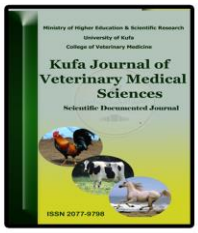
Kufa Journal for Veterinary Medical Sciences Vol.(5). No.(2) 2014

Kufa Journal for Veterinary Medical Sciences

www.vet.kufauniv.com



Isolation and identification of *vibrio fluvailis* from goldfish *Carassius auratus* In AL NajafAl-Ashraf Governorate

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Abstract

This study aimed to diagnose the *Vibrio fluvailis* and identify some of the virulence genes, Eighty six samples were obtained from gold fish *Carassius auratus* which were suffering from infection. Fourty one samples of gold fish *Carassius auratus* gave a positive result for the *Vibrio fluvailis*, in percentage (47.6%) . The biochemical tests were conducted to confirm the infection as well as the examination of API 20. Polymerase chain technique was used to identify virulence genes hupO that have a role in the pathogenesis of bacteria isolated.

Key words: *Vibrio fluvailis* , hupO, virulence gene ,golden fish.

عزل وتشخيص جراثيم *Vibrio fluvailis* من الاسماك الذهبية *Carassius auratus* في محافظة النجف

علي عدنان رديف

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الخلاصة:

هدفت الدراسه الى تشخيص جراثيم ***Vibrio fluvailis*** والتعرف على بعض جينات الضراوة, حيث تم اخذ 86 عينه من الاسماك الذهبية *Carassius auratus* من احواض اسماك الزينه كانت تعاني من الاصابه ,41 عينه من الاسماك الذهبية *Carassius auratus* اعطت نتيجه موجبه للجراثيم المذكورة اي بنسبة (47.6 %) حيث اجريت الفحوصات الكيموحيوية وكذلك فحص API 20 , كذلك تم استخدام تقنية سلسلة البلمرة للتعرف على جينات الضراوة *hupO* التي لها دور في امراضية الجراثيم المعزوله.

Introduction:

V. fluvialis is considered to be an emerging foodborne pathogen and has been implicated in outbreaks and sporadic cases of acute diarrhea [1]. Besides, *V. fluvialis* posed a significant economic threat for aquaculture for being pathogenic for cultured fish and lobsters [2].

Gastroenteritis caused by this organism is associated with drinking of contaminated water or consumption of raw or improperly cooked seafood [3].

V. fluvialis associated extra intestinal infections, such as hemorrhagic cellulites and cerebritis , peritonitis , acute otitis , biliary tract infection , bacteraemia and even ocular infections were also reported [4].

Several toxins that may be important in pathogenesis have been reported in *V. fluvialis* including a Chinese hamster ovary (CHO) cell elongation factor, CHO cell-killing factor, enterotoxin-like substance, lipase, protease, cytotoxin, and hemolysin [5]. The cell-free culture filtrates of *V. fluvialis* strains were capable of evoking distinct cytotoxic and vacuolation effects on HeLa cells [6].

The heme utilization protein gene *hupO* in *V. fluvialis* was induced under iron-restricted conditions and is associated with virulence expression through stimulation of *hemolysin* production and resistance to oxidative stress [7].

In spite of many pathogenic factors were characterized, their precise role in producing the clinical manifestations remains to be explored and little definitive information about the pathogenic mechanism of *V. fluvialis* has been achieved. The largest outbreak of *V. fluvialis* infection was reported in Bangladesh between October 1976 and November 1977, with more than 500 patients [8].

In the United States, *V. fluvialis* accounted for 10% of vibrio caused clinical cases along the Gulf Coast [9].

Material and methods :-

1-Bacterial strains and culture condition

Out of 86 samples from fish which collected from Al-najaf city, the positive samples of *V.fluvialis* were included in this study. All strains were maintained in Luria-Bertani (LB) broth supplemented 15% glycerol and stored at −80°C. Of these, eleven of positive samples were isolated from fin of goldfishes. The rest thirty positive samples were isolated skin of gold fishes .

2-Identification

Biochemical characteristics of *V. Fluvialis* strains were plated on LB agar and thiosulphate citrate bile salts sucrose agar (TCBS) followed by incubation at 37°C overnight. API 20E (bio Mérieux) identification strip was used to characterize the biochemical features. Each of the isolates were revived and identified using API20E miniaturized system. They were processed as in the manufacturer instructions.

3-Extrtaction of the total DNA

The organisms were in shake broth culture method at 37C in a shaker incubator for 18 hrs. Dense washed cell suspension were resuspend in TES buffer with 600 ml 25%SDS was added and incubated in 50C water bath for five mines. Cell lysates were cooled to room temperature and mixed with 2ml.5N Nacl through frequent inversions. The resultant
lysates were mixed V:V with chloroform gently by several inversions within 30mins.Then

mixtures were separated by centrifugation at 10 000 rpm in 4C for 15 mines. The aqueous phases were saved into polyethylene tubes. Chloroform treatments were repeated till protein layer was disappeared. The purified DNA extract was concentrated by isopropanol 1:0.6 V:V and the DNA spirals on pasteure pipette, the pellet was dissolved in 1ml.TE buffer and stored at -5C.The to be DNA preparations were loaded on a garose gel electrophoriesis to assure the presence of DNA[10],then transilluminated using the UV transilluminator[11].

4-Amplification of the total DNA

Specific primers and the amplification conditions were presented in tables 1 and 2.

Table(1) Primers and amplification conditions used in this study.

Primer Sequences (5'-3')	**Target size (bp)**	**References**
hupO-F ATTACGCACAACGAGTCGAAC	600	[12]
hupO-R ATTGAGATGGT AAACAGCGCC		

T**able (2) PCR Program That Apply In TheThermocyler**.

