

## First clinical isolation report of *Shewanella xiamenensis* from Chinese giant salamander

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### Abstract

*Shewanella xiamenensis*, a newly virulent zoonotic pathogen belonging to the genus *Shewanella* is the causative organism of emerging intra-abdominal infection, acute skin ulceration, rotten limbs and ascites in humans and animals. The global spread of *S. xiamenensis* entails severe economic impact. However, it was rarely reported as a cause of infection and no reports were found that *S. xiamenensis* isolated from clinical samples. The isolate was identified as a *S. xiamenensis* strain by 16S rDNA amplification and DNA sequencing identification method. Even if co-infection by other bacteria could not be ruled out, this is the first report of acute disease caused by *S. xiamenensis* in the Chinese giant salamander in China. By using the Kirby-Bauer disc diffusion method, the sensitivity of the isolate to clinical antibiotics was evaluated. Antibiotic susceptibility test indicated that the isolate was resistant to 32 antibacterial drugs such as kanamycin, florfenicol and ceftriaxone suggesting that the isolate was a multi-drug resistant strain.

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### Introduction

The Chinese giant salamanders (*Andrias davidianus*), which belongs to one of the most primitive orders of the urodele amphibians, is the largest extant amphibian species in the world.<sup>1</sup> However, the population of the Chinese giant salamanders has declined sharply with the deterioration of living environment and human disturbance resulting in reduced ecosystem function, exacerbated soaring incidence of emerging diseases in Chinese giant salamanders. It is crucial to study the bacterial infection of Chinese giant salamander in view of improving the artificial breeding technology and protect natural species resources.

*Shewanella* spp. are commonly known as environmental bacteria. They, were first reported in the coastal sediments in Xiamen, and were most frequently isolated from aquatic environments such as sea- and freshwater.<sup>2</sup> Currently, in clinical settings the most reported emerging infections in human are found to be caused by *Shewanella algae* and *Shewanella putrefaciens*.<sup>3,4</sup> *Shewanella xiamenensis*, a newly established species of the genus *Shewanella*, a zoonotic pathogen, is commonly found in aquatic ecosystem.<sup>5</sup> It was rarely reported as a

cause of infection and no reports were found that *S. xiamenensis* isolated from clinical samples.

In the present study, we reported a strain of *S. xiamenensis* was successfully isolated from an acute dead Chinese giant salamander with signs of skin ulceration, rotten limbs and ascites in Kaizhou, China. Also, the antibiotics sensitivity test was conducted for better understanding the drug resistance of the isolate.

### Case Description

A Chinese giant salamander was contracted with a disease that main clinical manifestations included deep hemorrhagic ulcers in the body, rotten limbs and ascites. The dead salamander was packaged into a sterile container and transported to the animal disease diagnostic laboratory immediately (within 3 hr) for diagnosis and pathogen isolation at low temperature. The heart, liver, lung and kidneys of the freshly dead Chinese giant salamander were harvested aseptically for bacteriological examination. Samples were streaked onto Luria-Bertani (LB) nutrient agar plate and cultured at 28.00 °C for 48 hr. After incubation, the dominant bacterial colonies were

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selected based on the size, shape and color, then were purified by streaking and re-streaking on the LB agar to obtain pure culture before being stored at  $-80.00\text{ }^{\circ}\text{C}$  with 20.00% glycerol prior to identification. The total DNA of the isolate was extracted using the Lysis buffer (Takara Biotech, Beijing, China) in accordance with the instructions in the user manual. The supernatant was collected and stored at  $-20.00\text{ }^{\circ}\text{C}$  until used as PCR templates. Amplified target fragment, primers and PCR amplification program based on 16S rDNA were conducted according to described previously.<sup>6,7</sup> Positive products were sequenced in the Huada Gene company (Shenzhen, China) and analyzed for the homology by DNASTar Software (DNASTAR Inc., Madison, USA).

**Table 1.** The results of antibiotic sensitivity test and average inhibition zone diameter (mm) against *Shewanella xiamenensis*.

Antibiotics	Disc content ( $\mu\text{g}$ )	Diameter	Results
Aminoglycosides	Gentamycin (30.00)	12.80	I
	Spectinomycin (25.00)	0	R
	Kanamycin (30.00)	12.80	R
	Neomycin (30.00)	15.00	I
	Amikacin (30.00)	-	-
	Tobramycin (30.00)	10.80	R
Tetracyclines	Tetracycline (30.00)	0	R
	Minocycline (30.00)	13.80	I
	Doxycycline (30.00)	9.30	R
	Chloramphenicol (30.00)	20.40	S
	Florfenicol (30.00)	0	R
Macrolides	Erythromycin (30.00)	0	R
	Medemycin (30.00)	0	R
	Clarithromycin (5.00)	9.60	R
Polymyxins	Vancomycin (5.00)	0	R
	Polymyxin B (30.00)	11.40	I
$\beta$ -lactams	Penicillin (2.00)	0	R
	Oxacillin (1.00)	0	R
	Pirbenicillin (1.00)	0	R
	Ampicillin (10.00)	0	R
	Amoxicillin (25.00)	-	-
	Piperacillin (30.00)	10.50	R
	Cefalexin (30.00)	22.80	S
	Mezlocillin (30.00)	0	R
	Cefoperazone (30.00)	11.50	R
	Ceftriaxone (30.00)	0	R
	Cefalotin (30.00)	0	R
	Ceftazidime (30.00)	0	R
	Cefazolin (30.00)	0	R
	Cefuroxime (30.00)	0	R
	Aztreonam (30.00)	0	R
	Quinolones	Enrofloxacin (5.00)	16.10
Pefloxacin (10.00)		16.50	S
Norfloxacin (2.00)		12.00	R
Ciprofloxacin (5.00)		17.10	I
Lomefloxacin (10.00)		15.30	I
Levofloxacin (5.00)		17.30	S
Lincosamides	Ofloxacin (5.00)	19.40	S
	Lincomycin (2.00)	0	R
	Clindamycin (10.00)	0	R

