, III, and XIVb of the clostridia (11). Based on its unique phenotypic and genotypic characteristics, a novel genus and species, C. hongkongensis, was proposed to describe this "catalasepositive bacterium," which may potentially belong to a new family, *Catabacteriaceae* (11). Since its first description, DNA sequences closely related to *C. hongkongensis* have been detected from environmental sources (1, 6, 16, 23). However, only one further case of human infection has been described, in France in a patient with intestinal perforation and secondary peritonitis (3). In this report, we describe five additional cases of *C. hongkongensis* bacteremia in Hong Kong and review the clinical characteristics of patients with *C. hongkongensis* bacteremia. The role of 16S rRNA gene sequencing in identifying more isolates and understanding the clinical significance of *C. hongkongensis* is also discussed.

MATERIALS AND METHODS

Patients and microbiological methods. Bacterial cultures and phenotypic identification were performed by standard conventional methods and with the API 20A system (bioMérieux Vitek) as described previously (8, 11, 17). Antibiotic susceptibility tests were performed by Etest and results interpreted according to the CLSI criteria for anaerobic bacteria (2).

Bacterial DNA extraction, PCR, and sequencing of 16S rRNA genes. Bacterial DNA extraction, PCR amplification, and DNA sequencing of the 16S rRNA genes were performed according to previously published protocols (11, 13). Bacterial DNA extracts were amplified with primers

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LPW398 (5'-GGCGTGCTTAACACATG-3') and LPW2523 (5'-GTGTG ACGGGCGGTGTGTA-3') (Gibco BRL, Rockville, MD). The PCR mixtures were amplified with 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 10 min in an automated 0.5-ml GeneAmp PCR system 9700 (Applied Biosystems). The sequences of the PCR products were compared with known 16S rRNA gene sequences in GenBank, and the phylogenetic relationships to closely related Gram-positive rods were determined using CLUSTAL X version 2.0 (7).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of the five blood culture isolates have been deposited in the GenBank sequence database under accession no. JF514883 to JF514887.

RESULTS

Patients. (i) Case 1. A 91-year-old woman was admitted to a hospital in 2008 because of fever and decreased general condition for 4 days. She had multiple medical problems, being bed bound and on Ryle's tube feeding. She also had suspected gastrointestinal malignancy which was managed conservatively. On admission, she was febrile with tachycardia. Her total leukocyte count was 22.9×10^{9} /liter (neutrophils, 93%; lymphocytes, 2%; monocytes, 4%), her hemoglobin level was 8.8 g/dl, and her platelet count was 369×10^{9} /liter. Her liver function was deranged, with alkaline phosphatase at 293 IU/liter, alanine aminotransferase at 59 IU/ liter, and aspartate aminotransferase at 56 IU/liter. Her serum albumin was 19 g/liter, globulin was 32 g/liter, urea was 13.6 mmol/liter, and creatinine was 46 µmol/liter. Blood culture performed on the day of admission before antibiotic treatment recovered a Gram-positive coccobacillus from the anaerobic bottle (isolate 1) after incubation for 3 days. Ultrasound of the abdomen showed a bilobar liver abscess and suspected carcinoma of the colon in hepatic flexure. Pus from image-guided drainage of the liver abscess recovered Escherichia coli and Bacteroides species. Her fever initially responded to intravenous ticarcillin-clavulanate and gentamicin. However, she subsequently developed nosocomial pneumonia and died 1 month after admission.

(ii) Case 2. A 21-year-old man was admitted to a hospital in 2009 because of fever and abdominal pain for 1 day. Examination of his abdomen revealed tenderness, guarding, and rebound tenderness over the right lower quadrant. A clinical diagnosis of acute appendicitis was made, and empirical intravenous cefuroxime and metronidazole were commenced. His total leukocyte count was 12.2×10^9 /liter (neutrophils, 92%; lymphocytes, 4%; monocytes, 4%), his hemoglobin level was 14 g/dl, and his platelet count was 172×10^{9} /liter. Emergency laparoscopic appendectomy showed an acutely inflamed appendix with gangrenous change and perforation. Purulent peritoneal fluid was noted over the pelvis and right paracolic gutter. Blood culture performed on the day of admission before antibiotic treatment recovered a Gram-positive coccobacillus from the anaerobic bottle (isolate 2) after incubation for 3 days. His case was complicated by postoperative intestinal obstruction with small bowel perforation, for which laparotomy, adhesiolysis, and small bowel repair were performed 8 days after appendectomy. He was discharged 1 month after admission.

