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## Research Article

# Detection of aerolysin and hemolysin genes in *Aeromonas hydrophila* isolates from in Al-Diwaniyah City, Iraq

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**Abstract:** The current work was aimed to identify the aerolysin (*aerA*) and hemolysin (*hlyA*) genes of *A. hydrophila* isolates separated from *Cyprinus carpio* in a farm in Al-Diwaniyah City, Iraq. Fish samples with external lesions were collected and *A. hydrophila* was isolated and characterized using traditional cultivation and biochemical techniques. Later, a total of 26 bacterial isolates were subjected to the identification of *aerA* and *hlyA* genes by employing PCR method. The clinical signs of the disease were reddish head, anal, and fin bases while the rest of the body was pale. External ulcerative and hemorrhage spots were noticed. In the detection of the bacterial genes, 19 (73%) were positive isolates for *aerA* and 13 (50%) positive for *hlyA* genes. *Aeromonas hydrophila* isolates from common carp contain aerolysin and hemolysin genes that increase the pathological effects on the infected fish.

**Keywords:** *AerA*, *hlyA*, Aquaculture, Carp.

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## Introduction

Common carp, *Cyprinus carpio,* is one of the important fishes in aquaculture industry of the world (Xu et al. 2021) and Iraq as well (Toffan et al. 2020). Common carp can be infected with a wide range of viruses, such as carp edema virus, carp virus, carp interstitial nephritis, and gill necrosis virus (Calif et al. 2002; Adamek et al. 2017a, b; 2019; Ortega et al. 2019; Dishon et al. 2021). Freshwater fish can be sick due to infections by bacterial species both Gram negative and positive; however, Gram-negative bacteria are the common with a wide-spectrum that are recovered from fish with clinical signs, including *Aeromonas* spp., *Pseudomonas* spp., *Flavobacterium* spp., and *Shewanella putrefaciens* and probably, *Plesiomonas shigelloides*, *Acinetobacter* spp., *Stenotrophomonas maltophilia*, and *Sphingomonas paucimobilis*. For the Gram positive bacterial species, *Streptococcus iniae*, *Lactococcus garviae*, and *Kocuria rhizophila* are among the important pathogenic isolates from different regions of the world (Pękala-Safińska 2018).

*Aeromonas hydrophila* is a bacterium with a high rate of pathogenesis in different types of fish, causing motile aeromonad septicemia in carp, perch, tilapia, salmon, and catfish, red sore disease in carp and bass, and ulcerative disease in catfish, carp, cod, and goby (Citterio & Biavasco 2015). This microorganism is a Gram negative bacteria (Janda & Abbott 2010) causing different infections in people, such as skin infections, gastroenteritis, bacteremia, peritonitis, meningitis, hemolytic uremic syndrome, cholera-like illness, and necrotizing fasciitis (Citterio & Biavasco 2015). The current work was aimed to identify the aerolysin (*aerA*) and hemolysin (*hlyA*) genes of *A. hydrophila* isolates from common carp collected

**Table 1.** PCR Amplification of *aerA* and *hlyA* Genes (13).

| Primer | | | size |
|---|---|---|---|
| Aer gene | F | 5′-AGC GGC AGA GCC CGT CTA TCC A-3′ | 412bp |
| | R | 5′-AGT TGG TGG GGG TGT CGT AGC G-3′ | |
| Hyl gene | F | 5′ GGC CCG TGG CCC GAA GAT GCA GG3′ | 597bp |
| | R | 5′ CAG TCC CAC CCA CTT C 3′ | |

**Fig.1.** Ulceration necrotic lesions and hemorrhages caused on the skin in *Cyprinus carpio*.

from Al-Diwaniyah City, Iraq.

## Materials and methods

Fish with external lesions were sampled from a farm in Al-Diwaniyah City, Iraq. Trypton soya agar (TSA, Oxoid) was employed and AIM for *Aeromonas* isolation. The plates were incubated at 25°C for one day, and sub-cultures were prepared by performing morphological and biochemical characterization viz. catalase, oxidase, oxidative-fermentative, production of gas and acid from sugars (mannitol, glucose, maltose, sucrose and lactose), furthermore, sulphide hydrogen and methyl-red forming, arginine hydrolysis, Esculin hydrolysis and Voges-Proskauer.

**Identification of *aerA* and *hlyA* genes:**

**DNA extraction:** The bacterial-based DNA was obtained utilizing the Wizard® genomic DNA purification kit (Promega, USA). Briefly, the purified bacterial culture from the nutrient broth was centrifuged at 13000rpm for 1min. Then, the suspension of the pellets was performed in 480µL of 50mm EDTA. Later, a 13000rpm centrifugation was done for 2min decanting the produced supernatant. After that, the kit components and steps were followed as demanded by the kit instructions (Table 1).

The total PCR reaction volume was 25µl, containing 5mM $MgCl_2$, 2.5µl reaction buffer, 10nmole dNTP, 10pmole primer, 2.5U Taq polymerase (Promega company), and 20ng DNA. Minicycler was used for PCR reaction (Table 2). The PCR products were tested by 1.5%-agarose-ethidium-bromide-gel using the electrophoresis and then observed under UV light.

## Results

In the pond, moribund fishes were detected with abnormal swimming. The clinical signs of the disease showed reddish head, anal, and fin bases while the rest of the body was pale. External ulcerative and hemorrhage spots were noticed (Fig. 1). The bacterial isolates showed the specific growth and biochemical characteristics of *A. hydrophila* (Table 3). For the detection of the bacterial genes, 19 (73%) positive isolates were found for the *aerA* gene, and 13 (50%) positive isolates were recognized for the *hlyA* gene (Fig. 2).

