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Epidemiological and bacteriological studies on tenacibaculosis in some Red Sea fishes, Egypt

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ABSTRACT

Tenacibaculosis is a serious bacterial disease known to affect many species of marine fish such as *Rhinecanthus assasi* (Picasso Trigger fish), *Neoglyphieodon meles* (Black damsel fish) and *Cheilinuslunu latus* (Broomtail wrasse). *Tenacibaculum maritimum* pathogen was recovered from ulcers, livers and spleens of clinically diseased fishes from coral reef in the marine site off the National Institute of Oceanography and Fisheries (NIOF) at Hurghada, Egypt. The obtained isolates were identified as *T. maritmum* by the morphological and biochemical characterization. The prevalence ratio of Tenacibaculosis among the clinically diseased Black damsel, Picasso Trigger and Broomtail wrasse fishes were 14.3, 13.3 and 19.4% respectively. The highest prevalence levels of the disease in the investigated clinically diseased Black damsel, Picasso Trigger and Broomtail wrasse fishes reached 20, 16.7 and 28.6% during winter and the lowest was during summer (0%). Also, FMM and Huso-Shotts media were the most effective media for the recovery of *T. maritimum* from diseased fish followed by MA and trypticas soya agar media.

1. INTRODUCTION

Tenacibaculosis is a serious bacterial disease affecting a great variety of marine fishes especially cultured species, where both adult and young are susceptible but the young fish are seriously affected (Toranzo *et al.,* 2005). It is worldwide disease affecting cultured marine fishes in Japan and Europe (Campbell & Buswell, 1982; Wakabayashi *et al.*, 1986; Bernardet, *et al*., 1990). In Galicia, northwest Spain, disease problems attributable to this pathogen have increased considerably during the last few years (Devesa *et al.*, 1989; Toranzo *et al*., 1990; Pazos *et al*., 1993).

Tenacibaculosis is often associated with environmental stress and/or mechanical failure (skin condition) and the disease often appears in association with environmental stress at temperature above 15ºC (Chen *et al*., 1995; Santos *et al*., 1999).

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It is caused by *Tenacibaculum 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 ; Toranzo *et al*. 2005).

Concerning Tenacibaculosis susceptible fish, it infects many cultured marine fish species including sole the *Solea solea* (L.) (Bernardet *et al.*, 1990); Senegalese sole (*Solea senegalensis*) (Cepeda and Santos, 2002); Japanese flounder (*Paralichthys olivaceous*) (Baxa *et al.*, 1986); turbot (*Psetta maxima*) (Avendaño, -Herrera *et al.*, 2004), sea bream and sea bass (Toranzo *et al.*, 2005). Also, Soltani *et al*. (1996) and Handlinger *et al.* (1997) recorded the disease in the Atlantic salmon (*Salmo salar* L.), Rainbow trout (*Oncorhynchus mykiss*), striped trumpeter (*Latris lineata*) and Greenback flounder (*Rhombosolea tapirina*).

In the southern hemisphere, T. maritimum had been identified as a pathogen of sea-caged Atlantic salmon, *Salmo salar* L., Rainbow trout, *Oncorhynchus mykiss* (Walbaum), captured striped trumpeter, *Latrislineata*, yellow-eye mullet, *Aldrichetta forsteri* (Valenciennes), and black bream, *Acanthopagrus butcheri* (Munro), (Schmidtke *et al.*, 1991). The first isolation of *T. maritimum* from wedge sole *Dicologoglossa cuneata*, was reported by Lopez, *et al*. (2009).

Specialized, non-selective, low-nutrient media such as Anacker and Ordal agar (AOA) prepared with 70% sea water had been advocated for the isolation of sea water *F. maritimus* (Bullock *et al*., 1986; Wakabayashi *et al*., 1986 ; Frerichs, 1993). Marine agar (MA) had also been described as suitable medium for the isolation of marine gliding bacteria (Frerichs, 1993). Bullock, *et al.* (1986) isolated *T. maritimum* on Huso-Shotts media and Pazos *et al*. (1993) isolated it from lesions and internal organs on *Flavobacterium Maritimus* medium (FMM) and the last two media contained antibiotic and considered as *T. maritimum* selective media.

