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Vibrio Species Isolated from Farmed Fish in Basra City in Iraq

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Abstract

Aim: This study was carried out to investigate the occurrence of potentially pathogenic species of *Vibrio* in seven types of fish sampled from fish farms located in different districts in Basra governorate, Iraq.

Methods and Results: A total of 153 live fishes was collected from fish farms during the period January-May 2016. Bacteria were isolated using selective medium thiosulfate citrate bile sucrose salt agar. Presumptive *Vibrio* colonies were identified using the VITEK 2 system and selected biochemical tests. In the present study *V. alginolyticus* (24 of 60) was the predominant species, followed by *V. cholerae* (10 of 60), *V. furnisii* (10 of 60), *V. diazotrophicus* (7 of 60), *V. gazogenes* (5 of 60) and *V. costicola* (4 of 60). The signs of vibriosis appeared in three types of fish, including *Cyprinus carpio*, *Coptodon zilli* and *Planiliza subviridius* in spite of the using Oxytetracycline in most fish farms.

Conclusion: The results of the present study demonstrated the presence of pathogenic *Vibrio* species nearly in all fish farms. So the farm owners should be concerned about the presence of these pathogenic bacteria which also contributes to human health risk and should adopt best management practices for responsible aquaculture to ensure the quality of fish.

Keywords: *Vibrio* spp; Vibriosis; *Cyprinus carpio; Coptodon zilli; Planiliza subviridius*

Introduction

The world fish production has grown recently- as a consequence of the decline production in capture fishery- with food fish supply increasing at an average annual rate of 3.2 percent, above the growth of the world population to 1.6 percent. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012. According to the latest available statistics collected globally by FAO, world aquaculture production attained another all-time high of 90.4 million tons in 2012, including 66.6 million tons of food fish and 23.8 million tons of aquatic algae [1].

But this worldwide growth of aquaculture is overwhelmed by catastrophic fish diseases and spoilage caused by pathogenic bacteria, which are introduced to the fish farm through natural or artificial food sources, treated inlet water or through vertical transmission from brood stock [2-4]. The most diseases are caused by *Vibrio* spp., which are considered the well-known cause of a significant problem for the development of a sector with strong economic losses worldwide because of its high morbidity and mortality rates (mortality ≥ 50%) [5-8]. The Centers for Disease Control and Prevention [9] estimates that vibriosis causes 80,000 illnesses each year in the United States. About 52,000 of these illnesses are estimated to be the result of eating contaminated food and about 80% of infections occur between May and October when water temperatures are warmer.

Several factors have been proposed to influence the survival, persistence and ability of vibrios to cause infection. These include water temperature, UV or sunlight and salinity [10]. Many studies have been conducted on seasonal variation of pathogenic *Vibrio* species in natural environments [11-15].

However, there is a little information pertaining to vibriosis and the presence of *Vibrio* species in the fish farms especially in Iraq. Hence, this paper attempts to describe the presence of *Vibrio* spp in different types of fish.

Materials and Methods

Fish samples collection

A total of 153 live fish was collected from fish farms located in different districts in Basra governorate, Iraq (Figure 1), over a five month period (January- May 2016). The parameters of water have been measured. Fish Samples included common carp (*Cyprinus carpio*) (65), silver carp (*Hypophthalmichthys molitrix*) (15), sea bream (*Acanthopagrus arabicus*) (6), green mullet (*Planiliza subviridus*) (23), molly fish (*Poecilia latipinna*) (15), Bue tilapia (*Oreochromis aureus*) (15) and redbelly tilapia (*Coptodon zilli*) (14). Live fish samples were transported to the laboratory within a few hours, the measurements of the total length and body weight are recorded (Table 1).