(iii) Case 3. An 81-year-old woman was admitted to a hospital in 2009 because of fever for 1 day. She was recently diagnosed to have metastatic carcinoma of the colon. Her total leukocyte count was 44.7 \times 10⁹/liter (neutrophils, 98%; lymphocytes, 2%), her hemoglobin level was 8.6 g/dl, and her platelet count was 339 \times 10⁹/liter. Her serum alkaline phosphatase was 209 IU/liter, bilirubin was 11 µmol/liter, urea was 10.6 mmol/liter, and creatinine was 47 μ mol/liter. Blood culture performed on the day of admission before antibiotic treatment recovered a Gram-positive coccobacillus from the anaerobic bottle (isolate 3) after incubation for 3 days. Despite intravenous amoxicillin-clavulanate and subsequently piperacillin-tazobactam treatment, she died 9 days after admission.

(iv) Case 4. A 76-year-old man with a history of gallstones was admitted to a hospital in 2009 because of epigastric pain, vomiting, and constipation for 3 days. On admission, he was afebrile but had tachycardia. Abdominal examination revealed tenderness at the right upper quadrant. His total leukocyte count was 15.2 imes10⁹/liter (neutrophils, 93%; lymphocytes, 3%), his hemoglobin level was 13.5 g/dl, and his platelet count was 113×10^{9} /liter. His liver and renal function tests were normal except for elevated serum aspartate aminotransferase at 84 IU/liter. Blood culture performed on the day of admission before antibiotic treatment recovered a Gram-positive coccobacillus from the anaerobic bottle (isolate 4) after incubation for 3 days. Ultrasound of the abdomen showed a distended gallbladder with thickened wall and gallstones, compatible with acute calculous cholecystitis. Emergency cholecystectomy revealed a grossly inflamed gallbladder with pus and adherence to omentum. He was given intravenous and subsequently oral cefuroxime and metronidazole and was discharged 3 weeks after admission.

(v) Case 5. An 81-year-old woman was admitted to a hospital in 2010 for management of newly diagnosed carcinoma of ascending colon with liver metastasis. She developed fever, chills, and rigor 2 days after admission. Abdominal examination revealed mild tenderness and a mass over the right flank, compatible with the clinical diagnosis of an infected tumor. Her total leukocyte count was 21.7×10^9 /liter (neutrophils, 90%; lymphocytes, 5%; monocytes, 4%), her hemoglobin level was 9.3 g/dl, and her platelet count was 475×10^9 /liter. Her liver and renal function tests were normal. Blood culture performed at the spike of fever before antibiotic treatment recovered a Gram-positive coccobacillus from the anaerobic bottle (isolate 5) after incubation for 3 days. Her fever responded to intravenous cefuroxime and metronidazole. However, she died 2 months later because of terminal malignancy and sepsis.

Phenotypic characteristics. All five strains exhibit phenotypic characteristics similar to those of C. hongkongensis. They are all strictly anaerobic, nonsporulating, Gram-positive coccobacilli which grow on sheep blood agar as nonhemolytic, pinpoint colonies after 48 h of incubation at 37°C in an anaerobic environment. They are motile and positive for catalase. They do not produce indole or reduce nitrate. They all produce acid from arabinose, glucose, mannose, and xylose, while interstrain variations were observed in esculin hydrolysis and glycerol and rhamnose fermentation (Table 1). All five isolates were "unidentified" by the whole API 20A system, with profile 40454042 or 40414052. They were susceptible to bile and kanamycin and resistant to colistin. All five strains were susceptible to penicillin, metronidazole, and vancomycin, with MICs of < 0.016 to $0.032 \mu g/ml$, $< 0.016 \mu g/ml$, and 0.75 to 1.5 µg/ml, respectively, but were resistant to cefotaxime, with MICs of $>32 \mu g/ml$.

Molecular characterization by 16S rRNA gene sequencing and phylogenetic characterization. PCRs of the 16S rRNA genes of all five isolates showed bands of about 1,400 bp. Their 16S rRNA gene sequences were identical. There was no difference between the 16S rRNA gene sequences of the five isolates and that of *C*.

	Type strain HKU16 ^T					
Test or characteristic	(11)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Esculin hydrolysis	_	-	-	+	+	-
Gelatin hydrolysis	_	-	-	-	-	-
Indole production	_	-	-	-	-	-
Urease	_	-	-	-	_	-
Reduction of nitrate	-	-	-	-	-	-
Oxidation/ fermentation of:						
Arabinose	+	+	+	+	+	+
Cellobiose	_	_	_	_	_	_
Glucose	+	+	+	+	+	+
Glycerol	+	_	_	_	_	_
Lactose	_	_	-	-	-	_
Maltose	_	_	-	-	-	_
Mannitol	_	_	-	-	-	_
Mannose	+	+	+	+	+	+
Melezitose	_	_	-	-	-	_
Raffinose	_	_	-	-	-	_
Rhamnose	_	+	+	-	-	+
Salicin	_	_	-	-	-	_
Sorbitol	_	_	-	-	-	_
Sucrose	_	_	_	_	_	_
Trehalose	_	_	_	_	_	_
Xylose	+	+	+	+	+	+

