

Full Length Research Paper

Identification, isolation and *in vitro* antimicrobial susceptibility testing of *Aeromonas veronii* associated with an acute death of Channel Catfish (*Ictalurus lunetas*) in China

Xiao-li Huang¹, Kai-yu Wang^{2*}, Du Zong-Jun¹, Yi Geng² and Yong-qiang Deng³

¹Department of Aquaculture, College of Animal Science and Technology, Sichuan Agricultural University, Ya'an 625014, Sichuan, China.

²College of Animal Science and Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, Sichuan, China.

³Sichuan Provincial center for Animal Disease Prevention and Control, ChengDu 610041, Sichuan, China.

Accepted 7 January, 2010

***Aeromonas veronii* is a common pathogen in both humans and animals. It exists in the environment we live. Many reports showed it could lead to human infection but few demonstrated its effect on aquatic animals, especially Channel Catfish (*Ictalurus lunetaus*). Here, *A. veronii* was isolated from an acute death case of Channel Catfish in Southwestern China. This Gram-negative bacillus was identified by 16S rRNA sequencing. Antimicrobial susceptibility was also conducted to guide the treatment of the disease.**

Key words: Channel catfish, *Aeromonas Veronii*, China.

INTRODUCTION

Channel catfish was introduced into China in 1984. After more than two decades of cultivation, this kind of fish was spread to most provinces in China, such as Hubei, Sichuan, Guangdong, Chongqing, Jiangxi and Anhui. The production reached 120,000 ton in 2006 and it is one of the major foreign exchange-earning export fish in China. *Aeromonas hydrophila* (Wang et al., 1999), *Edwardsiella tarda* (Meyer et al., 1973) and *Flavobacterium columnare* (Welker et al., 2005) were the main bacteria of channel catfish according to some reports, while *Aeromonas veronii* was rarely mentioned.

In April 2009, an infectious disease broke out at a channel catfish farm in Sichuan province of Southwestern China. At the beginning phase of the disease, the cultivated fish died in small scales. Dying fish swam alone near the surface of cultivated water without vigor. The color of body surface darkened and the ailing fish had little or no appetite. More and more fishes died as time went on.

Until we began to investigate this case, the death reached more than 1000 kg per day. For a rough estimation, the morbidity and the mortality were up to 30 and 50%, respectively. Furthermore, the temperature was 17 - 22 °C and the body weight of the dead fish was 1000 - 1500 g. The infected fish showed serious abdominal distention and the anus turned red, swollen and puffed out. Fins, including pectoral, ventral and anal fins displayed hyperemia and hemorrhage. A huge amount of ascites was streamed when dead fish was dissected. The ascites in the body cavity was clear, but sometimes yellowish or bloody. Hemorrhage existed in internal organs including fat, mucous membrane of gastrointestinal tract, hepar and kidney. No obvious parasites were found on the gills. The ammonia nitrogen of the water in the aqua farm was 0.1 mg/L and the nitrite was 0.05 mg/L which was suitable for the cultivation of freshwater aquatic animals. To prevent the acute death, fish farmers had used tetracycline for oral use and formalin for soaking. But this seemed to have little effect on the disease.

In order to investigate the cause of the disease, the pathogenic microorganisms were isolated, purified and

*Corresponding author. E-mail: hxlidyq@126.com. Tel: 086-08352885910.

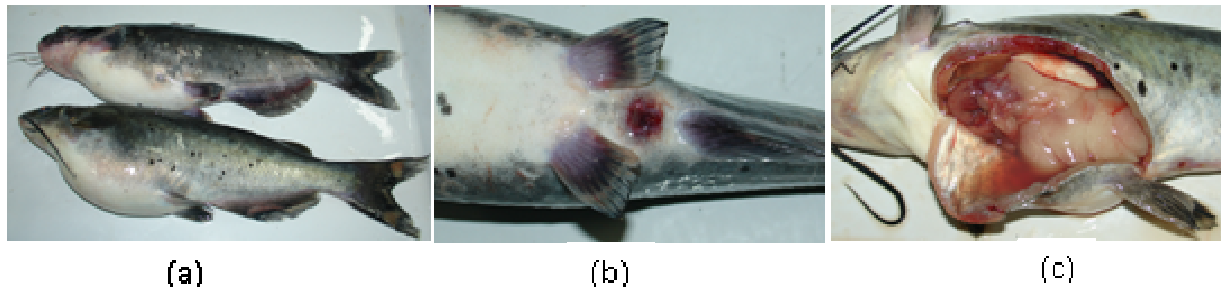


Figure 1. (a) Naturally infected channel catfish with serious abdominal distention; (b) the anus of the infected fish showed red, swollen and puffed out; and (c) a huge amount of ascites existed in abdominal cavity.

identified with 16S rDNA sequencing. And some effective drugs were screened for preventing and controlling based on antimicrobial susceptibility testing *in vitro*. This work is aimed at providing ways for stopping the death of Channel catfish caused by *Aeromonas veronii* and reducing the economic loss of the farmers.

