Case Report

Laribacter hongkongensis: The First Identification at Rajavithi Hospital

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Background: This is the first report of *Laribacter hongkongensis* that was isolated from the Rajavithi microbiology section at Rajavithi Hospital. *L. hongkongensis* is an emerging pathogen that causes bacteremia, community-acquired gastroenteritis and travel's diarrhea. *L. hongkongensis* is a gram-negative with seagull- or S-shaped rod, facultative anaerobic, non-fermentative bacillus. The distribution of *L. hongkongensis* had been described worldwide, but because of limitation of methods for accurate identification of *L. hongkongensis*, data reports about of them were few.

Objective: To present the phenotypic characteristics and antimicrobial susceptibilities of *L. hongkongensis* (LHRJ) strain.

Materials and Methods: *L. hongkongensis* had isolated from hemoculture of a Rajavithi's patient. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) method for *L. hongkongensis* identification, phenotypic characterization were described. The antimicrobial susceptibility of *L. hongkongensis* was determined.

Results: *L. hongkongensis,* Rajavithi (LHRJ) strain is motile, positive to urease, arginine dihydrolase, catalase, oxidase tests and reduce nitrate. The LHRJ strain was susceptible to most of antimicrobial agents, except ampicillin, cefotaxime, ceftriaxone, and ceftazidime.

Conclusion: *L. hongkongensis* is difficult to accurate identification by conventional biochemical method. Using the MALDI-TOF MS, which these bacteria database, has been identified accurately. However, some important characteristics of this bacterium have been reminded to microbiologists. The microbiology laboratory must be aware of accurate identification and antimicrobial susceptibility in the testing of *L. hongkongensis* isolates, especially in immuno-compromised patients, for appropriate treatment.

Keywords: *Laribacter hongkongensis*, MALDI-TOF MS, Antimicrobial susceptibility

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Laribacter hongkongensis is a gram-negative, facultative anaerobic, non-sporulating, seagull- or S-shaped bacillus and belongs to the family *Neisseriaceae* of the beta subclass of *Proteobacteria*[1]. *L. hongkongensis* is an emerging pathogen bacteremia, community-acquired gastroenteritis and traveler's diarrhea[1-7]. *L. hongkongensis* was first reported in Hong Kong isolated from blood and empyema pus of a 54-year-old Chinese man with alcoholic liver cirrhosis in 2001[1]. *L. hongkongensis* infections are global important pathogens, these isolations have been reported from a variety geographic areas such as Asia, Europe, Africa and Central America[6-8]. The most common isolates of *L. hongkongensis* are found in Asia[1-10].

The reservoirs of *L. hongkongensis* are freshwater fish and Chinese tiger[2-5]. The clinical syndrome of *L. hongkongensis* infections associated with gastroenteritis is similar to those of *Salmonella* or *Campylobacter*-infected patients[2]. Most patients have watery or bloody diarrhea and some of them may have systemic symptoms[6,7]. Fortunately, there are no reported deaths from *L. hongkongensis* infections.

Most *L. hongkongensis* strains have been found to be resist to most beta-lactams including ampicillin and cephalosporins and tetacycline, due to a chromosomal class C beta-lactamase and plasmid-encoded *tetA* genes[9,10]. All *L. hongkongensis* isolates were still susceptible to carbapenems[1,4,8,11,17]. However, there were some different antibiotic susceptibility profiles[1,4,8,11,17].

The prevalence, epidemiology and clinical pathology of *L. hongkongensis* infections have been unclear, because of limitations of *L. hongkongensis* identification in a microbiological laboratory. Although, *L. hongkongensis* isolate is a non-fastidious organism, easy to grow on blood agar and MacConkey agar in ambient temperature, it cannot correctly provide identification by conventional biochemical methods and automatic machines that do not show a database for *L. hongkongensis* strains[1,8]. Identification of

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L. hongkongensis is by using molecular, sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) methods, which are more accurate methods[1,8,12-14].

The aim of this report is to present the phenotypic characteristics and antimicrobial susceptibilities of the *L. hongkongensis* (LHRJ) strain.

Materials and Methods

Bacterial strain

At Rajavithi microbiology laboratory, the BD BACTEC™ FX (Becton Dickinson and Company, Sparks, MD) for hemoculture was used. On July 3, 2017, two aerobic-type hemoculture broth bottoms of blood specimens from a Rajavithi's patient a 38-year old Thai male, admitted to the neurosurgical ward, where he had been detected positive. Of them, were subcultured on chocolate agar (CA), sheep blood agar (SBA), and MacConkey (MAC) agar. Both CA and SBA have been incubated in 5% CO_2. All agar plates were incubated at 35°C for 24 h. There are bacterial colonies grow on CA, SBA, and MAC. The colony was identified by MALDI-TOF MS with phenotypic characteristics: colony morphology, Gram staining and conventional biochemical profile[15].

