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Catabacter hongkongensis bacteremia identified by direct metagenomic sequencing of positive blood culture fluid, first case report in the US

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ABSTRACT

Catabacter hongkongensis, an increasingly recognized bacteria in clinical samples, was identified by direct metagenomic sequencing of positive blood culture fluid from a 55-year-old patient with colonic perforation. The bacteremia was cleared by both antibiotic treatment and surgical intervention. This is the first case report of *C. hongkongensis* infection in the US.

1. Introduction

Catabacter hongkongensis is a strictly anaerobic, catalase-positive, motile, non-sporulating, gram-positive coccobacillus (GPCB) [1]. It is a causative etiology of sepsis, most commonly associated with intestinal or biliary sepsis such as perforated bowel and acute appendicitis [1–3]. First identified from blood cultures in Hong Kong and Canada in 2007, it remains rare and has only been described in a handful of patients in France, Italy, Scandinavia, New Zealand and South Korea [1,4–8]. It has also been isolated in environmental samples including water in Spain [9]. Clinical importance of this bacteria may be underrecognized due to the difficulty of identification by conventional methods since definitive identification requires sequencing [1,2]. We present the first case of a patient with sepsis in the US due to *C. hongkongensis* identified by metagenomic sequencing directly from a positive blood culture fluid.

2. Case description

A 55-year-old female with extensive medical history including subdural hematoma, pneumoperitoneum, gastrointestinal bleeding and colonic perforation after she underwent laparoscopic and subtotal colectomy a year ago presented to our hospital with acute abdominal pain, nausea and vomiting, and small bowel obstruction (SBO) as indicated by minimal stool output from colostomy for two days. She was admitted and treated for SBO with IV fluids and nasogastric tube for decompression with no improvement in symptoms, which was complicated by peritonitis and intra-abdominal fluid collection. Fig. 1 shows a summary of key events and antimicrobial treatment during her hospital stay. On hospital day 7, she was taken to operating room for colostomy takedown, right hemicolectomy, and ileostomy creation. Blood was collected from a central line for cultures on day of surgery, and on subsequent days (day 10, 11, 17 and 20). Her WBC count were within normal range until four days post-surgery, when it became elevated. She was put on IV antibiotics (vancomycin, metronidazole, and cefepime) on day 7, but with no symptom improvement. Due to intra-abdominal fluid collection, she underwent fluid drainage (on hospital day 14 and 19), which helped improve her symptoms. The patient continued to have leukocytosis despite being afebrile. On hospital day 20, the IV antibiotics were broadened to meropenem and caspofungin, in addition to vancomycin after the second abdominal fluid grew pan-sensitive *Pseudomonas aeruginosa* and *Clostridium clostridioforme*. On hospital day 30, 2 days before being discharged, repeat CT scan showed improvement in fluid collections with decreased bowel wall edema, WBC returned within

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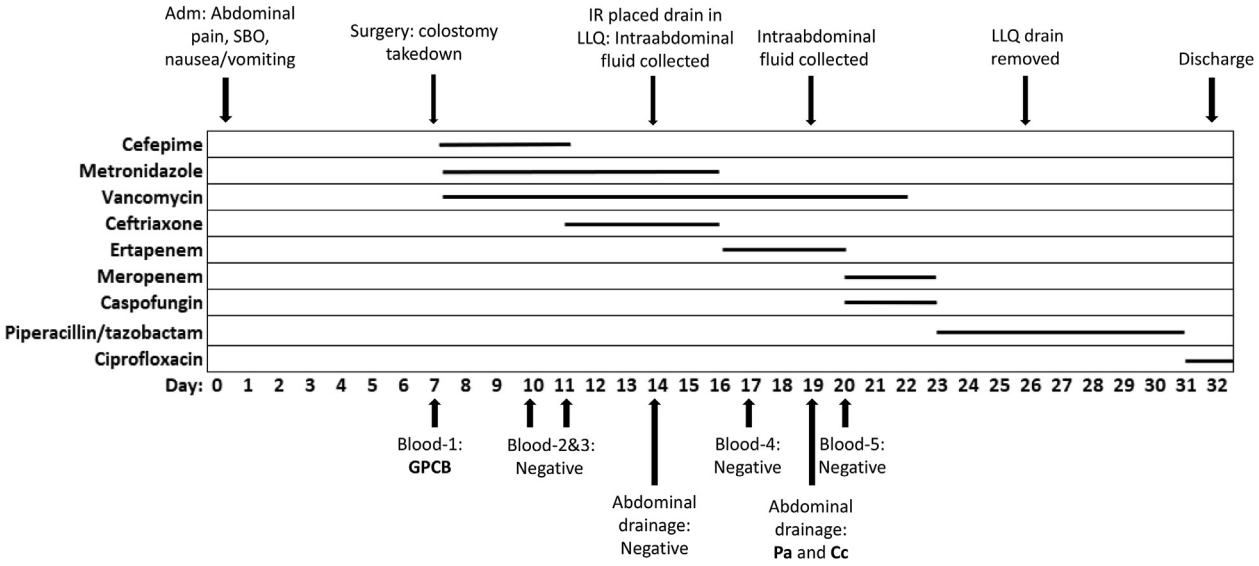

