Tenacibaculosis in Picasso Tigger Fish (*Rhinecanthus Assasi*) and Black Damsel Fish (*Neoglyphieodon Meles*) of Red Sea at Hurghada, Egypt

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Abstract: This study reported the first isolation and identification of *Tenacibaculum maritimum* (*T. maritimum*) in Egypt from Picasso Tigger Fish (*Rhinecanthus assasi*) and Black damsel fish (*Neoglyphieodon meles*) in the indoor aquarium of National Institute of Oceanography and Fisheries (NIOF) in Hurghada. The disease onset started after exposing the fish to catching and indoor rearing stress, the diseased fish manifested off food, lethargic and had external body lesions in the form hemorrhagic ulcers, ulcerated mouth and fin rot, in addition to 55 and 65% mortalities rate among the two fish species respectively. The pathogen was recovered from the body surface lesions and internal organs of the examined fish. Eleven isolates were isolated and identified as *T. maritimum* on the basis of morphological and cultural characters, API20E system tests and conventional biochemical tests. It is pathogenic strain caused clinical signs such as lethargic, off food and body surface lesions as white areas with hemorrhagic ulcers on all experimentally infected fish and 60% mortality. The experimentally infected fish could be treated by repetitive enrofloxacin at rate 30 ppm immersion bath for 1 h during three consecutive days.

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1. Introduction

Marine *Tenacibaculosis* is a serious bacterial disease affecting a great variety of marine fish especially cultured species, both adult and young are susceptible but the young fish are seriously affected (**Toranzo *et al.*, 2005)**. It is an ulcerative disease causes massive mortalities and severe economic losses in marine fish cultures in Japan (**Wakabayashi *et al.*, 1986**), Scotland (**Bernardet *et al.*, 1990**), Spain (**Alsina** & **Blanch, 1993**), France (**Bernardet *et al.*, 1994**) and North America (**Ostland *et al.*, 1999**).

Among the cultured fish, *Tenacibaculosis* has been reported in sole, *Solea solea* (L.) (**Bernardet *et al.*, 1990**); Senegalese sole (*Solea senegalensis*) (**Cepeda and Santos, 2002**); Japanese flounder (*Paralichthysolivaceous*) (**Baxa *et al.*, 1986**); turbot (*Psetta maxima*) (**Avendaño -Herrera *et al.*, 2004a**). Also, it is recorded in Atlantic salmon (*Salmo salar* L.,), Rainbow trout (*Oncorhynchus mykiss*), Striped trumpeter (*Latrislineata*), Greenback flounder (*Rhombosolea tapirina*) (**Soltani *et al.*, 1996** and **Handlinger *et al.*, 1997**) and sea bream and sea bass (**Toranzo *et al.*, 2005)**.

It is caused by *T. maritimum* (formerly *Flexibacter maritimus*) (**Suzuki *et al.*, 2001**) which is a filamentous gram negative bacterium and primarily attacks skin, mouth and fins of fish causing severe necrotic and ulcerative lesions on the body surface (**Baxa *et al.*, 1986** and **Toranzo *et al.*, 2005**).

It has been noted that, 55 and 65% mortalities occurred in Picasso Tigger Fish and Black damsel fish in the indoor aquaria of The National Institute of Oceanography and Fisheries (NIOF) at Hurghada, Egypt. The diseased fish were lethargic, off food and had body surface lesions as white area with hemorrhagic ulcers, eroded and haemorrhagic mouth and ulcerative skin lesions. A systemic disease can be also established in the form congestion of spleen and intestine.

The diagnosis of this disease is usually carried out by clinical signs, isolation and biochemical characterization (**Bernardet *et al.*, 1990; Pazos *et al.*, 1993**). Marine agar (MA), Anacker and Ordal agar (AOA) (**Anacker** & **Ordal, 1959**), Huso- Shotts medium (**Chen *et al.*, 1995**) and *Flexibacter maritimus* medium (FMM) has been advocated for the isolation of *T. maritimum* from infected fish **(Pazos *et al.*, 1996)**. However, *Flexibacter maritimus* medium (FMM) has been proposed to be the most appropriate medium for the successful isolation of *T. maritimum* (**Avendaño -Herrera *et al.*, 2005**).

The present study was designed to isolate and characterize the causative agent of *Tenacibaculosis* in Picasso Tigger Fish (*Rhinecanthus assasi*) and Black damsel fish (*Neoglyphieodon meles*) in the indoor

aquaria of National Institute of Oceanography and Fisheries (NIOF) at Hurghada. Moreover, pathogenicity assay and treatment trials with antibiotic were conducted.

2. Material and Methods

Fish :

Forty five clinically diseased and moribund Picasso Tigger Fish and Black damsel fish were collected from the indoor aquaria of The National Institute of Oceanography and Fisheries (NIOF) at Hurghada and subjected to clinical examination (**Kimberley, 2004)** and bacteriological isolation.

