

A new and potentially dangerous pathogen isolated in Hong Kong is suspected of causing severe gastroenteritis and traveller's diarrhoea. Here, Dr Richard A Collins looks at *Laribacter hongkongensis* and provides useful advice for clinical laboratory managers and microbiologists.

L. hongkongensis

A new foodborne pathogen?

In 2001, a new bacterium was isolated from the blood of a cirrhotic patient with community-acquired infection resulting in pus in the pleural space (empyema).¹ The bacterium was identified as a new genus and species and named *Laribacter hongkongensis* (*Laribacter* means seagull-shaped rod, *hongkongensis* because the bacterium was first isolated in Hong Kong). In the intervening period,

L. hongkongensis has been discovered in faecal specimens from patients with gastroenteritis.^{2,3} However, while studies have revealed the widespread nature of the new organism, its precise role as a major foodborne pathogen remains controversial. Therefore, this article examines the evidence for and against the role of *L. hongkongensis* in gastroenteritis.

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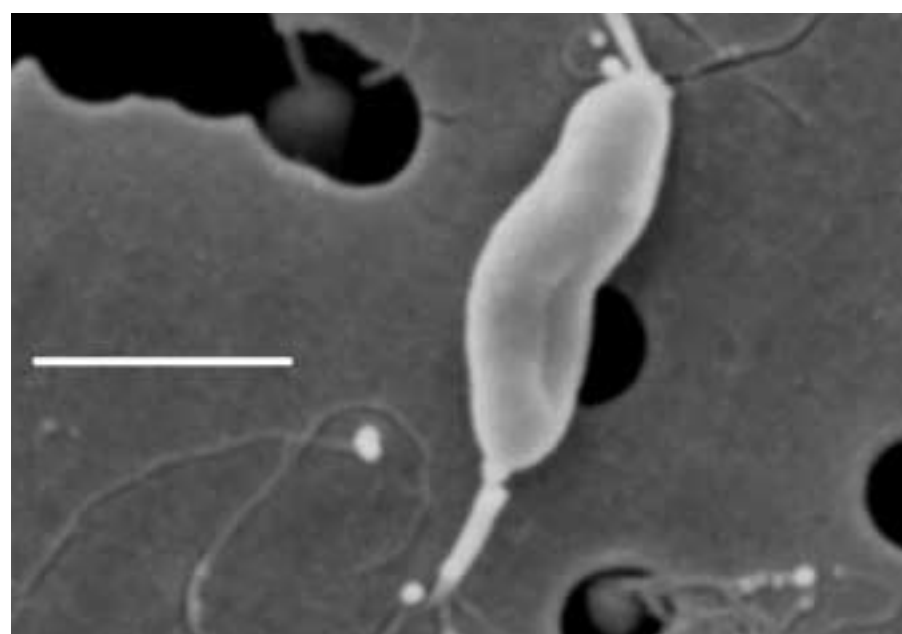


Fig 1. Scanning electron micrograph (SEM) of *L. hongkongensis*. Note the bipolar flagellae indicating that this isolate is not the type strain HKU1 (Bar = 1 µm).

Burden of disease

The most common bacteria implicated in outbreaks of foodborne illness are salmonellas, campylobacters, *Escherichia coli* 0157, *Listeria monocytogenes* and *Clostridium perfringens*.⁴ In the United States, foodborne diseases are estimated to cause 76 million illnesses, 325,000 hospitalisations and 5000 deaths each year.⁵ The most vulnerable in society to foodborne diseases are children, the elderly, pregnant women and those with a compromised immune system. Apart from human suffering, the economic costs of foodborne illness (eg medical expenses, lost productivity and recalls of contaminated food resulting in economic losses to industry) are considerable.

Properties of *L. hongkongensis*

The new bacterial strain (named HKU1) has a characteristic morphology, phenotype, genetic structure and biochemical profile.

Morphology

Under a scanning electron microscope (SEM) the bacterial cells are aflagellate, spiral, slender rods 0.8–2.5 µm long, which multiply by longitudinal division.

Phenotype

Strain HKU1 is a seagull-shaped, Gram-negative, facultatively anaerobic, non-spore-forming bacterium. It grows on sheep blood agar as non-haemolytic, grey colonies about 1 mm in diameter after 24-h incubation at 37 °C in ambient air. It can grow on 1–2% sodium chloride but not 3–5% sodium chloride. It is non-motile.

Genotype

The genome size as determined by pulsed-field gel electrophoresis (PFGE) is about 3 Mbp.¹ The G+C content is 68.0% + 2.43%. The 16S ribosomal RNA (rRNA) gene of *L. hongkongensis* has 91 base differences (6.2%)



Fig 2. Grass carp (*Ctenopharyngodon idellus*). This large freshwater fish is a popular food item in Hong Kong and Southern China and was a source of *L. hongkongensis* in a recent study.



Fig 3. A typical open-air fish market in Hong Kong. The fishmonger displays his skills by dissecting the fish so that the heart is still beating and the swim bladder is intact and inflated. The potential for cross-contamination with intestinal contents is obvious.

‘Sequencing the complete *L. hongkongensis* genome would clarify the genetic relationship with other organisms and identify potent new targets for therapeutic intervention’

compared with its closest known relative, *Microvirgula aerodentrificans*. Based on phylogenetic affiliation, HKU1 belongs to the *Neisseriaceae* family of the β -subclass of *Proteobacteria*.