Bacterial identification

Identification by MALDI-TOF MS

The colony was identified by the MALDI-TOF MS was done on a Microflex LT instrument (Bruker Daltonics, Bremen, Germany) using the FlexControl 3.4 software (Bruker Daltonics) which of the maldibiotyper 3.1 (Referencelibrary version 4.0.0.1 software, containing 5,627 species). Briefly, the colony was picked, smeared on target, dried in room temperature, and extracted by a formic acid-acetonitrile[12].

Identification by phenotypic characteristic[15]

The colony was identified by Gram staining and conventional biochemical tests, including Kligler's iron agar (KIA), H_2S production, motility, indole, citrate utilization, malonate utilization, urease, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, oxidation/fermentation (O/F) of glucose and manitol, NaCl broth (0, 1, 3, 6, 8, 10% NaCl), esculin hydrolysis, nitrate reduction, catalase, oxidase tests.

Antimicrobial susceptibility testing (AST)

AST by disk diffusion method

Both of *L. hongkongensis* isolates were performed by the Kirby-Bauer disk diffusion method. Disks of 14 antimicrobial agents were used as follows: Beta-lactams including Penicillins, ampicillin (AM); Cephalosporins, cefotaxime (CTX), ceftriaxone (CRO) and ceftazidime (CAZ); Carbapenems; doripenem (DOR) and meropenem (MEM), Beta-lactam combination agents, amoxicillin-clavulanate (AMC), piperacillin-sulbactam (TZP), cefoperazole-sulbactam (SCF), refer to the manufacturer's instructions; Non-beta-lactams including Aminoglycosides; gentamicin (GM), and amikacin (AN); tetracycline (TE); chloramphenicol (C); Folate pathway inhibitors, trimethoprim-sulfamethoxazole (SXT). The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines for *Enterobacteriaceae*[16].

AST by MICs using the gradient diffusion method E-test

Both of isolates were performed by the minimal inhibitory concentrations (MICs) of 6 antimicrobial agents were used as followed: ceftriaxone (CRO) and ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), ciprofloxacin (CIP), and levofloxacin (LEV). The results of MICs breakpoints were interpreted according to CLSI guidelines for other non-*Enterobacteriaceae*[16].

Results

Using the MALDI-TOF MS, these bacterial colonies were identified that *L. hongkongensis*, with a scores ranging from 2.405-2.437.

The phenotypic characterization of *L. hongkongensis* (LHRJ) strain was determined followed by colony morphology, Gram straining, and biochemical tests. Figure 1 shows the colonies of *L. hongkongensis* grow on CA, SBA, and MAC at 35°C, in ambient condition, a colorless colony 1 mm in a diameter. The colonies are non-hemolysis on SBA, and non-lactose fermenter on MAC. Figure 2 had shown Gram staining of *L. hongkongensis*, a gram-negative, curve-shaped rod. The biochemical tests of *L. hongkongensis* (LHRJ) isolate had shown in Table 1, it was a non-fermentative (K/N, K/K), non-oxidizer (both with glucose

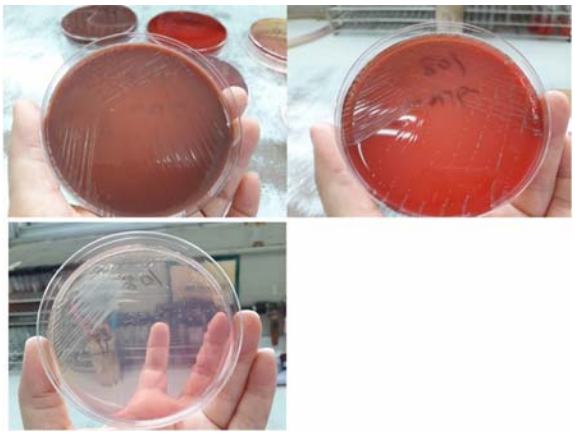

Figure 1. Characterization of *Laribacter hongkongensis* colony on chocolate agar (CA), sheep blood agar (SBA), and MacConkey agar (MAC). The colony is approximately 1 mm in a diameter, non-hemolysis on SBA, and non-lactose fermenter on MAC.