MATERIALS AND METHODS

Pathogenic organisms isolation

20 dying catfishes with obvious symptoms were collected from a pond in a commercial aquaculture farm in Sichuan province. The ascites, hepar and kidney were used for microbiological examination. Bacteria were isolated from ascites, hepars and kidneys under asepsis controlling. The specimens were inoculated onto Brain Heart Infusion (BHI) agar plate and incubated aerobically for 24 h at 28°C. Colonies of different shapes were chosen for Gram's staining and each colony was saved on one plate. All the purified isolates were stored at the BHI agar plates for later use.

Identification of the isolates

The purified isolates were amplified in BHI broth and DNA extraction was done with a DNA extraction kit. The bacteria were subjected to the PCR with universal 16S rRNA primers and the PCR products were detected by agarose gel electrophoresis and sequenced. The sequences were performed by comparative analysis with the Genbank databases for identification of the isolates.

In vitro antimicrobial susceptibility testing

In vitro antimicrobial susceptibility testing was done with paper sheets method to detect the bacteria sensitivity to different medicines. At first, the purified isolates were inoculated to BHI broth and incubated aerobically for 24 h at 28°C. Furthermore, the bacterial suspension was

smearred onto the BHI agar plates with an aseptic cotton bud. Third, commercial drug sensitive slips were stick on the surface of plates carefully and incubated for 24 h at 28°C to examine which medicine the bacteria is susceptible to.

RESULTS

Isolation and identification

After 24 h of incubation under aerobic conditions, colonies appeared on the BHI agar plate. The shape of colonies showed round or ellipse, diameter was 2 – 3 mm and the color was white. All the colonies were checked by Gram's staining under a microscope and same kind of pathogen was isolated from the diseased fish by identification with biochemical characteristics. The colonies showed gram-negative bacilli arranged in single or pairs after a Gram staining. The purified isolate was identified by 16S rDNA sequencing.

The PCR products of 16S rDNA was about 1500 bp after a agarose gel electrophoresis was done. And it was demonstrated that the 16S rDNA PCR products were 1523 bp by sequencing. And the pair-wise alignments of the 16S rDNA gene showed that the homology of the isolate to *A. veronii* was the most closest.

In vitro antimicrobial susceptibility testing

Antimicrobial susceptibility testing showed that the isolated strain *A.veronii* was susceptible to Levofloxacin, flornfenicol, sulfafurazole and tetracycline but was resistant to novobiocin, midecamycin and polymyxin. It suggested that we could select sensitive antibiotics to prevent the disease (Figures 1 and 2; Table 1).

DISCUSSION

A pathogen was isolated from channel catfish with acute death. It was identified as *Aeromonas veronii* by 16S



Figure 2. Gram's staining showed the isolate was gram-negative bacilli arranged in single and pairs.

Table 1. The results of in vitro antimicrobial susceptibility testing.

Antimicrobial agent	Disc potency (μg)	Diam of zone of inhibition (mm)			Diameter of zone of inhibition (isolate to antibiotics) (mm)
		Resistant	Intermediate or equivocal	Susceptible	
Polymyxin B	300	≤ 8	8-11	≥ 12	0
Novobiocin	30	≤ 12	13-16	≥ 17	0
Clindamycin	2	≤ 14	15-20	≥ 21	0
Midecamycin	30	≤ 13	14-17	≥ 18	9
Neomycin	30	≤ 12	13-16	≥ 17	11
Minocycline	30	≤ 14	15-18	≥ 19	12
Florfenicol	30	≤ 12	13-17	≥ 18	31
Lomefloxacin	18	≤ 10	19-21	≥ 22	30
Clarithromycin	15	≤ 13	14-17	≥ 18	18
Ciprofloxacin	5	≤ 15	16-20	≥ 21	29
Kanamycin	30	≤ 13	14-17	≥ 18	18
Tetracycline	30	≤ 14	15-18	≥ 19	18
Amikacin	30	≤ 14	15-16	≥ 17	16
Azithromycin	15	≤ 13	14-17	≥ 18	26
Tobramycin	10	≤ 12	13-14	≥ 15	16
Levofloxacin	5	≤ 13	14-16	≥ 17	32

rDNA sequencing. The stain was gram-negative and it could cause channel catfish severe ascites, extensive hemorrhage on the body surface and organs. The results demonstrated that *A. veronii* was a new pathogenic microorganisms to channel catfish and would lead to a serious death.

Infectious diseases in fish constitute a major obstacle in aquaculture. However, diseases caused by bacteria are main factors that led fish to die. *Aeromonas* spp. are ubiquitous inhabitants of aquatic ecosystems such as freshwater, coastal water and sewage. They are

increasingly being reported as important pathogens not only for human, but also for lower vertebrates, including fish (Janda and Abbott, 1998). Many reports demonstrated that *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* were frequently occurring species of *Aeromonas*. But relative reports about *A. veronii* were few in aquaculture.

