

First case of human bacteraemia by *Catabacter hongkongensis* in Scandinavia

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Abstract

Catabacter hongkongensis was isolated and cultured from human blood for the first time in Scandinavia. The patient, an 83-year-old man from Dalarna, Sweden, recovered without antibiotic treatment, although a high mortality rate associated with *C. hongkongensis* infection had been reported from China, Canada and France. The genome of the strain ABBA15k was sequenced, assembled and analysed. In contrast to the type strain of the species HKUI6^T, no antibiotic resistance was observed in Scandinavian strain ABBA15k. The strain was deposited as CCUG 68271, and the draft genome sequence is available from the DNA Data Bank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank under the accession number LLYX00000000.

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Introduction

Catabacter hongkongensis was isolated for the first time by Lau et al. [1] in Hong Kong in 2007. This bacterial species was also

detected in human specimens in British Columbia, Canada, in 2012 [2], and in Poitiers, France, in 2011 [3]. The isolation of *C. hongkongensis* in clinical laboratories is rare, and no case reports have been published after 2012. *C. hongkongensis* is supposed to be a human pathogen, with high mortality reported among patients with underlying disease [2]. Nothing is known about the transmission of this species, and little is known about its environmental occurrence. Codony et al. [4] detected 16S rRNA gene fragments of *C. hongkongensis* in three samples from wastewater inlets in Barcelona, Spain. This example might suggest undetected spreading of this species. *C. hongkongensis* probably remains undetected in many laboratory samples because this species grows very slowly and requires an anaerobic environment.

We report a case of human bacteraemia caused by *Catabacter hongkongensis* included with its genetic characterization based on genome sequencing. To our knowledge, the described case is the second case of a human bacteraemia in Europe and the first case of *C. hongkongensis* in Scandinavia.

Case Description

The patient was an 83-year-old man treated with acetylsalicylic acid for a prior transient ischaemic attack. He sought care in the emergency ward for sudden onset of chills and fever. There were no focal infection symptoms from the respiratory tract, urinary tract, stomach, intestine or skin. His general condition was fair, but his temperature was 39.6°C. No lymph nodes were palpable. Heart and lung sounds were normal, and the blood pressure was 150/60 mm Hg. The leukocyte count (white blood cell count) was $6.5 \times 10^9/L$. C-reactive protein was 58 mg/L and increased to 72 mg/L 4 hours later.

The patient was assessed as having a viral infection and was sent home without antibiotics. Three days later he was tired but had no fever, and 4 weeks later he was completely recovered. No antibiotic treatment was provided during the *Catabacter* infection episode.

Methods

Growth was detected after 80.5 hours (BACTEC 9240, Becton Dickinson [BD], San Diego, CA, USA) in an anaerobic blood culture bottle. From the positive bottle 1.5 mL of blood/blood culture broth was aspirated into an S-Monovette tube, and 10 µL from the S-Monovette tube was then inoculated onto agar plates. Gram staining was performed using PREVI Colour Gram (bioMérieux, Marcy l'Étoile, France) [5]. The bacteria were

incubated anaerobically on *Gonococcus* agar (BD) and on chocolate agar at 37°C. The results from matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) were inconclusive. Coincidentally, an undescribed *Clostridium* species was found in the same blood culture bottle from the patient.

The DNA of the grown bacteria was extracted using a MagNA Pure compact (Roche, Basel, Switzerland) according to the manufacturer's protocol. The libraries for whole-genome sequencing were prepared with a Nextera XT sample preparation kit. An Illumina HiSeq platform with a 2 × 100 paired end run was used for whole-genome sequencing (Illumina, San Diego, CA, USA). The single reads were assembled to contigs with Velvet [6]. The assembling of merging contigs was done with Geneious R8 [7]. GEGENES 2.0 software with a threshold of 20% was applied for determination of the average nucleotide identity with the type strain of the species HKU16^T [8]. The genome was annotated by the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline with GeneMarkS+, best-placed reference protein set method. Tandem repeat analysis was realized using Tandem Repeats Finder [9]. The antibiotic resistance genes were predicted using ResFinder 2.1 [10], and the species determination was accomplished by NCBI Basic Local Alignment Search Tool (BLAST) [11].

