Bacterial Infections Affecting Marine Fishes in Egypt

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Abstract: Some fish species are suffering from continuous depletion due to devastating environmental changes at their native aquatic environment. Qarun Lake and Suez Gulf are among the most vulnerable areas. Thus, representative fish samples from those areas were inspected for the presence of any fish pathogenic bacteria. The inspected samples included six different species; *Epinephelus tuvina*, *Siganus rivulatus*, and *Dedlechilus labiosus* native to Suez Gulf at Suez governorate; *Tilapia zilli*, *Mugil capito* and *Solea vulgaris* native to Qarun Lake at El-Fayoum governorate. A total of 600 samples were examined throughout the different year seasons. Gram positive and negative fish pathogenic bacteria were isolated from a total of 245 fish sample. Among those samples, the following bacteria were retrieved in the following percentages respectively, 17.55% (V. *anguillarum*), 16.73% (V. *alginolyticus*), 15.51% (P. piscicida), 15.91% (Ps. fluorescens), 13.46% (S. fecalis), 11.02% (A. hydrophila), 6.12% (A. sobria) and 3.67% were infected with Staph. aureus. The Siganus rivulatus was the highest infected fish species with a prevalence of 8.33%, while Mugil capito was the lowest infected species (5.67%). The highest total prevalence of bacterial infection was recorded in summer season (40.81%) while the lowest was recorded in winter (15.91%). The aforementioned bacterial isolates were successfully re-isolated from experimentally infected fish. The retrieved isolates were matched against standard isolates as well as confirmed to be positive using semi-automated (API 20 E) and conventional biochemical tests.

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1. INTRODUCTION

Fisheries represent an important sector in the Egyptian national income structure. In the fisheries economy, marine fishes represent the major investment choices for the national fishermen. Marine fishes are liable to variable number of environmental stressors, including chemical, natural and biological invaders. Such stressors are the main predisposing factors for the chronic immune-suppression of marine aquatic animals in the affected marine habitat. As an ultimate fate for the staggering immumo-suppression of fishes, bacterial invasion will be the most probable event (Ellis, 1999). Further, the bacterial invasion of any marine species could possibly exacerbate under the triggering effect of the fluctuating climatic changes (Wedemeyer, 1996).

The prevalence of bacterial pathogens have been well documented in several cultured and wild freshwater fish species, however; only a few bacteriological surveys have involved the marine species disease outbreaks (Alicia, et al 2005).

On the long run water resources will be the most limiting factor of aquaculture development in Egypt. Therefore, marine fisheries are the immediate alternative for water needed for mariculture (Sadek, 2000).

The present work was planned to isolate and identify the most predominant bacterial pathogens in some marine fishes, native to both Suez Gulf and Lake Qarun. Further, work aimed to evaluate the seasonal variation of bacterial isolates among the examined fishes.

2. MATERIALS AND METHODS

2.1. Sampling and processing

Six hundred (600) marine fishes of six different species were freshly captured from two localities in Egypt, (Suez Gulf and Lake Qarun) through the different seasons of the year.

On each season, twenty-five fish of each species were collected and examined. Fish species, numbers of fishes, average body weights and localities are shown in table (1). Clinical and P.M examination were carried out using the methods described by (Buller, 2004).

Samples from gills, liver, spleen, kidney and external lesions from fishes were cultured on general

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and selective media; tryptic soy agar and tryptic soy broth (Difco) supplemented with 1.5% (w/v) NaCl, marine agar (Difco), and thiosulphate—citrate—bile salt—sucrose agar (TCBS, Difco). Aeromonas agar base medium supplemented with ampicillin, pseudomonas agar base medium supplemented 2 % NaCl and Azide blood agar supplemented with 2 % NaCl. All the inoculated media were incubated at 22 °C for 48 hours.

2.2. Identification of the isolates

Pure cultures of the isolates were identified by biochemical characterization following the criteria proposed by those described in the Bergey's Manual of Determinative Bacteriology, (Garrity, 2001). Final confirmation of each strain was achieved using the analytical profile index of API20-E system (Buller, 2004).