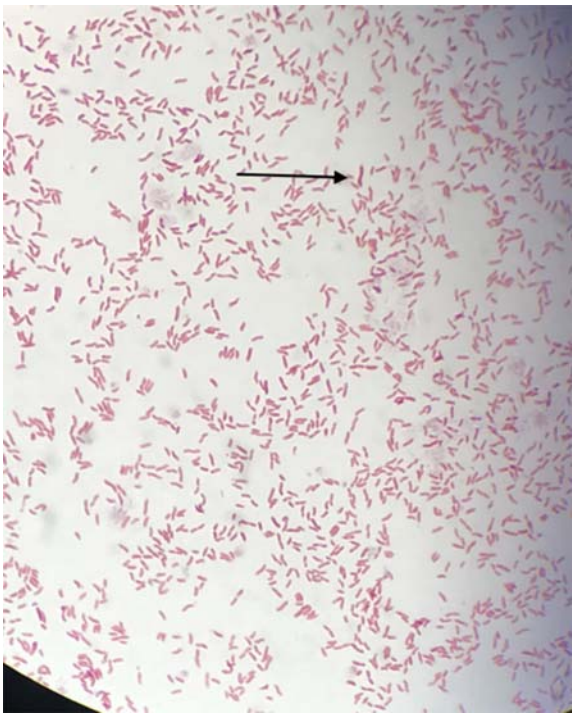

Figure 2. Gram's stain: *Laribacter hongkongensis* is a gram-negative, curved-shaped bacillus.

Table 1. Biochemical reaction or enzyme of *Laribacter hongkongensis* (RJLH strain)

Biochemical reaction or enzyme	Result
Kliger's iron agar (KIA)	K/N, N/N
H_2S	Negative
Motility medium test	Motile
Indole	Negative
Citrate utilization	Negative
Malonate utilization	Negative
Urease	Positive
Lysine decarboxylase	Negative
Arginine dihydrolase	Positive
Ornithine decarboxylase	Negative
Fermentation/Oxidation (O/F)	
- O/F glucose	Negative (Non-oxidizer)
- O/F mannitol	Negative (Non-oxidizer)
% NaCl	
0% NaCl	Positive
1% NaCl	Positive
3% NaCl	Negative
6% NaCl	Negative
9% NaCl	Negative
10% NaCl	Negative
Esculin hydrolysis	Negative
Catalase	Positive
Oxidase	Positive
Nitrate reduction	Positive

and manitol). It was tested positive for motility, urease, arginine dihydrolase, oxidase, catalase, and reduces nitrate. It showed growth in 0 and 1% NaCl, but not in 3, 6, 8, and 10% NaCl. It tested negative for citrate and malonate utilization, indole and H_2S production, lysine and ornithine decarboxylase and esculin hydrolysis.

This *L. hongkongensis* (LHRJ) strain was susceptible to amoxicillin-clavulanate (AMC), piperacillin-tazobactam (TZP), cefoperazole-sulbactam (SCF), doripenem (DOR), imipenem (IPM), meropenem (MEM), gentamicin (GM), amikacin (AN), chloramphenicol (C), tetracycline (TE), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), and levofloxacin (LEV), but resist to ampicillin (AM), cefotaxime (CTX), ceftriaxone (CRO), and ceftazidime (CAZ).

Discussion

The current methods for accurate identification of *L. hongkongensis* are based on a molecular method and the MALDI-TOF MS. It may be misidentified to other genuses and species by using conventional biochemical tests and the automatic machines which have no database of *L. hongkongensis* strains[1,8,12-14].

The clinical microbiology laboratory technician did not overlook the colonies of *L. hongkongensis*; because of its non-fastidious bacteria, it was easy to grow on CA, SBA (non-hemolysis), and MAC (non-lactose fermenter) at 35°C in ambient air and a colorless colony in 1 mm in diameter. There was misidentification because we do not know *L. hongkongensis*, which is not included in textbook bacterium[15].

The important phenotypic characteristics to differentiate other organisms are the morphology in Gram's stain: a gram-negative, curved-shaped rod; the biochemical tests: non-fermentative bacteria, the urease, motile, and catalase, oxidase and arginine dihydrolase tests are positive. However, suspected identification of bacterium should be confirmed by using molecular methods or MALDI-TOF MS.