The present study irevealed that *Tenacibaculum maritimum* is a causative agent of Tenacibaculosis in *Rhinecanthus assasi* (Picasso Trigger fish), *Neoglyphieodon meles* (Black damsel fish) and *Cheilinuslunu latus* (Broomtail wrasse) in The National Institute of Oceanography and Fisheries (NIOF) at Hurghada, Eygpt. Prevalence of the disease among the investigated fish species during different seasons from November 2010 to October 2011 was studied. Evaluation of five different bacteriological media for the successful isolation of the *T. maritimum* was carried out.

2. MATERIAL AND METHODS

2. 1 Fish

A total number of 360 fish of three different species namely damsel fish, Picasso Tigger fish and Broomtail wrasse were randomly collected from the coral reef of the Red Sea at Hurghada, Egypt. Thirty fish from each investigated species were collected every season from November 2010 to October 2011 to record the incidence of Tenacibaculosis all over the year. Fish were brought alive and kept in the indoor aquaria of NIOF at Hurghada for clinical examination and bacteriological isolation.

2.2 Clinical examination of fish

The fish was examined according to Santos *et al.* (1999) for the detection of the clinical signs on the external body surface and post mortem lesions on the internal organs.

3.3 Bacterial isolation and evaluation of different media

Samples were taken from the ulcers, liver and spleen of moribund and freshly dead fishes, each sample was inoculated on many culture media including *Flexibacter maritimus* medium (FMM) (Pazos *et al.*, 1996), Trypticas soya agar (TSA) (Difco), Marine agar (MA) (Difco), Nutrient agar (NA) (Difco) and Huso-Shotts media

(Bullock *et al*., 1986) were incubated at 25°C for 24: 96 hrs. All media except MA were prepared with sea water.

4. 4 Phenotypic characterization

The suspected *T. maritimum* colonies were isolated, purified and characterized using phenotypic tests as reported by Bernardet *et al.* (1990) and Avendan˜o-Herrera *et al*., (2004). Commercial miniaturized API 20E galleries (BioMerieux) were also used according to the manufacturer's instructions, but sterile sea water was used as a diluent and 20°C was used as incubation temperature.

5. 5 Pathogenicity assays

Two equal groups each of 10 fish from each fish species without a history of Tenacibaculosis, weighing 100± 10 gm were used. The first group of each fish species was challenged by bath immersion for 18 hrs with *T. maritimum* suspensions containing $1.5x10^6$ cell m L^{-1} according to Avendan˜o-Herrera *et al*., (2006). The second group of each fish species was used as control group. Each experimental and control fish group was kept in 110L glass aquarium with continuous flowing sea water at temperature 22 ± 2°C. The clinical signs and mortalities were recorded daily for 10 days.

3. RESULTS

3.1 Clinical signs on clinically diseased fishes

The main clinical signs observed on the affected fishes were varied according to the fish species, Picasso Trigger fish had hemorrhagic ulcers, eroded and ulcerated mouth and tail rot (Figs. 1 & 2). Moreover the diseased Black damsel fish showed ulcerated skin lesions surrounded with white batch of necrotizing tissues, corneal opacity and fin rot (Fig. 3) similarly the Broomtail wrasse fish showed rounded white batches of necrosis all over the body associated with tail and fin rot (Fig. 4). Generally, there were no clear post mortem lesions except hemorrhagic or pale liver was observed in few cases. The incidence of infection as detected by clinical examination of randomly collected black damsel, Picasso Trigger and broomtail wrasse fishes among the examined fishes were 29.2, 25 and 25.8% respectively (Table 1).

Fig. 1: Picasso trigger fish showing skin ulcer

Fig. 2: Picasso trigger fish showing eroded and ulcerated mouth

Fig. 3: Black damsel fish showing skin ulcer

Fig. 4: Broomtail wrasse fish showing white patches of necrosis all over the body

Table 1: Incidence of infection in randomly collected Red Sea fish at Hurghada

Fish species	Total No.	Apparently healthy fish		Clinically diseased fish	
		No.	%	No.	%
Black damsel fish	120	85	70.8	35	29.2
Picasso Tigger fish	120	90	75	30	25
Broomtail wrasse	120	89	74.3	31	25.8

3. 2 Prevalence of fish Tenacibaculosis in different species of Red Sea fishes randomly collected during different seasons

The prevalence of Tenacibaculosis among the clinically diseased Black damsel, Picasso Trigger and Broomtail wrasse fishes were 14.3, 13.3 and 19.4% respectively and among the examined samples (apparently healthy and clinically diseased) were 4.2, 3.3 and 5% respectively (Table 4). The prevalence of Tenacibaculosis among the examined fishes (apparently healthy and clinically diseased) and the clinically diseased fishes during different seasons of this study were presented in Tables (2 & 3).