Gene	**Initial denaturation**	**denaturation**	**Cycles annealing**	**elongatioin**	**Final elongation**
hupO-F	94ºC for 4 min	94ºC for 40 sec.	30 cycles 56ºC for 30 sec.	72ºC for 1 min	72ºC for 6 min
hupO-R					

RESULTS

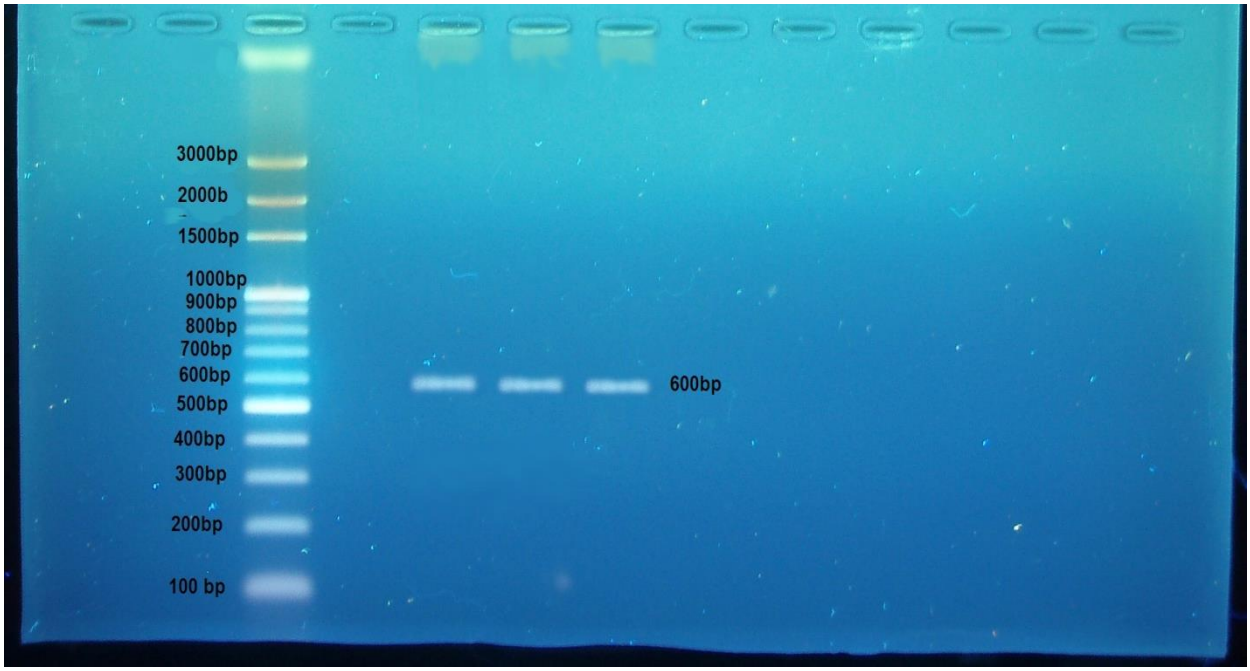
1-Organismic Identity:

Table (3) The vibrio is being identified as *Vibrio fluvialis*.

Tests	Vibrio
ONPG	+
Arginine dihydrolase	+
Lysine decarboxylase	-
Ornithine decarboxylase	-
Citrate utilization	-
H2S production	-
Urease	-
Gelatinase	+
Indole	+
Voges–Proskauer test	+
- Fermentation of	
Glucose	+
Mannitol	+
Inositol	-
Sorbitol	-
Rhamnose	-
Sucrose	+
Melibiose	-
Arabinose	+
Oxidase test	+
Growth in absence of NaCl	-
Growth in 7% NaCl	+
Growth in TCBS	+

3- V.fluvialis Virulence genes

The prepared ,purified and amplified total DNA this V. fluvialis was screened for the presence of the virulence gene *hupO* using their specific primers by PCR technique. It was positive for *hupO* gene Figure 1.



Detection of *hupO* gene by PCR technique is presented (Fig 1).

Fig. 1 Agarose Gel Electrophoresis 1.5% of PCR Amplify of *hupO* gene of *V. fluvialis* isolate for55 min at 100 Volt.

Discussion :-

Vibrio fluvialis is the pathogens which infected humans, as well as in vary aquatic species, either in the wild or in aquatic farms. Therefore, it is important that studies of etiology, as well as epidemiology be carried out for these species in order to establish whether some of their virulence gene. Results of this study showed *V. fl uvialis* strains isolated from infected golden fish and diagnosed by Epi 20 these results contrast with [13].

The clinical signs shown signs lethargy and a loss of appetite in agreement with[14] , the gross lesion include red spots on both skin and fin , fin is dark in color, ascites. The

intestine was distended with clear, viscous fluid, hemorrhage is common in the viscera and around the intestines, with inlargmant and necrosis of kidney , liver and spleen is identical with [15].

The identity the study organisms was consistent with; *Vibrio fluvialis* [16].

The obtained pure total DNA preparations were expected to

contain DNA segments of different sizes that might or might not bear specific genes . Virulence genes *hupO* was equally detected in bacterial isolates. The heme utilization protein gene hupO in V. *fluvialis* was induced under iron-restricted conditions and is associated with virulence expression through stimulation of hemolysin production and resistance to oxidative stress [17].

In spite of many pathogenic factors were characterized, their precise role in producing the clinical manifestations remains to be known and little definitive information about the pathogenic mechanism of V. *fluvialis* has been achieved. These results agreement with [18] and disagreement with [19].

The results indicate the higher risk and potential public health threaten of seafood contaminated by *V. fluvialis*.

Conclusions

The *hupO* was detected in isolate of *V. fluvialis*.

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