For the phenotypic characteristics of *L. hongkongensis* (LHRJ) strain, most of them were similar to those of Yuen KY[1], Ni XP[7] and Beilfuss HA[14]; however, some characteristics were different. The LHRJ isolates were motile, similar to those of Beilfuss HA[14] and Ni XP[7], which showed up to 5 bipolar tufted flagella, but differ from those of Yuen KY[1]. In contrast from the study of Beilfuss HA[14], the isolates deny malonate utilization. The LHRJ strain grew in 0 and 1% NaCl, but did not grow in 3, 6, 8, 10% NaCL which was similar to the strain of Yuen KY[1].

For antimicrobial susceptibility testing (AST), the LHRJ strain was resistant to AM, CTX, CRO, and CAZ, but susceptible to carbapenems (DOR, IPM, and MEM), AMC, aminoglycosides (GM and AN), and fluoroquinolones (CIP and LEV), which was similar to those of Lau SK[11] and TengJL[4]. However, the LHRJ strain was susceptible to TZP, which differ from those of Kim DS[8]. Nonetheless, previous

Table 2. Antimicrobial susceptibility of *Laribacter hongkongensis* (LHRJ) strain

Antimicrobial Class/Subclass		Antimicrobial agents		Interpretation		
				Disk Diffusion	MIC (mg/L)	
Beta-lactams class	Penicillins	Ampicillin	AM	R		
	Beta-lactam Combination agents	Amoxicillin-clavulate	AMC	S	ND	-
		Piperacillin-tazabactam	TZP	S	ND	-
		Cefoperazone-sulbactam	SCF	S	ND	-
	Cephalosporins	Cefotaxime	CTX	R	ND	-
		Ceftriaxone	CRO	R	>32	R
		Ceftazidime	CAZ	R	>256	R
	Carbapenems	Doripenem	DOR	S		
		Imipenem	IPM	ND	0.047	S
		Meropenem	MEM	S	0.004	S
Non-beta-lactams class	Aminoglycosides	Gentamicin	GM	S	ND	-
		Amikacin	AN	S	ND	-
	Quinolones	Ciprofloxacin	CIP	ND	0.012	S
		Levofloxacin	LEV	ND	0.008	S
	Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	SXT	S	ND	-
	Phenicol	Chloramphenicol	C	S	ND	-
	Tetracyclines	Tetracycline	TE	S	ND	-

S = susceptible; R = resistant; ND = not done

described studies of Yuen KY[1] and Kim DS[8], the isolates were susceptible to CTX and CAZ.

The report of Wu HK, had presented multidrug resistance (MDR) and resistance genes have been identified in *L. hongkongenis* isolates from Guangzhou, China, including resistant to AM, ampicillin-sulbactam, CTX, CRO, CAZ, CIP, LEV, SXT, TE, and streptomycin, but susceptible to IPM[17]. In addition, the study of Lau SK[9] and Wu HK[17], were reported about tetracycline resistance, from *tetA* plasmid-encoding and *tetG*.

To review this organism, searching the Pubmed by using *Laribacter hongkongensis* and human diseases, and English language, the reports of *L. hongkongensis* found 58 publications with 17 reports caused of human diseases. This case (LHRJ strain) is the first report isolated from Thailand, which was isolated in 2017. All cases found were varied in ages and not significant in children or elders.

Conclusion

This is the first report of *L. hongkongensis* identification in Thailand. The LHRJ strain was isolated from blood of 38-year-old Thai male. The LHRJ isolate was identified by using MALDI-TOF MS method. The phenotypic characteristic of *L. hongkongensis* isolate was described to encourage concern about this organism among microbiology laboratory in Thailand.

The LHRJ stain resists to AM, CTX, CRO and CAZ. However, the antimicrobial susceptibility of *L. hongkongensis* isolates should be tested by MICs method on each individual isolate, because there had been different antibiotic susceptible patterns among individuals.

What is already known on this topic?

1) Usual commercial biochemical tests cannot identify of *L. hongkongensis* correctly.

2) MALDI-TOF MS, which a has database of *L. hongkongensis*, can identify the strain correctly.

3) Awareness of *L. hongkongensis* should be concerned by using key phenotypic characterization and molecular confirmation method, especially laboratory without MALDI-TOF.

4) Antimicrobial susceptibility testing of *L. hongkongensis* should be done, because of individual susceptibility patterns.

What this study adds?

In the future, the correct data of *L. hongkongensis* should be studied, including prevalence, and antimicrobial susceptibility for appropriate treatment and infection control committee (ICC) prevention.

Potential conflicts of interest

The authors declare no conflict of interest.

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