VIBRIOSIS IN INDOOR AQUARIA AND OFFSHORE CAGE CULTURED TRIDACNA MAXIMA AND TRIDACNA GIGAS IN HURGHADA, RED SEA PROVINCE, EGYPT

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ABSTRACT

The Mariculture of *Tridacna* is greatly affected by pathogenic bacteria including those of vibrio species that result in high losses The present work was designed to investigate the most common bacterial infections of indoor aquarium and offshore cages culture *Tridacna maxima* and *Tridacna gigas* in Hurghada, red sea governorate, Egypt. Eighty Specimens of booth *Tridacna maxima* and *Tridacna gigas* in indoor aquarium and in offshore cages were subjected to clinical and bacteriological examination. The bacteria were recovered from gills, gut mantel and gonads of collected *Tridacna* samples. Decrease in movement and loss of attractive color pattern with 25 % and 20 % mortality rate were detected in indoor cultured *Tridacna maxima* and *Tridacna nagigas* respectively, while no changes in clinical picture or postmortem findings were detected in offshore cages culture *Tridacna*. 46 bacterial isolates were obtained and were identified by phenotypicall characterization and biochemical tests as Vibrio logei, V harveyi and unidentified Vibrio species. Experimental infection with V. logei proven its pathogenicity to *Tridacna* with 30% mortality rate.

Key words: Vibriosis, *Tridacna maxima, Tridacna gigas, V. logei and V. harveyi,*

INTRODUCTION

Mollusks aquaculture is affected globally by bacterial pathogens which cause great harms in hatcheries (Singh and Azam, 2013; Mahmoud *et al.,* 2013 and Paillard *et al.,* 2009). On of the main cause of the mortality is the genus Vibrio which represent a pathogen of major concern in aquaculture. Photobacterium (Vibrio) damsel, *V. harveyi, V. alginolyticus,* and V. campbelli caused 100 % mortality in *Tridacna gigas* larvae (Sutton and Garrick, 1993) *V. logei* was also reported as apathogenic microorganism to H. harid with 86.7% mortality rate (Mahmoud *et al.,* 2017). Different organs of squid, Sepiola spp. exhabited a mixed colonization population consisting of a dominant species (*V. logei*) and a minor species (*V. fischeri*) (Fidopiastis *et al.,* 1997). However, progressive scenes of high mortality have been recorded, over recent years, because of these microorganisms (Romalde *et al.,* 2013). One of the major serious problems in mollusks aqua farming is the high rate of mortality, which effectively decrease the production. larval and post-larval stages as well as juvenile and adults were affected with disease outbreaks in hatcheries and natural environment respectively. In hatcheries, the economic losses resulted from the massive mortalities that may involve the whole loss of the production. The etiological agent is not detected usually in the outbreak, which lead to high mortalities or even complete loss of the production stocks, usually (Prado *et al.,* 2005). The aims of current study were to evaluate bacterial diseases which infect the indoor aquarium and offshore cages cultured *Tridacna maxima* and *Tridacna gigas* and detect the effect of changes in environmental factors on the culture *Tridacna* spp. Alsoto determine the pathogenicity of the recovered bacteria.

MATERIALS AND METHODS

Tridacna spp. Sample

Forty reared specimens of *Tridacna maxima* and *Tridacna gigas* were collected from the cultured cages located in the reef area about 130 m from the shore of the National Institute of Oceanography and Fisheries (NIOF), Hurghada (27°17'37"N, 33°47'10"E). Also, forty reared specimens were taken from the indoor aquaria of the National Institute of Oceanography and Fisheries (NIOF), Hurghada and subjected for clinical and bacteriological examination.

Water quality

Water samples taken from the indoor aquarium and directly from the red sea at the study area in clean dry sterile dark brown bottles. pH and temperature were detected by digital combo pH meter and thermometer (Hanna instruments Inc., USA), total ammonia was detected and dissolved oxygen (DO) concentration were measured using a digital dissolved oxygen meter (HI 9142 - Hanna instruments Inc., USA).

