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# Environmental surveillance for *Laribacter hongkongensis*, a diarrhoeal pathogen discovered in Hong Kong

## Key Messages

1. *Laribacter hongkongensis* was isolated from the midguts and hindguts of 86 (24%) of 360 freshwater fish from retail markets, including grass carp (60%), bighead carp (53%), mud carp (25%), and large-mouth bass (5%).
2. This study is the first to demonstrate the presence of *L hongkongensis* in natural water environments, with the bacterium being isolated from the waters of six reservoirs, with higher recovery rates in summer and during days of higher water and ambient temperatures.
3. Molecular typing using pulsed-field gel electrophoresis revealed a heterogenous population of *L hongkongensis* in both the freshwater fish and drinking water reservoir isolates, suggesting that the bacterium is endemic in our freshwater environments.
4. Since freshwater fish are common food items for our population, the general public should be educated on the proper preparation and thorough cooking of freshwater fish before consumption to avoid *L hongkongensis*-associated gastroenteritis.
5. Although it is unlikely that treated drinking water is a significant source of *L hongkongensis*-associated gastroenteritis, it is important to be aware of the possibility of other contaminated water as a source of human infection.

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## Introduction

*Laribacter hongkongensis*, a novel genus and species, was first isolated in Hong Kong in 2001 from the blood and empyaema pus of a 54-year-old Chinese man with alcoholic cirrhosis, bacteraemia and empyaema.<sup>1</sup> Phenotypically, it is a facultative anaerobic, motile, non-sporulating, urease-positive, Gram-negative, S-shaped bacillus. By phylogenetic analysis using 16S rRNA gene sequences, *L hongkongensis* belongs to the *Neisseriaceae* family of the  $\beta$ -subclass of *Proteobacteria*. It was subsequently discovered in the stools of six patients with community-acquired gastroenteritis in Hong Kong and Switzerland.<sup>2</sup> Using cefoperazone MacConkey agar as the selective medium,<sup>3</sup> we confirmed that *L hongkongensis* is associated with community-acquired gastroenteritis and traveller's diarrhoea.<sup>4</sup> Furthermore, it was confirmed that freshwater fish are a reservoir for *L hongkongensis*.<sup>4</sup> The isolation of *L hongkongensis* from patients who either resided in or had histories of recent travel to Asia, Europe, America, and Africa implies that the bacterium is of global importance.

## Methods

This study was conducted from December 2004 to November 2006.

### Study design

To determine the prevalence of *L hongkongensis* in the freshwater fish population in our locality, we carried out a territory-wide animal and environmental surveillance study of commonly consumed freshwater fish purchased from local retail markets. We also investigated the presence of *L hongkongensis* in samples from drinking water reservoirs in Hong Kong. Freshwater fish from these reservoirs were also sampled. All *L hongkongensis* isolates were typed by pulsed-field gel electrophoresis (PFGE) and the patterns analysed and compared.

### Sample size

A total of 360 freshwater fish from the six different species commonly purchased for cooking in Hong Kong were obtained from retail food markets (six fish per species per market) in different districts of Hong Kong. The fish were grass carp (*Ctenopharyngodon idellus*), bighead carp (*Aristichthys nobilis*), mud carp (*Cirrhina molitorella*), large-mouth bass (*Micropterus salmoides*), Chinese perch (*Siniperca chuatsi*) and tilapia (*Oreochromis mossambicus*).<sup>5,6</sup>

Water samples were collected from 10 drinking water reservoirs located in different regions of Hong Kong, namely Pok Fu Lam, Tai Tam, Aberdeen, High Island, Shing Mun, Kowloon, Shek Lei Pui, Tai Lam Chung, Plover Cove and Shek Pik reservoirs. Samples were collected every 3 months over a 1-year period (October 2003 to September 2004). Where possible, fish were also obtained from these reservoirs during the non-spawning season (October 2003 to March 2004).<sup>7</sup>

### Sampling methods and analysis

Samples were obtained from the midguts and hindguts of the fish using sterile cotton wool swabs. All samples were plated onto cefoperazone MacConkey agar and incubated in aerobic conditions at 37°C for 48 h. Water samples (in

volumes of 2000 mL) were collected in pre-sterilised 1000 mL bottles submerged to a depth of 50 cm. Detection of *L hongkongensis* was performed using the membrane filtration technique. Volumes of 100 mL were filtered through membrane filters which were incubated on CMA at 37°C for 48 h. A total of 2000 mL water was tested from each reservoir each time and the colony counts were expressed as colonies/L. All suspected bacterial isolates were identified phenotypically using standard biochemical methods. Isolates suspected to be *L hongkongensis* were subject to 16S ribosomal RNA gene sequencing.<sup>1</sup> Pulsed-field gel electrophoresis was performed using bacterial plugs digested with *SpeI* in 0.5× TBE buffer and the CHEF Mapper XA System (Bio-Rad Laboratories, Hercules [CA], US). Digital images were stored electronically as TIFF files and analysed visually and with GelCompar II (version 3.0; Applied Maths, Kortrijk, Belgium).