Biochemistry

The HKU1 strain produces catalase, cytochrome oxidase, urease and arginine dihydrolase and it reduces nitrate. It does not ferment, oxidise or assimilate any sugar tested.¹ It is sensitive to a wide range of antibiotics but resistant to vancomycin, clindamycin, metronidazole and 0/129. As the bacterium is asaccharolytic but urease- and arginine dihydrolase-positive, it probably utilises proteins instead of carbohydrate as its source of energy.¹

Following initial isolation of the type strain, six other isolates were identified in the stools of patients with diarrhoea.² Unlike HKU1, all six were motile and had bipolar flagellae (Fig 1). Genomic analysis indicated that HKU1 and the six new *L. hongkongensis* isolates were genotypically unrelated strains. Each of the six new strains had a maximum of just two base differences from HKU1.²

Role of *L. hongkongensis* in gastroenteritis

Evidence for an association

L. hongkongensis was recovered from the stools of six patients with diarrhoea.² None of the stool samples was positive for enterohaemorrhagic *E. coli* or *Salmonella*, *Shigella*, *Vibrio*, *Aeromonas*, *Plestomonas* or *Campylobacter* species. In addition, rotavirus antigen detection, electron microscope examination for viruses and microscopic examination for ova and cysts were all negative.²

In a multicentre, case-controlled study, *L. hongkongensis* was recovered from 17 out of 3788 patients with community-acquired gastroenteritis over a four-month period.³ The bacterium was not recovered from 1894 controls. Of those who were culture-positive for *L. hongkongensis*, 10 (59%) had a recent history of travel compared with 2/34 (6%) matched controls. In addition, an association was observed with fish consumption (16/17 cases vs 19/34 controls) and of eating minced freshwater fish (5/17 cases vs 1/34 controls).

Further studies identified *L. hongkongensis*

in 25 samples of freshwater fish intestines and two from minced freshwater fish sold at retail markets in Hong Kong (Fig 2). In one case, PFGE pattern and ribotype of the bacterium recovered from one of the patients matched that found in a sample of minced freshwater fish obtained from the same market recently visited by the patient.

Evidence against an association

It should be noted that association does not mean causation. From the evidence presented by the team studying *L. hongkongensis*, two scenarios are equally valid: *L. hongkongensis* is a new enteric pathogen responsible for community-acquired gastroenteritis; or the occurrence of diarrhoea allows *L. hongkongensis* to colonise the intestine more readily, or allows it to survive better during passage through the intestine. In both cases, the bacterium would be present in the faeces.

If *L. hongkongensis* causes diarrhoea, high numbers of the bacterium should be present in the faeces during diarrhoea, with much lower numbers present after the diarrhoea has resolved.⁶ In addition, a rise in antibody titre to the patient's strain of *L. hongkongensis* and absence of antibodies to other commensal organisms in their faeces would also be expected. These analyses were not done in any of the studies reported in the literature.

A colony count of 10^6 – 10^9 *L. hongkongensis* per gram of faeces during acute diarrhoea and a four-fold (or more) increase in antibody titre would strongly suggest a causal role for the organism in diarrhoea. However, the studies reported to date, in which a highly selective growth media was used to isolate *L. hongkongensis*, show a colony count of only 10–100 organisms per gram of faeces. This strongly suggests colonisation or transient passage.

The failure to recognise *L. hongkongensis* from human stools in the past is probably due to a combination of misidentification and lack of an optimal selection medium. A special medium (cefoperazone MacConkey agar [CMA]) was developed to aid the identification and isolation of

L. hongkongensis.⁷ The CMA medium is prepared from MacConkey agar by the addition of 32 µg/mL cefoperazone. After isolation on charcoal cefoperazone deoxycholate agar (CCDA), several strains of *L. hongkongensis* were at first mistaken for *Campylobacter* species. After aerotolerance testing, however, all were found to grow in an aerobic environment. These strains would have been discarded as non-pathogens or wrongly reported as campylobacters in many clinical laboratories.

Prevention and treatment

In Chinese culture, consumption of undercooked freshwater fish is common, either as lightly-steamed whole fish or as raw minced fish added to hot rice porridge (congee). Flesh from the belly of the fish, which could easily be contaminated with intestinal contents, is especially prized (Fig 3). Cooking food thoroughly would reduce this potential source of contamination easily but would require a shift in culinary cultural attitudes.

Antibiotics are not required for patients with self-limiting gastrointestinal symptoms. For severely ill or immunocompromised patients, a quinolone and amoxicillin/clavulanate would be the antibiotics of choice in adults and children, respectively. Second- and third-generation cephalosporins, which are occasionally used to treat gastroenteritis associated with other

bacteria, are not expected to be useful.

Travellers to Hong Kong and Southern China in particular should be aware of the local culinary traditions surrounding fish dishes and the potential for foodborne contamination. Physicians and clinical laboratories should query the dietary and travel history of patients presenting with community-acquired gastroenteritis.

Future studies

Sequencing the complete *L. hongkongensis* genome would clarify the genetic relationship with other organisms and identify potent new targets for therapeutic intervention. Rapid diagnostic tests based on specific genomic regions could be developed. Epidemiological studies of community-acquired gastroenteritis would reveal the true global extent of the organism and the risk it presents as an enteric pathogen. ■

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