2.3. Experimental infection

A total of 70 apparently healthy O. niloticus fish, weighting 50 ± 5 gram were selected for determination of the pathogenicity of the most prevalent bacterial isolates. Fishes were divided into seven groups each contained 10 fish. The inocula prepared for bacterial isolates as I/P injections were prepared according to (Austin & Austin, 2007). Fish were observed daily for 15 days. Six groups were consistently inoculated I/P with the bacterial suspension of (Aeromonas hydrophila, Pseudomonas fluorescence, Vibrio anguillarum, Pasteurella piscicida, Streptococcus fecalis and Staphylococcus aureus) at a dose of 0.2 ml of (3 X 10⁷ CFU) while the control group (group 7) were injected I/P with 0.2 ml of sterile tryptic soy broth according to Hussain (2002).

3. RESULTS

3.1. Clinical examination:

Clinical signs included generalized hemorrhages (Fig 1). Some fishes showed bilateral exophthalmia and opacity (Fig 2), scale detachment and skin darkening. Gills were congested (Fig 3) while in others appeared to be pale with accumulation of excessive amount of mucus. Ascitis was observed in some fishes. Vent inflammation as well as prolapse were also observed.

Necropsy findings of naturally infected marine fishes exhibited sero-sanguinous fluid in the abdominal cavity. Liver was pale in some fishes (Fig 4), while in others was congest, haemorrhagic with numerous randomly scattered whitish nodules (Fig 5). Spleen and kidneys were congested and enlarged in some fishes. The intestines were haemorrhagic, inflamed with congestion of blood vessels. In other samples, the intestines were seen filled with gases.

3.2. Isolation and identification of the bacterial isolates:

Biochemical characteristics of the Gram negative bacterial spp. isolated from examined fishes are shown in Table (2).

Results indicated that 245 naturally collected fishes out of 600 were found to be infected with different types of bacteria. The culture results demonstrated that (203) fishes were found to be infected with Gram-negative bacteria and only (42) fishes were infected with Gram-positive bacteria. 17.50 % of the infected fishes were positive for *V. anguillarum*, (16.73%) for *V. alginolyticus*, (15.51%) for *P. piscicida*, (15.91%) for *Ps. fluorescens*, (13.46%) for *S. fecalis*, (11.02%) for *A. hydrophila*, (6.12%) for *A. sobria* and (3.67%) of the surveyed fishes were infected with *Staph. aureus*. The total prevalence of bacterial isolates is shown in table (3). Moreover, the results revealed that *Siganust rivulatus*

Moreover, the results revealed that *Siganust rivulatus* was the most infected fish spp (50 %), followed by *E. tuvina* (42 %), *S. vulgaris* (41 %), *Tilapia spp.* (40 %), *M.sahlae* (38 %), while *M. capito* was the lowest infected spp (34 %).

Prevalence of bacterial infections for fishes collected from both Suez Gulf and Lake Qarun was illustrated in table (4). The results revealed that: The total prevalence of bacterial infections for fishes collected from the Gulf of Suez (53.06%) was higher than that recorded for those collected from Lake Qarun (46.93%).

3.3. Seasonal prevalence:

Results indicated that, the highest total prevalence of bacterial infections among the naturally infected marine fishes was recorded in the summer season (40.81%), followed by autumn (25.71%), then spring (17.14%). On the other hand the minimal prevalence of infection was recorded in winter (15.91%). table (5).

The highest prevalence of bacterial infection among the naturally infected marine fishes in winter season, was recorded for Ps. fluorescens (7.75%) while the lowest one (0.40%) was recorded for V. anguillarum. on the other hand P. piscicida, S. fecalis and Staph. aureus were not recorded. For spring season, the highest prevalence of bacterial infection (3.67%) was recorded for A. sobria and V. anguillarum, while the lowest (1.22%) was recorded for Ps. fluorescens and S. fecalis.. The highest prevalence of bacterial infection (9.38%) in summer season was recorded for V. anguillarum, while the lowest (0.40%) was recorded for A. sobria. The highest prevalence of bacterial infection (5.30%) in autumn season was recorded for V. alginolyticus and P. piscicida while the lowest (0.40%) was recorded

for *A. sobria*. The Prevalence of different types of bacterial infections in the different seasons is illustrated in table (5).