Fig. 1. Key clinical events and microbiological findings during the hospitalization. Adm: admission; IR: Interventional Radiology; LLQ: Left Lower Quadrant; GPCB: gram-positive coccobacillus; Pa: *Pseudomonas aeruginosa*; Cc: *Clostridium clostridioforme*. The anaerobic blood bottle collected on hospital day 7 grew GPCB and abdominal drainage collected on hospital day 19 grew Pa and Cc.

normal range and she was tolerating regular diet with good ostomy output.

A total of five sets of blood bottles (aerobic and anaerobic) were collected during the hospital stay (Fig. 1). Only one anaerobic bottle (BACTEC FX anaerobic/F lytic, Becton Dickinson, MD) collected on the day of surgery (hospital day 7) grew GPCB, after 4 days of incubation. Non-hemolytic pinpoint colonies grew on Brucella blood agar plates (Becton Dickinson, MD) after 96h in strict anaerobic condition and incubated at 37 °C. The isolate was catalase positive, bile susceptible, nitrate negative and had rapid ANA II system (San Diego, CA, USA) profile of 040,401, which generated unidentifiable result. Antimicrobial susceptibility tests were performed using Etests (BioMérieux, France) on Brucella agar plated with a suspension prepared to 1 McFarland in pre-reduced Mueller Hinton broth and incubated for 48 hours. The results were interpreted according to the CLSI criteria. Antimicrobial drugs tested by Etest included clindamycin (1 μg/mL; susceptible); metronidazole (0.023 μg/mL; susceptible), meropenem (>32 μg/mL; resistant); and penicillin (1.5 μg/mL; resistant). Special potency disk test showed the isolate was sensitive to vancomycin (5 μg) but resistant to colistin (10 μg) and kanamycin (1000 μg). Attempt to identify the bacteria using the MALDI-TOF VITEK MS system with v3.2 FDA approved database (BioMérieux) was unsuccessful.

Metagenomic sequencing was performed directly from the blood culture fluid for a definitive bacterial identification using a laboratory developed test (LDT) based on next-generation sequencing (NGS), which is performed routinely in the clinical laboratory for isolates that cannot be identified by conventional methods and MALDI-TOF MS. Briefly, 200 μL of blood culture were obtained and the DNA extracted using Qiagen EZ1 tissue kit per manufacturer's recommendation (Qiagen, CA, USA). Sequencing was performed on Miseq (Illumina, CA USA) using 2 × 250bp standard protocol per manufacturer's recommendation. Metagenomics analysis was performed using IDseq (https://idseq.net), CLC genomic workbench (Qiagen, CA, USA), Geneious Prime software (Biomatters, New Zealand), and PlasmidFinder on Center for Genomic Epidemiology (CGE) web tools (https://cge.cbs.dtu.dk/services/PlasmidFinder/).