Bacteriological samples

Bacteriological samples from external lesions, liver and kidney of several clinically diseased fishes were directly inoculated onto freshly prepared plates of Flexibacter maritimus medium (FMM) (**Pazos *et al.*, 1996**) and Huso- Shotts medium (**Chen *et al.*, 1995**), the two media were prepared with 50% sea water and supplemented with antibiotic (tobramycine). All the inoculated plates were incubated at 25°C for up to 72hrs. Pure colonies of the presumptive *T. maritimum* isolates were picked up and inoculated on sloped FMM and preserved for further studies.

Identification of the isolates

The pure isolates were exposed to conventional bacteriological tests and API20E assay. API20E (BioMèrieux) was used according to the manufacturer's instructions and sterile 50% sea water was used as a diluents and the strips were incubated at 25°C for 24hrs. The eleven isolate were identified according to **Suzuki *et al.* (2001), Buller (2004), Mouriño *et al.* (2008)** and **Avendaño-Herrera (2009).**

Water quality

Water samples were taken from the investigated indoor aquaria and their water source from the red sea (control sample) in dark brown clean and bottles and subjected to DO, pH and total ammonia determination. Water temperature was determined in the aquaria.

Pathogenicity assay

Twenty Black damsel fish were acclimated for one week then subdivided into two equal groups each of 10 fish. The fish of the first group were experimentally infected by bath immersion in sea water bath for 18hrs (**Avendaño-Herrera *et al.*** (**2006a**) with *T. maritimum* suspension containing $1.5x10^6$ cellmL^{-1}.The fish of the second group were submitted to the same procedure without bacteria and used as control. Each fish group was preserved in 110L glass aquarium at water temperature 24±2°C and observed for 14days, the clinical signs and mortalities were recorded.

Treatment trial

Two groups of black damsel fish (each of 10 fish) were subjected to experimental infection with *T. maritimum* as had described in the pathogenicity assay. At the second day of infection, the fish of the first group were subjected to repetitive enrofloxacin treatment at rate 30 ppm immersion bath for 1 h during three consecutive days (**Avendaño -Herrera *et al.*, 2008)** and fish of the second group were left as infected control. The fish of the two groups were subjected to daily inspection for 14 days after treatment and the clinical signs and mortality were recorded.

3. Results

Clinical signs

Generally the diseased fish were off food, lethargic and had external body lesions, the diseased Picasso Tigger fish showed hemorrhagic ulcers **(Photo - 1)** and eroded and ulcerated mouth **(Photo - 2)** but the diseased Black damsel fish showed ulcerated skin lesions surrounded with white batch of necrotizing tissues **(Photos – 3- 5)** and fin rot, **photo (5).** 55 and 65% mortalities were reported among the two fish species respectively.

Photo (1) Picasso Tigger fish showed congested ulcer on the skin.

Photo (2) Picasso Tigger fish showed eroded and ulcerated mouths.

Photos (3 & 4) Black damsel fish showed haemorrhagic ulcer surrounded with necrotizing tissues

Photo (5) Black damsel fish showed haemorrhagic ulcer and fin rot

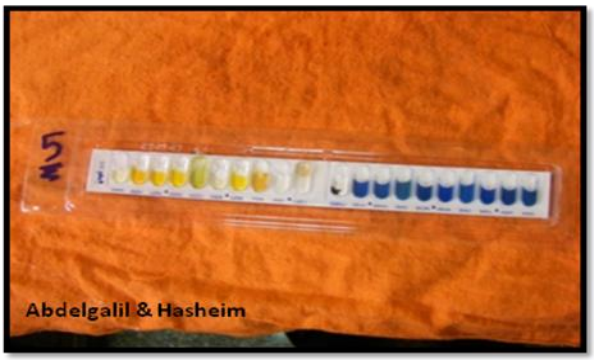

Photo (6) API20E results

Isolates identification

The eleven isolates were biochemically similar and identified through their morphology, conventional biochemical tests and API20E system tests as *T. maritimum* (Table, 1 and Photo, 6).

Water quality

The examination of water sample from the indoor aquaria revealed deterioration in the water quality in the form of increase total ammonia and decrease dissolved oxygen with alkaline pH comparing with the criteria of the control water sample from the red sea (Table 2).

Pathogenicity assay

The experimentally infected black damsel fish showed lesions similar to those of naturally infected fishes such as off food, lethargy, skin hemorrhagic ulcers, eroded and ulcerated mouth and these clinical signs started from the third day post-infection. By the end of the observation time (14 days) the mortalities of the experimentally infected

fish reached 70% and *T. maritimum* could be reisolated in pure culture from the experimentally infected fish.

Treatment trial

The enrofloxacin treated fish of the first group showed no mortality or clinical signs during the experiment time while all fish of the second group (no treated infected control) showed typical clinical signs as mentioned above and 60% mortality started from the third day post-infection.

Table (1): Shows the results of the conventional biochemical and API20E tests of the *T. maritimum* suspected isolate.