Bacterial isolation and identification

Samples for bacterial isolation were taken under aseptic procedures from gills, mantel, gonads and gut

of *T. maxima and Tridacna gigas.* These samples were cultured on plates of tryptone soya agar and brain heart infusion agar (Oxoid) supplemented with 1.5% (w/v) sodium chloride (TNA). The inoculated plates were incubated at 25°C for up to 48:72hrs. (Suttona and Garrick 1993).

The obtained bacterial colonies were purified using thiosulphate citrate bile salt sucrose agar (TCBS, Difco) and the conventional biochemical techniques were used for characterization according to Nicky (2004). API20E (bioMerieux). Galleries were used also for further biochemical identification according to the manufacturer's instructions.

Experimental infection (challenge)

Twenty *Tridacna gigas* individuals were acclimated to lab. Condition for 2 weeks in the fibro glass tanks and then subdivided into two groups (10 individuals each). The first group was infected by bath immersion in 5L glass aquaria containing $1.5x10^6$CFU of *V. logei* \ ml for 30 minutes as was previously described by (Martins *et al.*, 2010). The second group act as control non infected group. Both 2 groups were closely observed for tow months

RESULTS

Clinical findings

The infected *Tridacna maxima* and *Tridacna gigas* showed decrease in movement and remain stable at the tanks, the Characteristic combination of blue, green, brown, purple and yellow color patterns faded out (fig.1). Mortality rates were 25% and 20% in indoor culture maxima and gigas respectively. Postmortem examination revealed sever congestion in gut, mantel and gonads of culture *Tridacna*, ulceration in mantel and presence of green and dark lines in the inner surface of the shell (fig. 2), no changes in clinical picture or postmortem findings were detected in offshore cages culture *Tridacna* (fig.3).

Water parameter

The results revealed increase of pH and ammonia levels and decrease of dissolved oxygen in the water samples of indoor aquaria, table (1).

Results of bacteriological isolation and identification

Nine bacterial isolates were isolated from gut of offshore cages cultured maxima while 13 bacterial isolates were isolated from mantel, gonads, gills and gut of indoor aquarium *T. maxima* (table2). Ten bacterial isolates were isolated from gut of offshore cages cultured *T. gigas* while 14 bacterial isolates were isolated from mantel, gonads, gills and gut of aquarium gigas (table 3). Forty six bacterial strains were isolated from *T. gigas* and *T. maxima*. The 46 isolates were not biochemically similar and identified through their morphology, conventional biochemical tests and API20E tests *Vibriologei* was the most dominant pathogen (30 isolates), 12 isolates were identified as *Vibrio harveyi* and 4 isolates identified as unidentified *Vibrio species* (Table 4and 5).

Experimental infection (challenged)

The experimentally challenged *Tridacna gigas* showed sluggish hed in motility with mortality rate of 25**%**. The postmortem examination revealed sever congestion in gut and gonads and ulceration in mantel the recoverd bacteria from gut, gonads and mantel was identical to *V. logei***.**

Fig. 1: *Tridacnagigas* showed faint brown, purple color patterns

Fig. 2: *Tridacna gigas* showed green and dark lines in the inner surface of the shell

Fig. 3: *T. maxima* showed attractive color patterns

Table 1: Tested Water quality parameters

Item	Indoor aquarium sample	Red sea sample
Water temperature	27 °C	25°C
pH values	8.1	7.6
Dissolved oxygen	4.1 mg/ L	5.6 mg /L
total ammonia	0.004 mg /L	0.0007 mg /L

Table 2: Bacteriological isolation from *Tridacna Gigas*

Samples			**Growth on culture media**
Type		**No.**	**No.**
offshore cages culture *T. gigas*	Mantel	20	0
	Gut	20	10
	Gonad	20	0
	gills	20	0
Indoor aquarium *T. gigas*	Mantel	20	2
	Gut	20	9
	Gonad	20	1
	gills	20	2

Table 3: Bacteriological isolation from *Tridacna maxim*

Samples			**Growth on culture media**
Type		**No.**	**No.**
Offshore cages culture *T.maxim*	Mantel	20	0
	Gut	20	9
	Gonad	20	0
	gills	20	0
Indoor aquarium *T. maxima*	mantel	20	2
	Gut	20	7
	Gonad	20	1
	gills	20	3

Table 4: Identification of bacterial isolates from indoor aquarium and offshore cage cultured *t.gigas* and *T.maxima*

source	No. of isolates	identification
Offshore *T.gigas*	8	*V.logei*
	2	Unidentified vibrio species
Indoor *T.gigas*	9	*V.logei*
	5	*V.harveyi*
Offshore *T.maxima*	7	*V.logei*
	2	Unidentified vibrio species
Indoor *T.maxima*	6	*V.logei*
	7	*V.harveyi*

Table 5: Cultural and biochemical characterization of the *Vibrio logei* and *V.harveyi* isolates.