S: Sensitive; R: Resistant; and I: Intermediate.

The antibiotic susceptibility test of the isolate was evaluated using 42 antibiotics (Table 1) by the Kirby-Bauer (K-B) disc diffusion method<sup>8</sup> on LB nutrient agar using commercial antibiotic discs (Hangzhou Tianhe Microorganism Reagent Co., Hangzhou, China). After 24 hr of incubation at  $28.00\text{ }^{\circ}\text{C}$ , the size of the bacterial growth inhibition zones around each disc were measured, and the isolate was classified as sensitive (S), moderately sensitive (M) and resistant (R) according to standards suggested by the Hangzhou Tianhe Microorganism Reagent Company.

## Results

The typical signs of the diseased Chinese giant salamander were noticed as deep hemorrhagic ulcers and rotten limbs (Fig. 1A). Obvious post-mortem lesions included stomach swelling and ascites (Fig. 1B). From the liver, a motile Gram-negative bacillus was successfully isolated, and temporarily named CQ-SX1. Identification result of the isolate by 16S rDNA amplification method was shown in Figure 2. Based on the blast alignment of the 16S rDNA sequence, CQ-SX1 shared above 99.50% homology (Fig. 3) with *S. xiamenensis* strains registered in the GenBank. Phylogenetic tree showed that the isolate along with other *S. xiamenensis* strains formed a tight clade (Fig. 4). All these findings proved that CQ-SX1 belonged to *S. xiamenensis* and its nucleotide sequence data had been deposited in GenBank under accession number MK033616.



**Fig. 1.** Autopsy symptoms of the diseased Chinese giant salamander **A)** Typical signs including rotten limbs (yellow arrow) and deep hemorrhagic ulcers (blue arrow); **B)** Obvious postmortem lesions include stomach swelling (red arrow) and ascites (green arrow).

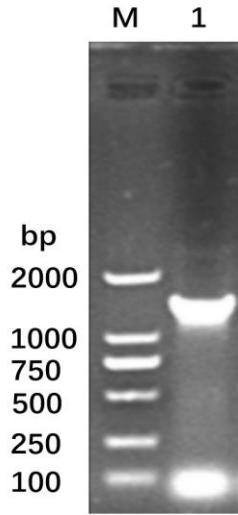


Fig. 2. Agarose gel electrophoresis of PCR products of 16S rDNA. Lane M: DL2000 marker; Lane 1: 16S rDNA amplification result.

The isolate was tested for sensitivity with 42 antibiotics by K-B disc diffusion method. Results revealed that CQ-SX1 was only highly sensitive to pefloxacin, levofloxacin and ofloxacin (Table 1). However, it proved resistant to the other most  $\beta$ -lactams and aminoglycosides drugs. The isolate was found resistant to three or more drugs, which indicated that CQ-SX1 was a multi-drug resistant strain.

**Discussion**

To the best of our knowledge, this is the first report in which *S. xiamenensis* was isolated from the Chinese giant salamander.

Despite the most *Shewanella* infections were caused by *S. algae* and to a lesser extent *S. putrefaciens*, *S. xiamenensis* has been reported as a pathogenic bacterium to humans.<sup>9,10</sup> In previous years, the potential of *Shewanella* infections increasingly recognized worldwide, by virtue of

		Percent Identity											
		1	2	3	4	5	6	7	8	9	10		
Divergence	1	■	100.0	100.0	100.0	99.9	99.8	99.7	99.7	99.5	99.8	1	Isolated bacteria Shewanella xiamenensis H3 HQ418493.1 Shewanella xiamenensis H31 MG428831.1 Shewanella xiamenensis H39 MG428836.1 Shewanella xiamenensis H51 MG428847.1 Shewanella xiamenensis H99 MG428891.1 Shewanella xiamenensis M21 KM268987.1 Shewanella xiamenensis M24 KM268990.1 Shewanella xiamenensis M50 MG428841.1 Shewanella xiamenensis RPK31 KX980453.1
	2	0.0	■	100.0	100.0	99.9	99.8	99.5	99.7	100.0	99.8	2	
	3	0.0	0.0	■	100.0	99.9	99.8	99.5	99.7	100.0	99.8	3	
	4	0.0	0.0	0.0	■	99.9	99.8	99.5	99.7	100.0	99.8	4	
	5	0.1	0.1	0.1	0.1	■	99.7	99.6	99.6	99.4	99.7	5	
	6	0.2	0.2	0.2	0.2	0.3	■	99.5	99.5	99.3	99.6	6	
	7	0.2	0.2	0.2	0.2	0.3	0.4	■	100.0	99.1	99.5	7	
	8	0.2	0.2	0.2	0.2	0.3	0.4	0.0	■	99.2	99.5	8	
	9	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.2	■	99.3	9	
	10	0.2	0.2	0.2	0.2	0.3	0.4	0.4	0.4	0.2	■	10	