Serial number	Tests	Result	
		Tested isolate	Standard *T. maritimum*
1	Colony shape	Uneven edge	Uneven edge
2	Colony colour	Pale yellow	Pale yellow
3	Gram stain	-ve rods	-ve rods
4	Motility test	Motile	Motile
5	Oxidase test	+ve	+ve
6	Catalase test	+ve	+ve
7	Nitrate reduction	+ve	+ve
8	Congo red reduction	+ve	+ve
9	KOH	-ve	-ve
10	Hydrolysis of Esculin	-ve	-ve
API20E			
12	ONPG (Ortho NitroPhenyle Galactopyranosidase)	-	-
13	ADH (Arginine Dehydrolase)	-	-
14	LDC (Lysine Decarboxylase)	-	-
15	ODG (Ornithine Decarboxylase)	-	-
16	CIT (Citrate test)	-	-
17	H2S (H_2S production test)	-	-
18	URE (Urase test)	-	-
19	TDA (Tryptophane Deaminase)	-	-
20	IND (Indole test)	-	-
21	VP (Vogus proskauer)	-	-
22	GEL (Gelatine liquefaction test)	-	±
23	Glu, Man, Ino, Sor, Rha, Sac, Mel, Amy, Ara	-	-

List of abbreviation

*(GLU) Glucose *(MAN) Mannitol * (INO) Inositol test * (SOR) Sorbitol *(RHA) Rhaminose *(SAC) Sucrose *(MEL) D-Melibiose *(AMY) Amygdaline * (ARA) L-Arabinose

Table (2): Shows water quality criteria

Item	Unit	Tested water sample	Control water sample
Water temperature	°C	24°C	27°C
pH values	-	9.2	7.7
Dissolved oxygen	mgL^{-1}	3.1	4.5
total ammonia	mgL^{-1}	0.4	0.03

4. DISCUSSION

This study reported the first isolation of the marine pathogen *T. maritimum* from Picasso Tigger Fish (*Rhinecanthus assasi*) and Black damsel fish (*Neoglyphieodon meles*) of red sea at hurghada, Egypt. The affected fish showed the classical clinical signs such as off food, leathergic, skin hemorrhagic ulcers (sometimes surrounded by white batch of necrotizing tissues), eroded and ulcerated mouth and fin rot. Similar lesions were recorded by Baxa *et al.* (1986); Santos *et al.* (1999); Toranzo *et al.* (2005) and López *et al.* (2009) in different fish species.

The *T. maritimum* virulence was exaggerated and the disease's clinical signs started by stressors including exposing the wild investigated fish to fishing and confinement in indoor aquarium, deteriorated water quality as high ammonia ($0.4mgL^{-1}$) and low dissolved oxygen ($3.1mgL^{-1}$) at temperature around 25°C and these results are in accordance with Magariños *et al.* (1995) who stated that the prevalence and severity of the disease

increased at temperatures above 15 °C with variable number of environmental stressors and host-related factors as skin surface condition and Mouriño *et al.* (2008) who stated that the presence of highly virulent strains of Flexibacter sp. is associated with the presence of organic matter dissolved in water. Such stressors are the main predisposing factors for the chronic immune suppression of marine fish (Santos *et al.*, 1999).

The eleven isolates had similar morphological and biochemical characterizations and identified as *T. maritimum.* Their biochemical tests were positive for cytochrome oxidase, catalase, motility, congo red reduction and nitrate reduction tests and they were negative for all tests of API20E system. These findings come in agreement with the results of *T. maritimum* identification established by Suzuki *et al.* (2001), Buller (2004), Mouriño *et al.*, (2008) and Avendaño-Herrera (2009).

The experimentally infected black damsel fish showed lesions similar to those of naturally infected fishes such as lethargy, external body lesions in the form hemorrhages and then ulcers on the skin, eroded and ulcerated mouth associated with 60% mortality, similar clinical signs were reported by Handlinger *et al.* (1997). These findings clearly demonstrated the potential pathogenicity of the isolated strain and confirmed the effectiveness of bath immersion challenge model to estimate the virulence of *T. maritimum*. Results of immersion challenge carried out in the present study demonstrated that *T. maritimum* can spread through water.

The treatment of the experimentally infected fish was successfully conducted by using repetitive 30 ppm enrofloxacin immersion bath for 1 h during three consecutive days, the treated fish showed no external lesions and no mortality recorded between them and these findings come in contact with the results reported by Avendaño-Herrera *et al.* (2008).

In conclusion, this study reported the first isolation and identification of the marine pathogen *T. maritimum* in Egypt from Picasso Tigger Fish and Black damsel fish reared in indoor aquarium of National Institute of Oceanography and Fisheries (NIOF) in Hurghada city. *Tenacibaculosis* could be treated by repetitive enrofloxacin immersion bath (30 ppm) for 1hr during three consecutive days. Further studies should be conducted on the molecular identification, immunity and control by using natural and safe alternatives such as probiotics or plant extracts.

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