Results and Discussion

Phenotypic and genetic strain characteristics

Gram-positive coccobacilli grew after an incubation period of 4 days. The colonies appeared smooth and glossy with a maximum diameter of 0.5 mm. The strain was determined with 100% 16S rRNA gene identity as *Catabacter hongkongensis*.

The draft genome sequence of the Scandinavian *C. hongkongensis* strain ABBA15k differs by 3.5% to the type strain of the species *C. hongkongensis*, HKU16^T. The G+C contents are 48.8% and 48.5%, respectively. The 28 tandem repeats that were detected had a length of 12 to 45 bases with a repeat number of 1 to 5.

The genome of strain ABBA15k consists of 2 797 114 bases with 2516 coding sequences (CDSs). Three prokaryotic rRNA types—5S, 16S and 23S—with a total number of five operons and one noncoding RNA as well as 49 tRNA operons exist.

In total, there are 2645 genes and 74 pseudo genes present in the genome. The type strain of the species *Catabacter hongkongensis* HKU16^T consists of 3161 CDSs [12]. The majority of the missing genes in strain ABBA15k were predicted as hypothetical proteins with unknown function.

Some genes coding for bacterial chemotaxis (*cheA*) and flagellar assembly (*flhA*, *MotA*) which were detected in strain

HKU16^T were not present in the genome of strain ABBA15k. In contrast to the type strain of the species, strain ABBA15k is not motile and has no flagella because it lacks genes *flhA* and *MotA*.

Genes that code for the multidrug ABC transporter ATP binding protein (NCBI WP_046442587) as well as genes coding for cationic antimicrobial peptide resistance (*amiA*, *amiC*) were absent in the genome of strain ABBA15k. The tetracycline resistance gene *tet(32)* was detected in the genome of *Catabacter hongkongensis* HKU16^T on position 698 906 to 700 825, which corresponds to 1920 bases with an identity of 100%. Furthermore, cefotaxime resistance was reported for strain HKU16^T [2]. No resistance genes were detected in the genome of strain ABBA15k. Read mapping with the complete cds of the *tet(32)* gene (NCBI accession no. EF626943) as reference genome confirmed the absence of the tetracycline resistance gene in strain ABBA15k.

Medical strain characteristics

Catabacter hongkongensis strain ABBA15k might be less pathogenic compared to the strains that were described from Asia, Canada and France. In these studies five out of ten patients died during the *Catabacter* infection, although they all had underlying diseases [1–3]. The patient in our study never received any antibiotic treatment but recovered completely. Although he was elderly, he was in good condition, and there was growth in only one out of four blood culture bottles. According to recommended guidelines, blood cultures were taken from two separate venipuncture sites. The likelihood of contamination being the cause of growth is low, even if only one blood culture bottle was positive. As far as we know, *Catabacter* does not belong to the normal human skin flora and has not previously been detected anywhere in Scandinavia.

Because the patient in our report did not show any focal signs or symptoms, it is difficult to draw conclusions about the source of the bacteraemia.

Perspectives

The natural reservoir and transmission route of *Catabacter hongkongensis* are still unknown. The occurrence of *C. hongkongensis* in Scandinavia leads to the necessity of considering this species in cases of bacteraemia even outside of China and Canada in the future. As a result of the high mortality reported in the French, Chinese and Canadian cases, we recommend the implementation of diagnostic methods such as cultivation followed by a species-specific PCR [4] for the detection of *C. hongkongensis*. In the case we describe here, the patient recovered completely without any antibiotic treatment. No resistance genes were detected in the described Scandinavian strain. In contrast to this observation, strain HKU16^T is tetracycline and cefotaxime resistant.

Accession numbers

Catabacter hongkongensis strain ABBA15k was deposited in the Culture Collection of the University of Gothenburg under deposition number CCUG 68271.

The draft genome of *Catabacter hongkongensis* strain ABBA15k was deposited at the DNA Data Bank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL) and GenBank under accession number LLYX00000000. The version we describe here belongs to NCBI Bioproject PRJNA299543 and NCBI Biosample SAMN04209960.

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Conflict of Interest

None declared.

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