Bacterial isolation and identification

The fish were killed by physical destruction of the brain, in order to prepare the samples for bacterial isolation. Initially a swab was taken from skin, fins and eyes while 1 gm of the gills and intestine were incised aseptically using a sterile scalpel. These samples were homogenized in 9 ml of sterile normal saline solution using a sterilized glass homogenizer (Brand- Germany). One milliliter aliquots of the homogenate solutions were serially diluted (10^{-1} to 10^{-7}). Aliquots of 0.1 ml of the serial dilutions were inoculated onto thiosulfate citrate bile sucrose salt agar (TCBS) (Hi media- India) in duplicate using the spread plate method and the plates were incubated at 30°C for 24-72 h.

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The presumptive *Vibrio* colonies, yellow- greenish- blue, on TCBS agar were picked and subjected to bacterial identification using VITEK 2 system (Biomerieux- USA) and biochemical tests [16] such as: oxidase test, H_2S production, urease, indole production, Voges- Proskauer, fermentation of: glucose, lactose, inositol, raffinose, mannitol, dextrose, adonitol, fructose, dolcitol, xylose, arabinose, trehalose, salicin, rahmnose, milibiose, galactose, sorbitol, sucrose, mannose and inuline.

Results and Discussion

Water quality parameters

The study was conducted during January- May, 2016 in which there is a fluctuation in water quality parameters in the aquaculture throughout the sampling sites (Table 2). The mean of temperature fluctuated from 24.5°C to 30.1°C. Meanwhile the mean of salinity was recorded to range from 1.23 to 6.22 ppt. The pH was relatively from 6.5 to 7.2.

The fish in a culture system always exposed to a variety of stressors which including high stocking density, handling, transportation and poor water quality [17]. On the other hand, fish immunity is reduced during a stressful event which causes the fish to become susceptible to disease infection [18]

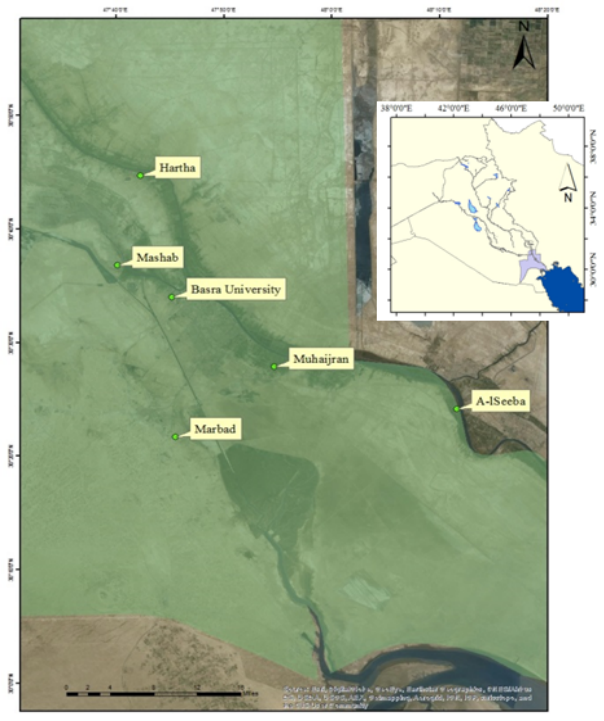

Figure 1: A map of sampling sites.

Fish Species	Total Length, cm Range (Mean)	Body Weight, gm Range (Mean)
Cyprinus carpio	16-49 (36.44)	13.0-2353.9 (890.4)
Hypophthalmichthys molitrix	32-54 (32.66)	65.0-1360 (653.3)
Acanthopagrus aerobics	15-29 (28.3)	13.0-123.8 (22.4)
Oreochromis aureus	12.3-14.2 (12.62)	10.0-62.0 (42)
Coptodon zilli	12-14 (12.23)	10.0-65.0 (40)
Planiliza subviridus	11.3-16.4 (14.23)	12.0-650 (42)
Poecilia latipinna	5.2-6.5 (4.21)	4.0-6.44 (3.88)

Table 1: Physical measurements taken at the time of collection.