TABLE 1 Phenotypic characteristics of C. hongkongensis type stra	iin
HKU16 ^T and the five blood culture isolates	

TABLE 2 Clinical reports of Catabacter hongkongensis bacteremia

Reference and case	Place of origin	Sex/age (yr) ^a	Underlying condition ^b	Diagnosis	Antimicrobial therapy	Complication	Outcome
11	Hong Kong, China	M/48	ESRF on HD, TB peritonitis, recurrent IO	IO with secondary sepsis	Cefuroxime + metronidazole		Recovered
11	Hong Kong, China	M/39	None	Acute perforated appendicitis	Cefuroxime + metronidazole		Recovered
11	British Columbia, Canada	M/74	Plasmacytoma, biliary obstruction with stenting	Biliary sepsis	Ciprofloxacin		Recovered
11	British Columbia, Canada	F/66	Metastatic CA lung	Sepsis	Cefuroxime + ciprofloxacin		Died
3	Poitiers, France	M/52	Hypertension	Intestinal perforation with pneumoperitonitis	Amoxicillin-clavulanate + gentamicin	Septic shock	Died
Present study							
1	Hong Kong, China	F/91	Hypertension, CVA, anemia, suspected GIT malignancy	Sepsis, liver abscess	Ticarcillin-clavulanate + gentamicin	Nosocomial pneumonia	Died
2	Hong Kong, China	M/21	None	Acute gangrenous perforated appendicitis	Cefuroxime + metronidazole	Intestinal obstruction with small bowel perforation	Recovered
3	Hong Kong, China	F/81	Hypertension, OA knee, metastatic CA colon	Sepsis	Amoxicillin-clavulanate, piperacillin-tazobactam	•	Died
4	Hong Kong, China	M/76	Gallstones	Acute calculous cholecystitis	Cefuroxime + metronidazole		Recovered
5	Hong Kong, China	F/81	Hypertension, metastatic CA colon	Infected tumor	Cefuroxime + metronidazole		Responded but died 2 mo later

^a M, male; F, female.

^b CA, carcinoma of; CVA, cerebrovascular accident; ESRF, end-stage renal failure; GIT, gastrointestinal tract; HD, hemodialysis; IO, intestinal obstruction; OA, osteoarthritis of; TB, tuberculous.

hongkongensis type strain HKU16^T (GenBank accession no. AY574991), a 20.7% difference from that of *Atopobium minutum* (GenBank accession no. X67148), a 21.6% difference from that of *Eggerthella lenta* (GenBank accession no. AF292375), a 23% difference from that of *Bifidobacterium dentium* (GenBank accession no. D86183), a 21.8% difference from that of *Propionibacterium acnes* (GenBank accession no. AB097215), and a 22.7% difference from that of *Actinomyces odontolyticus* (GenBank accession no. AJ234047). The phylogenetic positions of the five isolates were in line with that of *C. hongkongensis* type strain HKU16^T as described previously (11).

DISCUSSION

Despite several reports describing the detection of C. hongkongensis in environmental sources, only one additional case of human infection was reported since its first description (Table 2) (3, 11). Apart from the five previously reported cases, a sequence from a ruminococcus-like clinical isolate, CCUG 37327, has been deposited in GenBank (accession no. AJ318864). It is most likely that this isolate is also C. hongkongensis, although its true identity has not been validated (11). In this report, we describe five additional cases of C. hongkongensis bacteremia. Although only one set of blood cultures was performed during the episodes of bacteremia in the present patients, the bacterium was always isolated in pure culture, which was associated with evidence of clinical sepsis with a systemic response. Similar to the earlier report (11), fever and leukocytosis were common. This supported that the C. hongkongensis isolates were clinically significant. Of the total of 10 reported cases, eight patients had underlying diseases and the other two

cases occurred in previously healthy young individuals with perforated acute appendicitis. Six patients presented with infections associated with either the gastrointestinal or biliary tract. Three of the previous five cases recovered with appropriate treatment, while one with terminal malignancy and the other who presented with intestinal perforation died (3, 11). Of the present five cases, three patients with gastrointestinal malignancy died within 2 months of admission. Although the other two patients survived, both had prolonged hospitalization, and the young patient with acute appendicitis had postoperative complications. These findings suggest that C. hongkongensis bacteremia may be associated with a poor prognosis, especially in patients with advanced malignancies. C. hongkongensis is susceptible to metronidazole but may exhibit variable susceptibility to penicillin (3, 11). Patients who recovered from C. hongkongensis infections have responded to intravenous cefuroxime-metronidazole combinations, and one responded to oral ciprofloxacin (11). Further studies in other countries may reveal more cases of C. hongkongensis infections and help in understanding its epidemiology and pathogenicity.

