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Bacterial Shrimp Disease in Iraqi Marine Waters

Abdul Amer R. Jassim, Eman A. Al-Imara* & Nadia A.H. Al-Shammari

Marine Science Centre, University of Basrah, Basrah, Iraq

*Corresponding author: eman.abdalali@uobasrah.edu.iq

Abstract: A sample of 116 shrimps *Metapenaeus affinis* (H. Milne-Edwards, 1837) were collected from local fish markets in Basrah Province, Southern Iraq. Height, weight, and pathological signs of the shrimps were recorded. Both morphological and biochemical examination by VITEK identification system were undertaken. Pathological infections were seen on the cuticle of abdominal segments in addition to a severe infection in uropod (tail segment), rostrum and pleopods (walking legs). Results of biochemical identification showed the presence of *Aeromonas sobria* and *A. salmonicida* from the infected organs and this study is considered as the first record of this bacterial infection in shrimps in Iraq.

Keywords: Bacteria, Shrimp, *Metapenaeus affinis*, *Aeromonas sobria*, *A. salmonicida*, Iraq

Introduction

Shrimps are considered as one of the most important seafood distributed in the Iraqi water and consumed by people in southern portion of the country. It has a significant role in the aquatic ecosystem with respect to biodiversity and its place in the food pyramid in the environment (Jassim, 2013). Shrimps are low in calories with a balance of healthy proteins and fats (Dayal et al., 2013). They also contain vitamins and mineral treasure trove. On the negative side, many bacterial diseases infect them to affect their health and compromise the hygiene. They can also be charged as a vector of foodborne infections ranging from mild gastrointestinal upset to life-threatening diseases (Fadel & El-Lamie, 2019).

In the last decade, the prevalence of viral and bacterial diseases in farmed shrimps has been high, probably due to fluctuations in temperature, salinity, oxygen, pH and water nutrients (Morales-Covarrubias et al., 2018). Environmental conditions and containment are two important triggers for the rapid proliferation of opportunistic bacteria in the digestive tract, gills and shrimp cuticles as well as in the sediments of water, feed and pond (Alfiansah et al., 2018). Important constraints of shrimp aquaculture include improper farm management and lack of services like technical support, quality of shrimp seeds and infrastructure. Moreover, bad weather, conflict among local people and risk of disease has a big impact as well (Xiong et al., 2015). Exoskeleton provides an efficient physical

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shield against pathogens that attempt to penetrate the outer surface of shrimps, while the intestine is another defensive shield to keep pathogens out. However, certain chitinoclastic (or chitinolytic) bacteria are associated with exoskeletal disease and can also enter via wounds (Mancuso et al., 2010).

Aeromonas is a genus of Gram-negative rods that are widely distributed in freshwater, estuarine and marine environments. They are increasingly identified as important pathogens for marine, terrestrial and human animals (Janda & Abbott, 2010). Its species grow at a range of temperatures, although in warmer months (from May through October in the northern hemisphere) they are isolated with increasing frequency. *Aeromonas* species also causes a wide range of syndromes between homeothermic and poikilothermic animals. This includes fishes, reptiles, amphibians, mammals and even humans (Morris et al., 2021). These bacteria cause hemorrhagic septicemia, fin rot, rot in the soft tissue and furunculosis. Epizootic ulcerative syndrome (EUS) in fish farms of various parts of Southeast Asia, India and Bangladesh are the major examples (Aberoum & Jooyandeh, 2010).

According to Sivasankar et al. (2017), the major marine wild species of prawns and shrimps are the white prawn (*Penaeus indicus*), tiger prawn (*Penaeus monodon*), pink shrimp (*Metapenaeus dobsoni*), Jinga shrimp (*Metapenaeus affinis*) and marine shrimp (*Paraenaeopsis stylifera*). Most health problems of shrimps belong to viruses and bacteria. Flegel (2012) mentioned that approximately 60% of disease loses in shrimp aquaculture is caused by viral pathogens and 20% by bacterial pathogens. In comparison to these, loses to fungi and parasites are relatively low. Available studies on shrimp diseases in Iraq are rare. There is only one study (Jassim & Al-Salim, 2015) on shrimp viral diseases. The first report of the infection of the shrimp (*Macrobrachium nipponense*) by the *Vibirio* bacterial infection in the Iraq waters was by Abbas et al. (2021).