Station	Temp (°C) Range (Mean)	Salinity ppt Range (Mean)	PH Range (Mean)
Hartha	18.5-32.4°C (24.5)	1.32-4.00 (1.34)	7.9-8.2 (7.1)
Mashab	13.0-33.0°C (29.8)	0.50-1.80 (1.23)	6.9-7.7 (6.8)
Basra University	19.0-32.0°C (26.4)	1.60-4.50 (2.1)	7.3-7.9 (7.1)
Muhaijran	16.0-30.0°C (26.2)	1.80-3.70 (1.54)	7.5-8.4 (6.5)
Seeba	12.0-30.0°C (28.2)	3.20-12.60 (6.22)	7.1-8.4 (7.2)
Marbad	18.0-34.0°C (30.1)	4.10-5.30 (4.5)	7.0-7.4 (7.1)

Table 2: The water parameters of studied stations.

Tests	*V. gazogenes*	*V. alginolyticus*	*V. costicola*	*V. furnisii*	*V. diazotrophicus*
Oxidase	-	+	+	+	+
Nitrate Reduction	-	+	+	+	+
Indole	-	+	-	-	+
V-P	-	+	+	-	-
H_2S	ND	-	-	-	-
Urease	ND	-	ND	-	ND
Fermentation of Glucose	-	-	+	+	-
Lactose	+	-	-	-	+
Inositol	-	-	-	-	-
Raffinose	-	-	-	-	-
Mannitol	+	+	+	+	+
Dextrose	+	+	+	-	+
Adonitol	-	-	-	-	-
Dolcitol	+	-	-	-	-
Xylose	+	-	-	-	+
Arabinose	+	-	-	+	+
Trehalose	-	+	+	+	+
Salicin	+	-	-	-	+
Rahmnose	-	-	-	-	-
Galactose	+	+	-	+	+
Sorbitol	+	-	-	-	-
Sucrose	+	+	+	+	+
Mannose	+	+	-	+	-
Inuline	-	-	-	-	-
Milibiose	-	-	-	-	-
+: Positive, -: Negative, ND: Not Determined					

Table 3: Biochemical profile of *Vibrio* sp.

The growth of *Vibrio* in water is increased by high levels of organic matters, high salinity, high water temperature (25°C to 32°C) and pH (5-9). While the low salinity and high pH (>9.5) had shown to reduce the growth of this bacterium [19]. These favorable conditions for *Vibrio* were also observed in the present study.

In the present study, three types of aquaculture systems have been studied, the net cage aquaculture in Hartha station, which lies in the north of Shatt Al-Arab River. The second type is the terrestrial pond, which takes water either from Shatt Al-Arab River (Basra University, Muhaijran and Seeba stations) or from artesian wells as Marbad station. The third is Mashab station (net cage), which represent as a part of Hor Al-Hammar marsh and either take water from Shatt Al-Arab River and general downstream. There is an obvious effect of the temperature and salinity on the infected fish, especially in Seeba and Marbad stations. The present study agreed with Le Roux et al. [20] who reported that, *Vibrio* abound in the warm (>15°C) and saline aquatic environments.

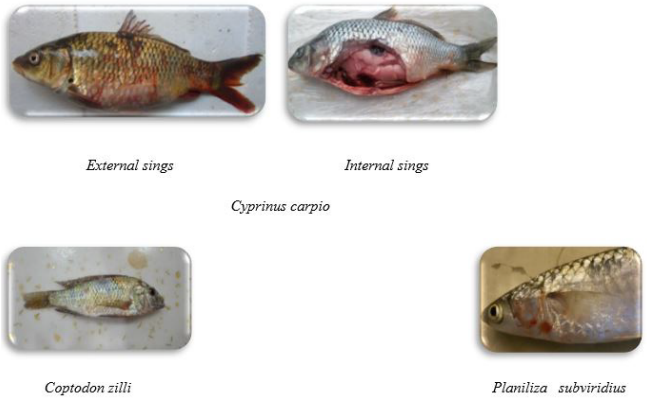

Figure 2: Vibriosis in an infected fish.