Similarly to other nonsporulating anaerobic Gram-positive bacilli, C. hongkongensis may be part of the human gut flora. As most cases of C. hongkongensis bacteremia have occurred in patients with underlying diseases in or infections associated with the gastrointestinal or biliary tract, the source was most likely the gastrointestinal tract. Since its first report, 16S rRNA gene sequences related to C. hongkongensis have also been detected in various environmental samples. In a study on urban aerosols collected in the United States, 16S rRNA gene sequences belonging to "class Catabacter" were detected, although the degree of sequence similarity was not mentioned (1). Similar findings have been obtained in mangrove sediment in China (16). In a study from Japan, a 16S rRNA gene sequence with 93% sequence identity to that of C. hongkongensis has also been detected among microbial communities from rice paddy field soil (6). However, it remains to be determined if C. hongkongensis or other, undescribed Catabacter species were present in these environments. Nevertheless, in a recent study on the fecal microflora of a dugong (Dugong dugong), an aquatic herbivorous mammal, in Japan, a 16S rRNA gene sequence clone that possessed 100% identity with C. hongkongensis was identified (23). This suggests that C. hongkongensis may be a gut commensal in this animal. Further epidemiological studies are required to understand the reservoir of C. hongkongensis.

The rarity of reports of C. hongkongensis infections may be a reflection of its fasitidious growth and difficulties in accurately identifying anaerobic Gram-positive bacilli. Traditional methods for identification of these bacteria, such as analysis of cell wall fatty acids and metabolic end products by gas-liquid chromatography, which require special equipment and expertise, are often not available in clinical microbiology laboratories, and therefore such data for C. hongkongensis are currently lacking. Commercially available identification systems are also associated with problems when used for these bacteria (14, 27), and they do not include the recently described species in their databases. As a result, these bacteria are poorly identified in clinical laboratories, often not even to the genus level. Isolation of a catalase-positive, motile, nonsporulating, anaerobic Gram-positive bacillus or coccobacillus that is negative for indole production and nitrate reduction but positive for arabinose, glucose, mannose, and xylose fermentation should raise the suspicion of C. hongkongensis, and the isolate should be subject to molecular identification. As 16S rRNA gene

Description of *Catabacter* gen. nov. *Catabacter* (Ca.ta.bac'ter. Arbitrary name. N.L. cata- [abbreviation], catalase positive, derived from Gr. *kata*, down; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Catabacter*, catalase positive rod).

Cells are obligately anaerobic, Gram-positive coccobacilli or straight bacilli. It does not produce spores. It is motile with flagella (11). It produces catalase. The G+C content of the DNA of the type strain HKU16^T of the type species is 40.2 mol% (11).

The type species is *Catabacter hongkongensis*, a member of the order *Clostridiales*, phylum *Firmicutes*, according to 16S rRNA gene analysis (11).

Description of *Catabacter hongkongensis* **sp. nov.** *Catabacter hongkongensis* (hong.kong.en'sis. N.L. fem. adj. in honor of Hong Kong, the place where the type strain was isolated).

The bacterium displays the following characteristics in addition to those listed in the genus description. Cells are approximately 0.5 to 1.5 μ m in length and 0.3 to 0.6 μ m in diameter (11). It grows on sheep blood agar as nonhemolytic, pinpoint, grayish colonies after 48 h of incubation at 37°C in an anaerobic environment. It does not grow in an aerobic or microaerophilic environment. It produces acid from arabinose, glucose, mannose, and xylose but does not produce indole or reduce nitrate. In the API 20A system, reactions are as follows: positive reactions are production of acid from arabinose, glucose, mannose, and xylose; negative reactions are hydrolysis of gelatin, production of indole and urease, production of acid from cellobiose, lactose, maltose, mannitol, melezitose, raffinose, salicin, sorbitol, sucrose, and trehalose; and positive or negative reactions are hydrolysis of esculin and production of acid from glycerol and rhamnose. The bacterium is susceptible to metronidazole (MIC, $<0.016 \mu g/ml$) and variably resistant to penicillin (MIC range, <0.016 µg/ml to 4 μ g/ml) (11). The type strain, HKU16^T (= CCUG 54229^T = JCM 17853), was isolated from the blood culture of a patient with intestinal obstruction and sepsis in Hong Kong, China (11).

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