A. veronii belongs to *Aeromonas* genus. It was first found by Hichman et al in 1987. Reports showed that it was discovered from wounds, feces and sputum, an endotracheal tube and a lung biopsy specimen. And it

could also be isolated from freshwater and saltwater environments (Neves et al., 1990). It usually infects humans and causes diseases like septic arthritis, bacteraemia (Roberts et al., 2006), spontaneous bacterial empyema (Wang et al., 2000) and severe pneumonia (Li et al., 2008). Besides infected human beings, *A. veronii* also infects fish and make diseases to spread quickly. Mokhlasur et al. (2002) reported that the pathogen of epizootic ulcerative syndrome in fish in Bangladesh was *A. veronii*. But analogous case reports were few. Our study found that it could infect channel catfish and cultured fish appeared severe death. The main symptom of the infected fish was serious ascites and septicemia. Our result was almost the first to find that *A. veronii* could infect channel catfish in China and it not only increase the data of diseases in channel catfish but also gave reference to fish farmers for diagnosis and prevent similar symptoms of the disease.

The 16S rRNA contain regions that are conserved among all organisms so far investigated (Jill and Clarridge, 2004). Comparison of gene sequences of bacterial species showed that the 16S rRNA gene is highly conserved within a species and among species of the same genus (Patrick et al., 2002). By analyzing the partial 16S rRNA sequence, it is possible to find group-, species-, and even serotype-specific sequence patterns. Therefore, besides the common bacteria, noncultivable organisms and organisms with ambiguous biochemical profiles can be classified and identified (Relman et al., 1990; Relman et al., 1992). Following bacteria isolation, our study aimed at the identification of pathogen by sequence analysis of 16S rRNA. The bacterium was subjected to the PCR with universal 16S rRNA primers and the PCR product was sequenced. The sequence was performed by comparative analysis with the Genbank. The homology of the unknown bacterium to *A. veronii* was 99.9%. We considered *A. veronii* was the pathogen of infected fish in Sichuan province in Southwestern China.

According to the antimicrobial susceptibility testing, we could select susceptible antibiotic to prevent and treat the disease. To consider the residue and harm of antibiotic, sufficient withdrawals period should be performed. For another way, careful cultivation and management were needed to increase the resistance of aquatic animal to pathogenic microorganism.

REFERENCES

- Hichman-Brenner FW, MacDonald KL, Steigerwalt AG, Fanning GR, Brenner DJ, Farmer JJ (1987). *Aeromonas Veronii*, A new ornithine decarboxylase-positive species that may cause diarrhen. J. Clin. Microbiol. 25: 900-906.
- Janda JM, Abbott SL (1998). Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clin. Infect. Dis, 27: 332-334.
- Jill E, Clarridge III (2004). Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. Clin. Microbiol. Rev. 17: 840-862.
- Li L, DU ZZ, Sun X, Shao XG, Li JG, Zhang LX, Wang YM, Wu Q (2008). Severe pneumonia caused by *Aeromonas veronii*, *Biovar sobria*: A case report and review of the literature. Zhonghua Jie He He Hu Xi Za Zhi, 31: 736-739.
- Meyer EP, Bullock GL (1973): *Edwardsiella tarda*, a new pathogen of channel catfish (*Ictalurus punctatus*). Appl. Microbiol. 25: 155-156.
- Mokhlasur R, Patricia CN, Inger K, Geert H, Jean S, Roland M (2002). Identification and characterization of pathogenic *Aeromonas veronii*, *Biovar sobria* associated with epizootic ulcerative syndrome in fish in Bangladesh. Appl. Environ. Microbiol. 68: 650-655.
- Neves MS, Nunes MP, Milhomem AM, Ricciardi ID (1990). Production of enterotoxin and cytotoxin by *Aeromonas veronii*. Braz. J. Med. Biol. Res. 23: 437-440.
- Patrick CYW, Ami MYF, Susanna KPL, Yuen KY (2002). Identification by 16S rRNA gene sequencing of *Lactobacillus salivarius* bacteremic cholecystitis. J. Clin. Microbiol. 40: 265-267.
- Patrick CYW, Ami MYF, Susanna KPL, Yuen, KY (2002). Identification by 16S rRNA gene sequencing of *Lactobacillus salivarius* bacteremic cholecystitis. J. Clin. Microbiol. 40: 265-267.
- Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS (1990). The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N. Engl. J. Med. 323: 1573-1580.
- Relman DA, Schmidt TM, MacDermott RP, Falkow S (1992). Identification of the uncultured bacillus of whipple's disease. N. Engl. J. Med. 327: 293-301.
- Roberts MT, Enoch DA, Harris KA, Karas JA (2006). *Aeromonas veronii*, *Biovar sobria* bacteraemia with septic arthritis confirmed by 16S rDNA PCR in a immunocompetent adult. J. Med. Microbiol. 55: 241-243.
- Wang C, Silva JL (1999). Prevalence and characteristics of *Aeromonas* species isolated from processed channel catfish. J. Food. Prot. 62(1): 30-34.
- Wang JT, Fang CT, Hsueh PR, Chang SC, Luh KT (2000). Spontaneous bacterial empyema caused by *Aeromonas veronii biotype sobria*. Diagn. Microbiol. Infect. Dis. 37: 271-273.
- Welker TL, Shoemaker CA, Arias CR, Klesius PH (2005): Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. Dis. Aquat. Organ. 63(2-3): 129-138.