Fig. 3. The homologous analysis results of reference strains and the isolate.

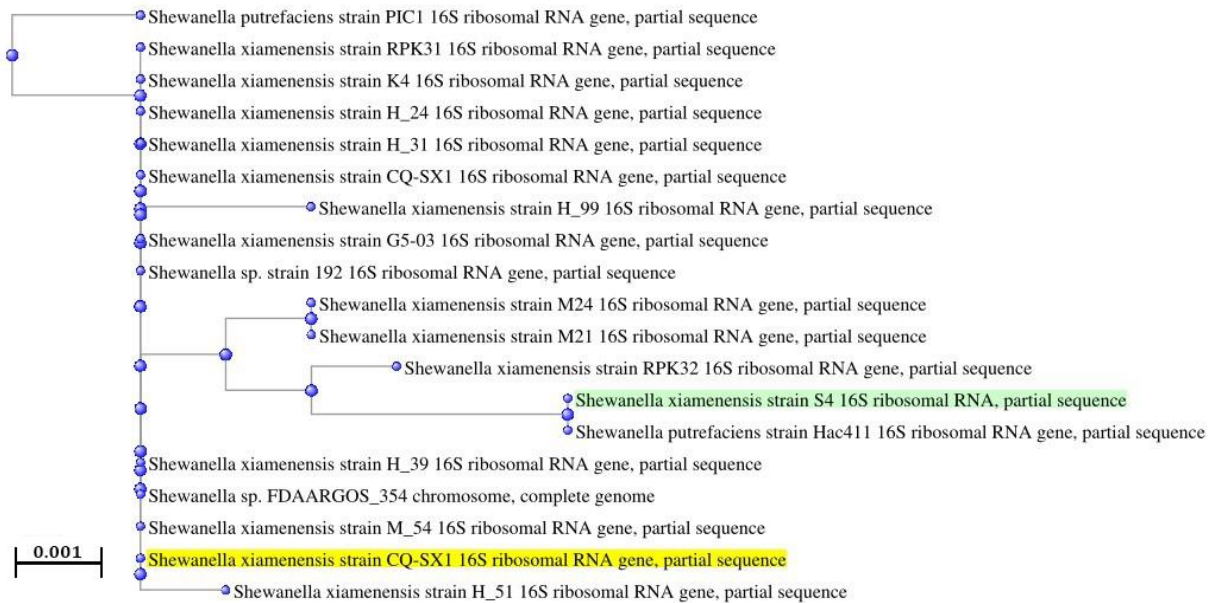


Fig.4. Phylogenetic tree of *Shewanella* species based on 16S rDNA nucleotide sequences.

the *Shewanella* genus, which is abundant in marine environments and infection can be occurred via contact with seawater or consumption of raw seafood.<sup>11,12</sup> In the present study, we cautiously concluded that the Chinese giant salamander was infected by eating the unsterilized fish and shrimp. We could reasonably speculate that the bacteria may cause food-borne infections.

As reported before, the typical antibiotics for humans infected with *Shewanella* were  $\beta$ -lactams, aminoglycosides and quinolones.<sup>2</sup> In the present Chinese giant salamander, quinolones, such as pefloxacin, levofloxacin and ofloxacin were found effective against the *S. xiamenensis* isolate which it was in line with what had been reported. Yet aminoglycosides and  $\beta$ -lactams (cefoxitin excluded) were all ineffective. A previous study indicated that antibiotic resistance was on the rise to *Shewanella* and the genus has been regarded as a reservoir and vehicle of antimicrobial resistance.<sup>2</sup> Our findings were in accordance with that report. The isolated *S. xiamenensis* displayed an extensively drug-resistant to 32 kinds of antibacterial drugs such as kanamycin, florfenicol, ceftriaxone and so on (Table 1) indicating that it was a multi-drug resistant strain. The accurate mechanisms of drug resistance were still the subject of further research.

In summary, no previous *S. xiamenensis* infection had been reported in our area. Therefore, understanding the disease caused by this bacterium is significant. Also, the multi-drug resistant *S. xiamenensis* strain could be a serious threat to the Chinese giant salamander breeding industry, food safety and human public health. Legal restriction and the prudent use of antibiotics and continual stringent surveillance by the government could be some effective measures in order to control the issue of the resistance.

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### Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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