The NGS data of UCLA549 were uploaded to IDSeq for the initial analysis, which revealed *Catabacter hongkongensis* as the top result with pairwise identity (PI) of 99.5 %, followed by *Christensenella minuta* which had a lower overall score and PI of 93.5 %. Mapping of the UCLA549 sequences to *C. hongkongensis* 16S rRNA (accession LT223646.1) and BLAST analysis against NCBI nucleotide database (https://blast.ncbi.nlm.nih.gov) produced 100 % query coverage and PI. *K-mer* analysis was used to construct a phylogenetic tree comparing UCLA549 to *C. hongkongensis* and *C. minuta* genomes. We used *Clostridium* and *Eubacterium* as outgroups. UCLA549 and *C. hongkongensis* strains were clustered together in the same branch of the phylogeny, with *C. hongkongensis* strain DSM 18959 as the most closely related strain (Fig. 2). We then performed SNP analysis using *C. hongkongensis* strain HKU16 genome as the reference. Only 34 SNP differences between UCLA549 and *C. hongkongensis* strain DSM 18959 were identified, confirming the close genetic relatedness of these two strains. Of note, there were >800 SNP difference between UCLA549 and *C. minuta* strains DSM 22607 and MGYG-HGUT-02523, further confirming *C. minuta* is not the correct species of our isolate.

Antimicrobial resistance (AMR) analysis using CLC microbial suite, CARD database revealed UCLA549 carried *tet*32, a tetracycline resistance gene, which was consistent with the phenotypic result. There were no plasmids detected by PlasmidFinder. However, it is important to note that PlasmidFinder database contains plasmids mainly found in Enterobacterales and may not have all plasmids that might be present in anaerobes.

3. Discussion

Although *C. hongkongensis* bacteremia is associated with high mortality, only a few cases have been reported and most of these patients had underlying conditions in the gastrointestinal (GI) tract. To date, there have been 14 cases reported including ours, in which 9 (64 %) patients recovered. Patients who did not recover tended to be older and have GI malignancy or intestinal perforation [2,4]. Most importantly, similarly to our case, treatment in most patients resulted in rapid bacterial clearance. For those who did not recover, it was not clear whether their demise was mainly due to bacterial infection or co-morbidities [2,6,7]. Interestingly, one Scandinavian

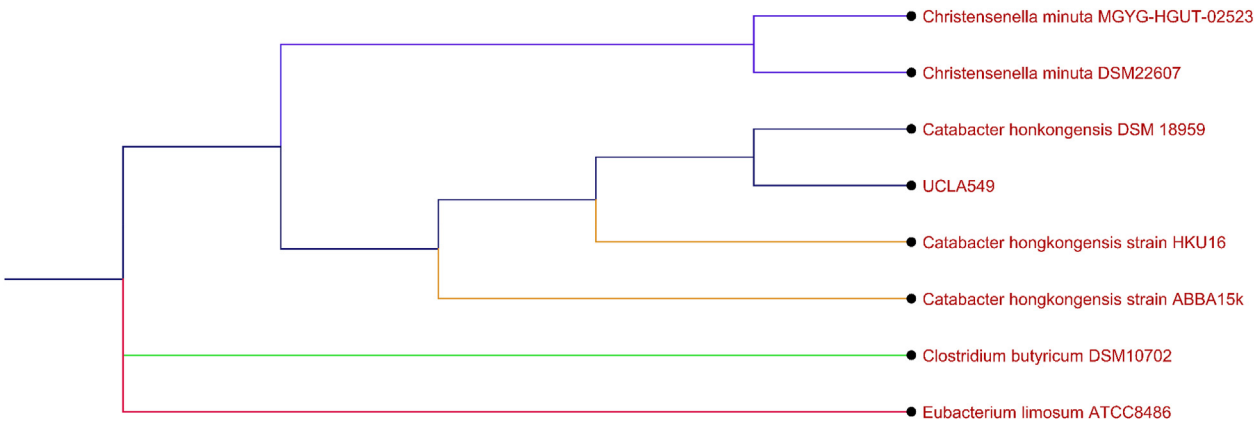

Fig. 2. *K-mer* tree showed UCLA549 is most closely related to *C. hongkongensis* strain DSM 18959. A *K-mer* tree was constructed using three strains of *C. hongkongensis* (strain ABBA 15k, assembly ID GCA_001507385.1; strain DSM 18959, assembly ID GCA_004342745.1; and strain HKU16, assembly ID GCA_000981035.1) and two strains of *C. minuta* genomes (strain DSM 22606 accession SRR5260860; and strain MGYG-HGUT_025,323 accession ERS3640553). *Clostridium* and *Eubacterium* were used as outgroups.