Table 2: Prevalence of fish *Tenacibaculosis* among the examined fish (apparently healthy and clinically diseased) during different seasons

Fish species	**Autumn**			**Winter**			**Spring**			**Summer**		
	No. of examined fish	Tenacibaculosis		No. of examined Fish	Tenacibacul.		No. of examined fish	Tenacibacul.		No. of examined fish	Tenacibacul.	
		No.	Fish		No	%		No	%		No	%
Black damsel	30	2	6.7	30	3	10	30	0	0	30	0	0
Picasso Tigger	30	2	6.7	30	2	6.7	30	0	0	30	0	0
Broomtail wrasse	30	1	3.3	30	4	13.3	30	1	3.3	30	0	0

Table 3: Prevalence of fish *Tenacibaculosis* among different clinically diseased fishes during deferent seasons

Fish species	**Autumn**			**Winter**			**Spring**			**Summer**		
	No. of clinically diseased fish	Tenacibaculosis		No. of clinic dis. fish	Tenacibaculosis		No. of clinic. Dis. Fish	Tenacibacul.		No. of clinic. Dis. Fish	Tenacibacul.	
		No.	%		No	%		No.	%		No	%
Black damsel	12	2	16.7	15	3	20	4	0	0	4	0	0
Picasso Tigger	12	2	16.7	12	2	16.7	4	0	0	2	0	0
Broomtail wrasse	10	1	10	14	4	28.6	7	1	14.3	0	0	0

Table 4: Prevalence of *Tenacibaculosis* among different examined fishes and clinically diseased fishes during this study.

Fish species	No. of examined fish	*Tenacibaculosis*		No. of clinically diseased fish	*Tenacibaculosis*	
		No.	%		No.	%
Black damsel fish	120	5	4.2	35	5	14.3
Picasso Tigger fish	120	4	3.3	30	4	13.3
Broomtail wrasse fish	120	6	5	31	6	19.4

3. 3 Isolation and characterization of *T. maritimum*

Fifteen suspected *T. maritimum* strains were isolated from the skin' ulcers, liver and spleen of the clinically diseased fishes. The isolated strains were subjected to morphological and biochemical identification. The colonies of suspected *T. maritimum* were flat with irregular edges, pale yellow in colour and in some cases adhered to the agar. All strains were gram-negative, long rods, cytochrome oxidase positive and catalase positive, grew at 25 °C but did not grow at 35°C and did not grow in absence of sea salts. All isolates absorbed Congo red, but did not produce flexirubin-pigments. They were negative for all API20E tests (Fig. 5).

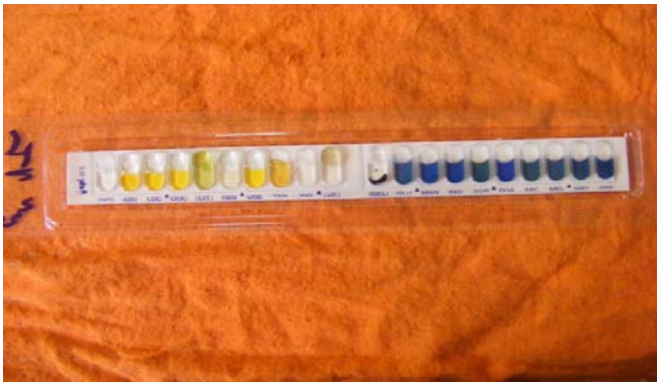

Fig. 5: API20E strip showing results of *T. maritimum*

3.4 Evaluation of different culture media for the successful recovery of *T. maritimum* from clinical specimens

The recovery rate of marine agar, trypticase soy agar, Huso-Shotts medium, FMM and nutrient agar for *T. maritimum* was 7.2, 6.8, 7.8, 7.8 and 0% respectively (Table 5).

Table 5: Evaluation of media for the successful culture of *Tenacibaculum maritimum* from clinical specimens

Samples	No.	Positive culture									
		MA		TSA		HSM		FMM		NA	
		No.	%	No.	%	No.	%	No.	%	No	%
Ulcers	96	12	12.5	12	12.5	13	13.5	13	13.5	0	0
Liver and spleen	96	2	2.1	1	1.04	2	2.1	2	2.1	0	0
Total	192	14	7.3	13	6.8	3	7.8	15	7.8	0	0

MA = Marine agar HSM = Huso- Shotts medium TSA = Trypticas soya agar
NA = Nutrient agar FMM = *F. maritimus* medium

3.5 Pathogenicity assay

The experimentally infected Black damsel fish, Picasso Trigger fish and Broomtail wrasse showed lesions similar to those of naturally infected fishes such as off food, lethargy, skin hemorrhagic ulcers, eroded and ulcerated mouth. The mortality of the experimentally infected fish species reached 60, 45 and 55% respectively and *T. maritimum* could be reisolated in pure culture from these fish.