The goal of this work was to chronicle the first case of this bacterial disease in shrimp in Iraqi marine water, as well as isolate and identify the causal bacterial species.

Materials and Methods

A total of 116 shrimps, *Metapenaeus affinis* (H. Milne-Edwards, 1837) were collected from local fish markets in Basrah province (Central Basrah Market, 5 Mile market and Al-Jumhouria Market). The samples were transported to the laboratory under cooling conditions within few hours. Length, weight and morphological examination were undertaken. Pathological infections were recorded.

Ten gm of infected tissues from uropod (tail segment), rostrum and pleopods (walking legs) were taken, put in homogenizer (Brand- Germany), 90 ml of normal saline were added to the tissues and homogenized, serial tenfold dilutions (10^{-1}-10^{-6} ml) were done and inoculated on two types of growth media: TCBS agar (Oxoid, England) for isolating *Vibrio* species and Tryptic Soy agar (TSA) (Himedia, India). The plates were incubated for 48 hours at 37 °C. Grown colonies were subcultured to obtain pure colonies. Both morphological (Cappuccino et al., 2019) as well as biochemical examinations were performed by VITEK II compact cards in Bayan Group for Advanced Laboratory Diagnostics, Basrah, Iraq.

Results and Discussion

Gross Signs

Lengths of shrimp specimens ranged between 7 and 11 cm, whereas weights ranged between 4 and 14 gm. As shown in Figures 1-4, pathological infections were seen on the cuticle of abdominal rings in addition to a severe infection in uropod (tailed segment), rostrum and pleopods (walking legs).

Identification of *Aeromonas* Species

All of the isolates which obtained from the infected organs were belonged to the genus *Aeromonas*, which were Gram-negative, rod-shaped bacteria grown on TSA plates. They also developed yellow colonies of TCBS agar. *A. salmonicida* as well as *A. sobria* have been biochemically identified by VITEK GN cards (Tables 1 & 2).

Figure 1: Severe infection in rostrum.

Figure 2: The severe infection in uropod.

Figure 3: Severe infection in the abdominal rings.

Figure 4: Severe infection in pleopods.

Table 1: Results of biochemical identification of *Aeromonas sobria* by VITEK system.

2	APPA	+	3	ADO	+	4	PyrA	_	5	IARL	_	7	dCEL	+	9	BGAL	+
10	H2S	_	11	BNAG	+	12	AGL Tp	_	13	d GLU	+	14	GGT	(+)	15	OFF	+
17	BGLU	_	18	DMAL	+	19	dMAN	+	20	Dmne	+	21	BXYL	_	22	BAlap	_
23	ProA	+	26	LIP	+	27	PLE	_	29	TyrA	+	31	URE	_	32	dSOR	_
33	SAC	+	34	d TAG	_	35	d TRE	+	36	CiT	+	37	MNT	_	39	SKG	_
40	ILATK	_	41	AGLU	_	42	SUCT	+	43	NAGA	_	44	AGAL	+	45	PHOS	_
46	GlyA	_	47	ODC	_	48	LDC	_	53	IHISa	_	56	CMT	+	57	BGUR	_
58	0129R	+	59	GGAA	+	61	IMLTa	+	62	ELLM	+	64	ILATa	_			

(+) Indicative of week reaction that is too close to the test threshold.

Table 2: Results of biochemical identification of *Aeromonas salmonicida* by VITEK system.