patient recovered from *C. hongkongensis* bacteremia without antibiotic treatment [8]. The clinical symptoms in our patient did not improve with antibiotic treatment alone until after intra-abdominal fluid was drained. This indicates *C. hongkongensis* may have contributed to her illness but is less likely a direct cause for her symptoms. Indeed, the presence of *C. hongkongensis* in the blood likely indicates a transient bacteremia due to intestinal perforation and GI surgeries in some cases, which seems to be cleared quickly with metronidazole-based combination therapy as observed in our case and other cases [6].

Based on the phenotypic tests, our isolate was susceptible to metronidazole, which was part of the initial empirical treatment for our patient. The isolate had similar key phenotypic characteristics and antibiotic profile as those previously reported for *C. hongkongensis* [2,4]. However, with exception of vancomycin and metronidazole, *C. hongkongensis* isolates have demonstrate variable susceptibility to some of the drugs tested [2–7]. For example, our isolate was found to carry *tet*32, a ribosomal protection type tetracycline resistance gene, which is also present in *C. hongkongensis* strains HKU16 and DSM 18959, but not in ABBA 15k [10].

We used a number of different genetic analysis to confirm that our isolate was *C. hongkongensis*. Based on the SNP analysis, UCLA549 was highly identical to *C. hongkongensis* strain DSM 18959 with only 34 SNP difference in a genome of about 3 million base pairs. Generally, the 16S rRNA gene sequence identity of 95 % and 98.7 % is used to delineate bacterial genus and species, respectively [10]. Currently, *C. hongkongensis* belongs to family *Catabacteriaceae* in the order *Clostridiales*, but its 16S rRNA gene sequence identify is 96–97 % similar to that of *Christensenella* species in the family *Christensenellaceae*, supporting these two species belong to the same genus [10]. Based on whole genome comparisons, *Christensenella* and *Catabacter* were recently re-classified to the same family *Christensenellaceae*, within a new order *Christensenellales* as shown in the Genome Taxonomy Database [11]. A name change of *Catabacter hongkongensis* to *Christensenella hongkongensis* comb. nov. was proposed [10]. *Christensenellaceae* has recently garnered interest as part of human gut microbiome that is widely prevalent, heritable and associated with unfavorable health conditions such as obesity and inflammatory bowel disease [12]. Additional studies will provide new insight regarding the role *C. hongkongensis* in human health and disease.

The initial attempt to subculture the blood bottle after it turned positive failed after 5 days of incubation due to the fastidious nature of this bacteria which required strict anaerobic conditions. In the second attempt, it took 96h for the bacteria to grow on solid medium but the MALDI-TOF MS system used in this study (Vitek MS) failed to identify it because the spectra for this organism is not in the database. To acquire a definitive species identification to help with accurate diagnosis, we sequenced the bacteria directly from blood bottle utilizing a NGS test routinely performed in our laboratory for microbial identification. With this metagenomics approach, not only were we able to provide the definitive identification, we were also able to provided certain level of genomic AMR profile that could potentially be helpful in guiding treatment. Notable, in this case, the metagenomics results became available after the patient was discharged, and thus did not actually impact the clinical management. It is also important to point out that MALDI-TOF MS may still be able to detect *C. hongkongensis* if the microbial database is updated to include this species.

In summary, we reported the first case of *C. hongkongensis* bacteremia associated with colonic perforation in the US. The patient recovered with both antibiotic treatment and surgical drainage of intra-abdominal fluid. The *C. hongkongensis* strain isolated showed similar phenotypic characteristics and antibiotic susceptibility profile as those previously published [2–7]. The importance of *C. hongkongensis* in health and disease is yet to be defined and requires further studies. The re-classification of its taxonomy may continue to pose challenge for its correct species identification in the clinical setting. We also demonstrated a proof of concept for the clinical utility of direct metagenomic sequencing of positive blood fluid for definitive identification of fastidious bacteria that are challenging to grow and cannot be identified using conventional methods or MALDI-TOF MS.