## Results

### *Isolation of Laribacter hongkongensis from retail freshwater fish*

*Laribacter hongkongensis* was isolated from the midguts and hindguts of 86 (24%) of 360 freshwater fish. It was isolated from 36 (60%) of 60 grass carp, 32 (53%) of 60 bighead carp, 15 (25%) of 60 mud carp, and three (5%) of 60 large-mouth bass. Overall, 67 different PFGE patterns were found in the 86 *L hongkongensis* isolates from freshwater fish.<sup>5</sup>

### *Isolation of Laribacter hongkongensis from water of reservoirs*

*Laribacter hongkongensis* was isolated from the waters of six (60%) of 10 drinking water reservoirs, with numbers ranging from 1 to 12 cfu/L. The numbers ranged from 0 to 1 cfu/L in autumn and winter, 0 to 9 cfu/L in spring and 0 to 12 cfu/L in summer. There was a significant difference in the mean numbers of *L hongkongensis* in reservoir waters in different seasons ( $P=0.046$ ). Higher numbers were observed in summer than in autumn ( $P\leq 0.04$ ) and winter ( $P=0.046$ ). There was a positive correlation between the numbers of *L hongkongensis* and the water temperature (Pearson correlation 0.379,  $P=0.016$ ), and between numbers and ambient temperature (Pearson correlation 0.39,  $P=0.013$ ). A total of 27 freshwater fish from 10 species were collected from six reservoirs, including 15 during the autumn and 12 during the winter. *Laribacter hongkongensis* was recovered from the intestines of two of the 27 fish, a Goldfish (GC2) and a Nile Tilapia (NT4), collected in autumn from Pok Fu Lam reservoir and Plover Cove reservoirs respectively. Overall, 35 different PFGE patterns were identified among the 59 isolates of *L hongkongensis* recovered from water and the two isolates from freshwater fish.<sup>7</sup>

## Discussion

This report demonstrates the existence of *L hongkongensis* in natural water environments. Its presence in the water

samples of six of the 10 sampled reservoirs suggests that the bacterium is prevalent in the drinking water reservoirs of Hong Kong, although the significance of *L hongkongensis* in freshwater environments is yet to be determined. Drinking water is subjected to purification procedures in advanced treatment plants before being distributed to residents. The risk of acquiring *L hongkongensis* from drinking tap water is therefore likely to be low, but the significance of other water-borne sources of *L hongkongensis* in humans warrants further investigation.

The presence of *L hongkongensis* in drinking water reservoirs is also likely to be related to the freshwater fish reared in the reservoirs. *Laribacter hongkongensis* was not detected in the water from the three reservoirs where water from Dongjiang is stored. This suggests that water from Dongjiang is probably not an important source of *L hongkongensis* in the reservoirs. The waters collected from local streams are mainly from rainfall and there are no notable contamination sources in the catchment areas. Therefore, the most likely source of the bacterium is the freshwater fish in the reservoirs.

Freshwater fish, especially carp, is probably the major reservoir for human infections with *L hongkongensis*. Molecular typing showed that a heterogeneous population of *L hongkongensis* was present in both freshwater fish and natural waters in Hong Kong, suggesting that the bacterium is endemic in our locality. It is probably also endemic in southern China, where over half of the patients reported in our previous study had recently travelled prior to developing *Laribacter* gastroenteritis. Since *L hongkongensis*-associated gastroenteritis is associated with eating fish and the bacterium can be found in diverse freshwater fish species; caution should be taken when handling and cooking any freshwater fish to prevent infections associated with *L hongkongensis* and other freshwater fish related pathogens.

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## References

1. Yuen KY, Woo PC, Teng JL, Leung KW, Wong MK, Lau SK. *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. *J Clin Microbiol* 2001;39:4227-32.
2. Woo PC, Kuhnert P, Burnens AP, et al. *Laribacter hongkongensis*: a potential cause of infectious diarrhea. *Diagn Microbiol Infect Dis* 2003;47:551-6.
3. Lau SK, Woo PC, Hui WT, et al. Use of cefoperazone MacConkey agar for selective isolation of *Laribacter hongkongensis*. *J Clin Microbiol* 2003;41:4839-41.
4. Woo PC, Lau SK, Teng JL, et al. Association of *Laribacter hongkongensis* in community-acquired gastroenteritis with travel and eating fish: a multicentre case-control study. *Lancet*

- 2004;363:1941-7.
5. Teng JL, Woo PC, Ma SS, et al. Ecoepidemiology of *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis. *J Clin Microbiol* 2005;43:919-22.
  6. Lau SK, Woo PC, Fan RY, Lee RC, Teng JL, Yuen KY. Seasonal and tissue distribution of *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis, in retail freshwater fish in Hong Kong. *Int J Food Microbiol* 2007;113:62-6.
  7. Lau SK, Woo PC, Fan RY, et al. Isolation of *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis, from drinking