Fish spp	Total Exam. Fish	Total Infected Fish	Disease
Cyprinus carpio	65	47	Vibriosis, Spring Viraemia of carp Virus (SVCV), Fin rot, Dropsy, Bacterial gill disease
Hypophthalmichthys molitrix	15	2	Viral hemorrhagic septicemia
Acanthopagrus arabicus	6	2	Pox disease
Oreochromis aureus	15	-	
Coptodon zilli	14	6	Vibriosis
Planiliza subviridius	23	5	Vibriosis
Poecilia latipinna	15	5	Red mouth

Table 4: The types of fish and their disease signs.

Finlay and Falkow [21] observed that, the occurrence of high total *Vibrio* count in the biofilm at high temperature concurred with the occurrence of disease outbreaks. Albert and Ransangan [17] revealed that, the water temperature has shown to play an important role in enhancing the growth of *Vibrio* spp, causing fish to stress and inducing severe vibriosis outbreak. Kaspart and Tamplin [22] noticed that, the optimal temperatures of survival of *V. vulnificus* 4965 was between 13 and 22°C in 10-ppt (noninhibitory salinity) sterile seawater, while the temperature outside this range reduced the time of survival.

Bacterial diagnosis

A total number of 153 fish were sampled during the study. Almost all the primary isolates from sampled organs (skin, round mouth, fin base, gill cover and intestine) showed green and yellow colonies on TCBS. From the samples of fish examined, the average rate of infection was 43.79%, including 15.03% of them as a bacterial infection and identified as *Vibrio* spp. Two methods were used for identification of *Vibrio spp.*, the VITEK 2 system which detect only *V. cholerae* (10 isolates) with probability 98% and confidence, excellent identification, while the other isolates were failed to identify with it, so its identified depending upon their biochemical profiles (Table 3) as *V. gazogenes* (5), *V. alginolyticus* (24), *V. costicola* (4), *V. furnisii* (10) *and V. diazotrophicus* (7).

The results of the present study indicated that, the infected fish display skin discoloration, red patches around the base of the fins and mouth and necrotic intestine. These signs have appeared in three types of fish, including *Cyprinus carpio, Coptodon zilli and Planiliza subviridius* (Figure 2 and Table 4). The infected fish were sampled from all stations (except Basra university station).

In the present study, although the managers of the farms stated that, they use Oxytetracycline (1%) in all stations. Oxytetracycline is widely used to treat bacterial infections in aquaculture farms, such as vibriosis and furunculosis [23,24]. In spite of that, many infected cases have been detected among fish, particularly common carp, and this is may be related to that, the an extensive use of antibiotics can cause the development of antibiotic-resistant pathogens which can infect both cultured animals as well as humans [25-27].

V. cholerae was the most species isolated from intestinal necrosis in common carp and the infections were distributed in all stations (except Basra University station), while other species of *Vibrio* were isolated from external infections.

In the present study *V. alginolyticus* (40%) was the predominant species, followed by *V. cholerae* (16.6), *V. furnisii* (16.6%), *V. diazotrophicus* (11.6), *V. gazogenes* (8.3%) and *V. costicola* (6.66%). The species of *Vibrio* were different between farms and this is may be related to the different source of larvae or different source of water. This agreed with Bhaskar et al. who reported the presence of *V. alginolyticus* as the most common, followed by *V. cholerae, V. parahemolyticus*, and *V. vulnificus* in *P. monodon* culture system. Sanjoy et al. [28] isolated five species of *Vibrio* from shrimp farm and found that, *V. cholerae* was the most common species.

Many researchers found that, the virulence level of Vibriois dependent on fish species, doses of infection, the time of exposure and age of host species and pathogenic factors of the bacterial strains [29-32].

Conclusion

The results of the present study demonstrated the presence of pathogenic *Vibrio* species as *V. cholerae, V. gazogenes, V. alginolyticus, V. costicola, V. furnisii* and *V. diazotrophicus* nearly in all fish farms. So the farm owners should be concerned about the presence of these pathogenic bacteria which also contributes to human health risk and should adopt best management practices for responsible aquaculture to ensure the quality of fish.

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