4. DISCUSSION

The prevalence levels of *Tenacibaculosis* among the clinically diseased wild Black damsel, Picasso Trigger and Broomtail wrasse fishes were 14.3, 13.3 and 19.4% respectively and the diseased fish manifested classical clinical signs of Tenacibaculosis such as off food, leathergic, skin hemorrhagic ulcers (sometimes surrounded with white batch of necrotizing tissues), eroded, ulcerated mouth and fins rot. Similar lesions were reported by Baxa *et al*. (1986), Wakabayashi *et al.* (1986);

Bernardet *et al.,* (1990); Santos *et al.* (1999), Toranzo *et al.* (2005) and López *et al.* (2009).

The susceptibility of the three investigated wild marine fish species to Tenacibaculosis may be attributed to the lack of host specificity of *T. maritimum* and their living habit in the coral reef and their exposure to skin injuries by the sharp edges of the coral reef, in agreement with Neulinger *et al*. (2009) who reported that *T. maritmum* have long been known to be associated with feeding, gastrodermal, and tentacle cells and mucosal secretions in cnidarians, notably coral polyps. Coral mucus harbors specific populations of these bacteria.

Also this result was supported by Santos *et al*. (1999) who stated that the disease is influenced by a multiplicity of environmental (stress) and host-related factors (skin surface condition) and Toranzo *et al.*(2005) who reported that *T. maritmum* had a lack of strict host specificity.

The highest prevalence ratio of Tenacibaculosis among the three investigated clinically diseased Black damsel, Picasso Trigger and Broomtail wrasse fishes were 20, 16.7 and 28.6% respectively, during winter season and followed by 16.7, 16.7 and 10% during Autumn, which may be attributed to the previously mentioned factors and the suitable temperature during winter and autumn (15 - 30ºC) at Hurghada. This result was supported by Lopes *et al*. (2009) who recorded three outbreaks of Tenacibaculosis during March, April–May and January in wedge sole cultured in southwestern Spain at water temperature of 20.5°C+ 1.5°C.

The isolated strains had similar morphological and biochemical characterizations and identified as *Tenacibaculum maritimum*, their biochemical tests were positive for cytochrome oxidase, catalase, motility and Congo red reduction and they were negative for all tests of API20E. The biochemical, physiological and enzymatic characteristics of the isolates showed no discrepancies and agreed with the findings of Bernardet *et al*. (1990), Ostland *et al.* (1999), Avendan˜o-Herrera *et al*. (2004) and Buller (2004).

The comparative study of the culture media on field samples showed that FMM and Huso-Shotts media were the most effective media for the recovery of *T. maritimum* from diseased fish followed by MA and trypticas soya agar media, while the ordinary nutrient agar was completely ineffective for recovery of *T. maritmum*. These findings agree with those of Pazos *et al*. (1993); Frerichs (1993) ;Fijan (1969), Hsu-Shotts and Waltman (1983) ; Bullock *et al.* (1986) and Austin and Austin (1993). moreover Pazos *et al.(*1996),who indicate that our earlier failure to recover *T. maritimum* from field samples using MA and AOA media may have been a result *T. maritimum* of the ability of these media to favor growth of heterotrophic halophytic bacteria that were inhibitory for *T. maritimum*. Therefore although both MA and FMM media and Huso-Shotts can be used in the laboratory for the routine culture of *T. maritimum*, FMM medium and Huso-Shotts are more appropriate for the successful isolation of this species from environmental samples.

Concerning the experimental infection of the isolated *T. maritimum* in the investigated fishes clearly demonstrated the pathogenic potential of the isolate and confirmed the effectiveness of this immersion bath challenge model to estimate the virulence of *T. maritimum*.

In conclusion, Tenacibaculosis is caused by *T. maritimum* that lacked host specificity and affected Black damsel, Picasso Trigger and Broomtail wrasse fishes. It is influenced by a multiplicity of environmental stress factors especially temperature and host-related factors especially skin surface condition. FMM and Huso-shotts media are the most suitable media for its isolation.

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