2	APPA	+	3	ADO	_	4	PyrA	_	5	IARL	_	7	dCEL	_	9	BGAL	_
10	H2S	_	11	BNAG	_	12	AGL Tp	_	13	d GLU	_	14	GGT	_	15	OFF	_
17	BGLU	_	18	DMAL	_	19	dMAN	_	20	dMNE	_	21	BXYL	_	22	BAlap	_
23	ProA	+	26	LIP	_	27	PLE	_	29	TyrA	_	31	URE	_	32	dSOR	_
33	SAC	_	34	d TAG	_	35	d TRE	_	36	CiT	_	37	MNT	_	39	SKG	_
40	ILATK	_	41	AGLU	_	42	SUCT	_	43	NAGA	_	44	AGAL	_	45	PHOS	_
46	GlyA	_	47	ODC	_	48	LDC	_	53	IHISa	_	56	CMT	_	57	BGUR	_
58	0129R	_	59	GGAA	_	61	IMLTa	_	62	ELLM	_	64	ILATa	_			

Results of VITEK II system showed the presence of *Aeromonas sobria* in infected shrimp. Also, the results of VITEK recorded *A. salmonicida*. This indicated that the shrimp is a new host for these two bacterial species. A total of 24 recognized species are currently known for the genus *Aeromonas*. Amongst them, *A. hydrophila*, *A. salmonicida*, *A. veronii*, *A. caviae*, *A. bestiarum*, as well as *A. jandaei* have been identified worldwide as primary or secondary fish pathogens (Liu et al., 2020).

Aeromonas species are ubiquitous aquatic ecosystem inhabitants and reported as important pathogens for both aquatic and terrestrial animals including humans (Janda & Abbott, 2010). *Aeromonas* species are Gram-negative and optional anaerobic bacteria with a wide range in several ecosystems. At refrigeration temperature (4 °C), these bacteria can grow and produce enterotoxin and haemolysin. *Aeromonas* are potential agents of foodborne diseases since they have been isolated from fresh and raw foods. Cases of gastroenteritis, septicaemia, wound, soft tissue and skin or blood infections in humans have been associated with different species of *Aeromonas* (Martins et al., 2002). The reason behind the spread of this bacterium may be attributed to injuries, seasonal changes, drastic variations in water temperature, and inadequate sanitation or nutrition can all put the fish in a vulnerable position to germs.

Prevalence of *Aeromonas* Infections

Table 3 showed that among the 116 examined samples, the overall prevalence of *Aeromonas* was 21.55% (25 samples). These results are higher than what was recorded by Fadel & El-Lamie (2019) who recorded prevalence of infection as 16 (9.41%) of 170 tested shrimp.

Table 3: Prevalence of infection with *Aeromonas* in *M. affinis* samples tested.

Infected region	No. of infected shrimps
Swimming feet	12
Abdominal rings	4
Rostrum and uropod	9

Total samples= 116
Total infected samples= 25

$$\text{Prevalence} = \frac{\text{Infected number}}{\text{Total number}} \times 100 = 25/116 \times 100 = 21.55\%$$ (Fadel & El-Lamie, 2019).

Infection is found most severely in pleopods, uropod and rostrum, respectively. The reason is that the shrimp is a benthic animal, and therefore the parts that come into contact with the bottom and places with a high bacterial content are more vulnerable to infection. In a study carried out by Nimrat et al. (2008) about the effect of different shrimp pond bottom soil treatment on the change of physical characteristics and pathogenic bacteria in pond bottom soil, they found that treatment and improving the quality of the bottom is of great importance in removal of *Vibrio* and *Pseudomonas* from shrimp pond bottom soil containing organic sludge. Recording and monitoring bacterial infections in shrimp, in addition to its importance in the field of shrimp health, gives an important indication of the extent of the deterioration in the quality of the environment. The available studies in the field of shrimp diseases in Iraq are almost non-existent, and this study is the first of its kind to record this disease. These results were in agreement with what was recorded by Alavandi et al. (1995) who attributed the black colour of infected organs to the presence of *Vibrio* and *Aeromonas* species.

In conclusion, study of bacterial diseases in shrimp in Iraq is still in the beginning because the attention in shrimp breeding field in Iraq is absent, so the researches have focused on fish bacterial disease. The distinct prevalence of infection and the damage resulted from the infection may be considered as an indicator for microbial load which exist in marine environment.

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