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References

[1] S.K. Lau, A. McNabb, G.K. Woo, L. Hoang, A.M. Fung, L.M. Chung, P.C. Woo, K.Y. Yuen, Catabacter hongkongensis gen. nov., sp. nov., isolated from blood cultures of patients from Hong Kong and Canada, J Clin Microbiol 45 (2007) 395–401, https://doi.org/10.1128/JCM.01831-06.

[2] S.K. Lau, R.Y. Fan, H.W. Lo, R.H. Ng, S.S. Wong, I.W. Li, A.K. Wu, K.H. Ng, S. Tseung, R.A. Lee, K.S. Fung, T.L. Que, K.Y. Yuen, P.C. Woo, High mortality associated with Catabacter hongkongensis bacteremia, J Clin Microbiol 50 (2012) 2239–2243, https://doi.org/10.1128/JCM.00128-12.

[3] S.K.P. Lau, J.L.L. Teng, Y. Huang, S.O.T. Curreem, S.K.W. Tsui, P.C.Y. Woo, Draft genome sequence of Catabacter hongkongensis type strain HKU16T, isolated

from a patient with bacteremia and intestinal obstruction, Genome Announc 3 (3) (2015), https://doi.org/10.1128/genomeA.00531-15 e00531-15.
[4] Y.J. Choi, E.J. Won, S.H. Kim, M.G. Shin, J.H. Shin, S.P. Suh, First case report of bacteremia due to Catabacter hongkongensis in a Korean patient, Ann Lab Med 37 (2017) 84–87, https://doi.org/10.3343/alm.2017.37.1.84.
[5] A. Torri, F. Delbianco, F.D. Baccarini, M. Fusari, S. Bertini, F. Congestrì, et al., First report of sepsis due to Catabacter hongkongensis in an Italian patient, New Microbes New Infect 9 (2015) 54–55.
[6] A. Elsendoorn, R. Robert, A. Culos, F. Roblot, C. Burucoa, Catabacter hongkongensis bacteremia with fatal septic shock, Emerg Infect Dis 17 (2011) 1330–1331, https://doi.org/10.3201/eid1707.101773.
[7] K. Smith, S.K. Pandey, J.E. Ussher, Bacteraemia caused by Catabacter hongkongensis, Anaerobe 18 (2012) 366–368, https://doi.org/10.1016/j.anaerobe.2012.03.006.
[8] R. Kaden, M. Thelander, L. Engstrand, B. Herrmann, First case of human bacteraemia by Catabacter hongkongensis in Scandinavia, New Microbes New Infect 15 (2016 Oct 4) 6–8, https://doi.org/10.1016/j.nmni.2016.09.015. PMID: 27830080; PMCID: PMC5094672.
[9] F. Codony, B. Adrados, L.M. Pérez, M. Fittipaldi, J. Morató, Detection of Catabacter hongkongensis in polluted European water samples, J Zhejiang Univ Sci B 10 (12) (2009 Dec) 867–869, https://doi.org/10.1631/jzus.B0920218. PMID: 19946949; PMCID: PMC2789520.
[10] X. Liu, J.L. Sutter, J. de la Cuesta-Zuluaga, J.L. Waters, N.D. Youngblut, R.E. Ley, Reclassification of *Catabacter hongkongensis* as *Christensenella hongkongensis* comb. nov. based on whole genome analysis, Int J Syst Evol Microbiol 71 (4) (2021 Apr), https://doi.org/10.1099/ijsem.0.004774. PMID: 33881979.
[11] D.H. Parks, M. Chuvochina, D.W. Waite, C. Rinke, A. Skarshewski, P.A. Chaumeil, P. Hugenholtz, A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life, Nat Biotechnol 36 (10) (2018 Nov) 996–1004, https://doi.org/10.1038/nbt.4229. Epub 2018 Aug 27. PMID: 30148503.
[12] J.L. Waters, R.E. Ley, The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health, BMC Biol 17 (1) (2019 Oct 28) 83, https://doi.org/10.1186/s12915-019-0699-4. PMID: 31660948; PMCID: PMC6819567.