Items	*Vibrio logei*	***V.*** harveyi
Colony shape	Round	Round
Colony colour	White creamy	White creamy
Motility	+	+
Gram stain	-Ve rods	-Ve slightly curved rods
Cytochrome oxidase	+ Ve	+ Ve
Catalase	+ Ve	+ Ve
0% NaCl	-	-
1.5% NaCl	+	+
3% NaCl	+	+
6% NaCl	+	+
ONPG	+	-
ADH	-	-
ODC	-	+
LDC	+	+
CIT	-	+
URE	+	+
H2S	-	-
TDA	-	+
Indole	-	+
VP	-	-
GEL	-	v
Xylose	-	+
Raffinose	-	-
Manitol	-	+
Glucose	-	+
Inositol	-	-
Sorbitol	-	+
Rhaminose	-	-
Sucrose	-	+
Malonate	-	-
Arabinose	-	-
Adonitol	-	-
Lactose	-	-
Salicin	-	v

VP = Voges-Proskauer, GEL = gelatin hydrolysis, TDA= tryptophanedeaminase, ONPG, o-nitrophenyl-b-d-galactopyranoside, v= variable
LDC = lysine V= variable decarboxylase, ADH = arginine dihydrolase, ODC = ornithine decarboxylase, CIT = citrate, URE = urea hydrolysis,

DISCUSSION

Since a few is known about the disease affecting both cultured and wild *Tridacna* spp. so this study focuses on the one of main problems in *Tridacna* spp. aqua culture through plan a map to the bacterial infection affecting the highly economic value and beauty maxima and gigas in Hurghada, Red Sea governorate, Egypt. A total number of 80maximaand gigas were collected from indoor aquarium of NIOF and offshore cage cultures in Red Sea at Hurghada during the period from Januray 2017 to December 2018 .The Clinical and postmortem pictures revealed a decrease in movement and changes in coloration with severe congestion in gut, mantel and gonad in addition to appear once of green and dark lines in the inner surface of the shell of the affected indoor cultured *Tridacna* spp while no postmortem changes were observed in offshore cages cultured *Tridacna* spp., Mortality rates were 25% and 20 % in indoor cultured and gigas respectively. These changes associated with decrease in DO and increase in ammonia level in water the results agreed with Tubiash and Otto (1986), who recorded that the typical signs of *vibriosis* in clam included reduction of motility and movements in circles, velum disruption, visceral atrophy and lesions in the other organs. *Vibriosis* in clam is characterized by changes in the calcification process on the inner surface of the valves and the appearance of a characteristic brown deposit between the edge of the shell and the pallial line (Borrego *et al.*, 1996). The pathogenesis of the disease is favored by the susceptibility of the bivalves, but external stress factors, as poor water quality, high organic matter and other stressors facilitate the propagation of potential pathogenic bacteria. So, in many episodes mortalities can resulted from the overgrowth of opportunist bacteria (DiSalvo *et al.,* 1978, Tubiash and Otto 1986). Regarding the bacteriological investigations, 46 bacterial isolates were recovered from guts mantel, gills and gonad of the indoor cultured spp. and gut only of the offshore cages cultured *spp.* These results indicate that the optimum enviroment for bivalve aquaculture induce the growth of bacteria (Brown and Tettelbach, 1988). Thirty isolates were identified as *V. logei* while 12 isolates were identified as *Vibrio harveyi* by the culture, morphological character and biochemical character including the API20E tests. Similar findings were described by Austin, (2009); Al-Sunaiher *et al***.** (2010), Mahbub *et al*. (2011) and Mahmoud *et al*. (2017). The bacteria belonging to the genera Vibrio and Aeromonas are often pathogenic to larval clams and those in other genera are less frequently pathogenic (Romalde and Barja, 2010, Paillard *et al*., 2009 and Sutton and Garrick 1993) In the present study *V. logei* was the dominant isolates from gut of both indoor aquarium and offshore cages cultured spp. and mantel and gonad and gills of indoor tank culture spp. only followed by *V.harveyi* these findings were in accordance with those of Fidopiastis *et al.* (1998) and Edward and Kyu -Ho (1998) who stated that *V. fischeri, V.logei,* Photobacterium phosphoreum, and P. leiognathi form a variety of pathogenic and cooperative associations with marine animals, these pathogens are increasingly recognized as causes of marine invertebrate diseases where they are a common constituent of intestinal tract microbial inhabitant. Also current results agreed with of Mahmoud *et al.* (2017) who declared that *V. logei*, was dominant bacterial pathogen to *H. harid. V. harveyi* is considered as a caustive agent of summer mortalities in oyster and adult clam since it was isolated from most samples of affected oysters, and it induce mortality in the experimental infection. Mortalities cannot be attributed to the infection with the bacterial pathogen only, but to a complex interaction between the genetic and or physiological state of the bivalves, environmental state and the presence of more than one opportunistic pathogenic *Vibrio species* (Pruzzob *et al.,* 2005 and Labreuche *et al*., 2006). The challenge test show that *V. alogei* was pathogenic to gigas. The experimentally infected individuals revealed clinical picture and P.M lesions similar to those of the naturally infected ones with 25% mortality. Similar findings were reported in experimental infection with different Vibrio spp. in juvenile and adult clams (Beaz-Hidalgo, 2010). In conclusion the offshore cages are much more convenient for *Tridacna* spp. aquaculture in comparison with indoor aquaria. Opportunistic pathogen as *V. logei* and *V. harveyi* must be taken in consideration in T. spp. aqua culture as they may cause serious losses specially when associated with deterioration in environmental factors.

