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 *Cheilinus lunulatus* (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. maritimum* 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.
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, Latris lineata, 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 revealed that Tenacibaculum maritimum is a causative agent of Tenacibaculosis in Rhinecanthus assasi (Picasso Trigger fish), Neoglypheodon meles (Black damsel fish) and Cheilinus latus (Broomtail wrasse) in The National Institute of Oceanography and Fisheries (NIOF) at Hurghada, Egypt. 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 Trigger 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 Avendañó-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 Avendañó-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).
Table 1: Incidence of infection in randomly collected Red Sea fish at Hurghada

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Total No.</th>
<th>AppARENTLY HEALTHY FISH</th>
<th>CLINICALLY DISEASED FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Black damsel fish</td>
<td>120</td>
<td>85</td>
<td>70.8</td>
</tr>
<tr>
<td>Picasso Tigger fish</td>
<td>120</td>
<td>90</td>
<td>75</td>
</tr>
<tr>
<td>Broomtail wrasse</td>
<td>120</td>
<td>89</td>
<td>74.3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fish species</th>
<th>AUTUMN</th>
<th>WINTER</th>
<th>SPRING</th>
<th>SUMMER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of examined fish</td>
<td>Tenacibaculosis No.</td>
<td>%</td>
<td>No. of examined fish</td>
</tr>
<tr>
<td>Black damsel</td>
<td>30</td>
<td>2</td>
<td>6.7</td>
<td>30</td>
</tr>
<tr>
<td>Picasso Tigger</td>
<td>30</td>
<td>2</td>
<td>6.7</td>
<td>30</td>
</tr>
<tr>
<td>Broomtail wrasse</td>
<td>30</td>
<td>1</td>
<td>3.3</td>
<td>30</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fish species</th>
<th>AUTUMN</th>
<th>WINTER</th>
<th>SPRING</th>
<th>SUMMER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of clinically diseased fish</td>
<td>Tenacibaculosis No.</td>
<td>%</td>
<td>No. of clinically diseased fish</td>
</tr>
<tr>
<td>Black damsel</td>
<td>12</td>
<td>2</td>
<td>16.7</td>
<td>15</td>
</tr>
<tr>
<td>Picasso Tigger</td>
<td>12</td>
<td>2</td>
<td>16.7</td>
<td>12</td>
</tr>
<tr>
<td>Broomtail wrasse</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fish species</th>
<th>No. of examined fish</th>
<th>Tenacibaculosis No.</th>
<th>%</th>
<th>No. of clinically diseased fish</th>
<th>Tenacibaculosis No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black damsel fish</td>
<td>120</td>
<td>5</td>
<td>4.2</td>
<td>35</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Picasso Tigger fish</td>
<td>120</td>
<td>4</td>
<td>3.3</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Broomtail wrasse fish</td>
<td>120</td>
<td>6</td>
<td>5</td>
<td>31</td>
<td>6</td>
<td>19.4</td>
</tr>
</tbody>
</table>
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](image)

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>
<thead>
<tr>
<th>Samples</th>
<th>MA No.</th>
<th>MA %</th>
<th>TSA No.</th>
<th>TSA %</th>
<th>HSM No.</th>
<th>HSM %</th>
<th>FMM No.</th>
<th>FMM %</th>
<th>NA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcers</td>
<td>96</td>
<td>12</td>
<td>12</td>
<td>12.5</td>
<td>13</td>
<td>13.5</td>
<td>13</td>
<td>13.5</td>
<td>0</td>
</tr>
<tr>
<td>Liver and spleen</td>
<td>96</td>
<td>2</td>
<td>2.1</td>
<td>1.04</td>
<td>2</td>
<td>2.1</td>
<td>2</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>14</td>
<td>7.3</td>
<td>6.8</td>
<td>3</td>
<td>7.8</td>
<td>15</td>
<td>7.8</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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, leathery, 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. maritimum* 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. maritimum* 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 - 30°C) 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 trypicas soya agar media, while the ordinary nutrient agar was completely ineffective for recovery of *T. maritimum*. 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.
5. REFERENCES