## Discussion

Carp is one of the primary food resources in Iraq and like in many global countries, aquaculture industry faces challenges in Iraq, and one of them health issues caused by bacterial diseases. Understanding

**Table 2.** Showed stages, the temperature and period of PCR thermocycler.

| The stage | The temperature (°C) | The period (min) |
|---|---|---|
| The initial stage | 95 | 5 |
| The denaturation | 95 | 2 |
| The annealing | 55 | 1 |
| The extension | 72 | 1 |
| The final extension | 72 | 7 |

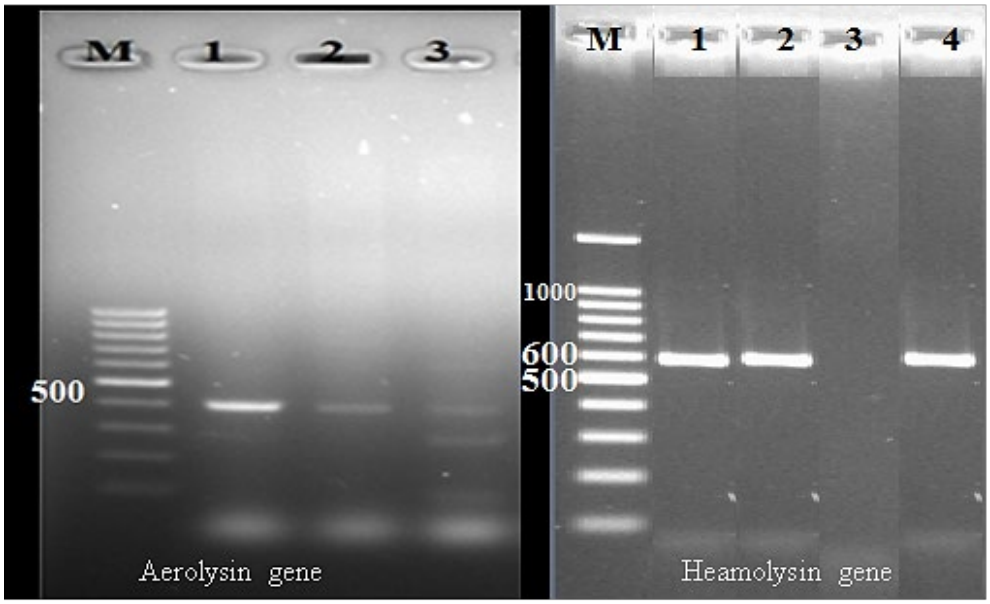


**Fig.2.** PCR-Agarose electrophoresis of aerosylin and hemosylin.

the biological components of the disese causing bacteria may help control them. The present work identified the presence of the virulence factors, *aerA* and *hlyA* genes, in the genome of *A. hydrophila* isolated from common carp. *Aeromonas hydrophila* is an anaerobic chemoorganoheterotroph bacterium of mostly freshwater and food that infects different species, such as fishes, birds, humans, reptiles, and many mammals causing septicemia, gastroenteritis, and necrotizing fasciitis (Rasmussen-Ivey et al. 2016). In addition, *A. hydrophila* can be source of some virulence factors, including generating biofilms, cytotoxins, adhesins, lipases, hemolysins, and proteases (Rasmussen-Ivey et al. 2016a). The bacterium is a habitat of different virulent secretion systems. Some isolates of the bacterium have the type III secretion system (T3SS), a powerful virulence component of these bacteria (14). However, some isolates from different animal species, such as fish that were genetically identified to have another secretion system that gives the super-virulence activity of this bacterium in these aquatic creatures (Hossain et al. 2013; Pang et al. 2015). It has been detected that some super-virulent isolates from freshwater fishes have a complete T6SS (Rasmussen-Ivey et al. 2016b).

Aerolysin is a key toxin of *A. hydrophila* pathogenicity in fish encoded by a 1482-bp gene. Singh et al. (2008) used specific primers to detect the gene by targeting a 326-bp region. They identified the gene in 85% of the fish and water bacterial isolates. It has been reported that not all *A. hydrophila* isolates have the *aerA* gene that can occur due to evolutionary processes that may have happened on the bacterium leading to deletion of this gene and the acquisition of other required genes (Watanabe & Morita 2020).

As conclusion, *A. hydrophila* isolates from carp

**Table 3.** Characteristics of *Aeromonas hydrophila* isolates.

| Characters | Isolates |
|---|---|
| Gram stain | - |
| Motility | + |
| Shape | Red |
| Oxidase | + |
| Catalase | + |
| Test | Fermentative |
| Dextrose | + |
| Source | + |
| Maltose | + |
| Lactose | + |
| Acid production Manitol | + |
| $H_2S$ production | + |
| Voges-proskauer | + |
| Methyl red test | - |
| Esculin hydrolysis | + |
| Growth at | |
| 4°C | - |
| 37°C | + |
| 40°C | - |
| Growth in Nacl solution | |
| 0% | + |
| 1% | + |
| 2% | + |
| 5% | - |
| 4% | - |

fish in Al-Diwaniyah City, Iraq, contain aerolysin and hemolysin genes. Aerolysin and hemolysin genes increase the pathological effects on the infected fish. Such a problem may accelerate more losses in fish production in the country.

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