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عدوى الفيبريو فى الترايدكنا ماكسيما والترايدكنا جيجس المستزرعه فى الاحواض الزجاجية والأقفاص البحرية فى الغردقة - البحر الأحمر- مصر

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يتأثر الاستزراع البحري للترايدكنا بصوره كبيره بالبكتريا الممرضة خاصة أولئك المنتمين لسلالات الفيبريو والتي ينتج عن العدوى بها خسائر كبير . تهدف الدراسة الحالية الى بحث ومعرفة العدوى البكترية الأكثر انتشارا وسط مزارع الترايدكنا ماكسيما وترايدكنا جيجس في الأحواض زجاجيه والأقفاص البحرية بالغردقة بالبحر الأحمر بمصر. تم اخذ ٨٠ عينه من كل من الترايدكنا ماكسيما وترايدكنا جيجس من الأحواض الزجاجية والأقفاص البحرية وتم فحصها اكلينيكيا ودراستها بكتريولجيا . اظهرت النتائج عزل البكتريا من الخياشيم والامعاء والمنتل والغدد التناسليه لعينات الترايدكنا المجمعة .لوحظ انخفاض معدل الحركه وفقد الالوان الجذابه المميزه للترايدكنا مصحوب بمعدل نفوق ٢٥ % و٢٠ % فى كل من الترايدكنا ماكسيما وترايدكنا جيجس على التوالى كانت اهم التغيرات فى التريدكنا المستزرعه فى الاحوض القيرو زجاجيه بينما لم يكن هناك اى تغيرات فى الصورة الاكلينيكيه او التشريحيه فى الترايدكنا المستزرعة فى الأقفاص البحرية. تم الحصول على ٤٦ عزله بكتريه وتم تعريفها باستخدام الخواص الظاهرية والاختبارات البيو كيميائيه على انها *Vibrio logei* و *V. harveyi* و *Vibrio* unidentified *species* وبإجراء العدوى التجريبه باستخدام *Vibrio logei* أكدت الإعراض السابقة في الترايدكنا مع معدل نفوق ٣٠ %.