3.4. Results of experimental infection:

Mortality patterns in experimentally infected *O. niloticus* with the different bacterial isolates (Table 6). Experimentally infected O. niloticus with the different isolates were characterized by septicemic lesions nearly similar to those of naturally infected fishes. Experimentally infected fish showed haemorrhagic patches distributed on different parts of the body surfaces, fin and tail rot (Fig. 6). Some fish exhibited typical ulcers (Fig.7). Some cases showed inflammation of the vent (Fig. 8). Necropsy findings showed, congestion of Liver. Spleen and kidneys were congested and enlarged. Gall bladder was distended. The gut was haemorrhagic and filled with yellowish content. Serous to sero-sanguinous

fluid in the abdominal cavity was noticed in some cases.

Re-isolation of all inoculated bacterial isolates was obtained from dead and sacrificed fish. Moreover, the results of the culture and biochemical characteristics of the re-isolated bacterial isolates revealed the same morpho-chemical characteristics of the inoculated bacterial isolates.

Table (1): Fish species, locality, number and weight of examined fish

| Fish Species | Locality | Number | weight |
|---------------|------------|--------|--------|
| E. tuvina | Suez Gulf | 100 | 95±20 |
| S. rivulatus | Suez Gulf | 100 | 70±10 |
| M. sahla | Suez Gulf | 100 | 50±5 |
| S. vulgaris | Lake Qarun | 100 | 75±15 |
| M capito. | Lake Qarun | 100 | 85±10 |
| Tilapia zilli | Lake Qarun | 100 | 65±5 |

Table (2): Biochemical characteristics of the bacterial isolates retrieved from naturally infected marine fishes.

| | A.hydrophila | A. sobria | Ps. fluorescens | V. anguillarum | V. alginolyticus | P.piscicida |
|---|--------------|-----------|-----------------|----------------|------------------|-------------|
| B –Galactosidase production (OPNG) | + | + | - | + | - | - |
| Arginine dihydrolase production (ADH) | + | + | + | + | - | + |
| Lysine decarboxylase production(LDC) | - | + | - | - | + | - |
| Ornithine decarboxylase production(ODC) | - | - | - | - | - | - |
| Citrate utilization (CIT) | - | V | - | V | + | - |
| H2S production(H2S) | - | - | - | - | - | - |
| Urease production(URE) | - | - | - | - | - | - |
| Tryptophane deaminase production (TDA) | - | - | - | - | - | - |
| Indole production(IND) | + | + | - | + | + | - |
| Acetoin production(VP) | + | + | + | + | V | + |
| Gelatinase production(CEL) | + | + | - | + | + | - |
| Acid from glucose(GLU) | + | + | V | + | + | + |
| Acid from manitol(MAN) | + | + | - | + | + | - |
| Acid from inositol(INO) | - | - | - | - | - | - |
| Acid from Sorbitol(SOR) | - | - | - | + | - | - |
| Acid from rhamnose(RHA) | + | - | - | - | - | - |
| Acid from sucrose(SAC) | + | + | - | + | + | - |
| Acid from Melibiose (MEL) | - | - | V | - | - | - |
| Acid from amygdalin (AMY) | V | - | - | - | V | - |
| Acid from arabinose (ARA) | V | = | V | V | - | - |
| Cytochrome oxidase (OX) | + | + | + | + | + | + |

V: variable result.

Table (3): Prevalence of bacterial infections in the examined marine fishes.

| Type of M.O | No. Of Exam | NO Inf. fish | A. hydrophila | | drophila A. sobria | | Ps.fluorescens | | V.anguillarum | | V.alginolyticus | | P.piscicida | | S. fecalis | | Staph. aureus | |
|---------------|-------------------|--------------------|---------------|-------|--------------------|------|----------------|-------|---------------|-------|-----------------|-------|-------------|-------|------------|-------|---------------|------|
| Fish spp | fish | | No. inf | % | No inf | % | No inf | % | No. inf | % | No. inf | % | No inf | % | No. Inf | % | No .inf | % |
| E. tuvina | 100 | 42 | 2 | 4.76 | 4 | 9.52 | 7 | 16.66 | 10 | 23.8 | 8 | 19.04 | 6 | 14.28 | 2 | 4.76 | 3 | 7.14 |
| S. rivulatus | 100 | 50 | 4 | 8 | 5 | 10 | 6 | 12 | 7 | 14 | 9 | 18 | 10 | 20 | 8 | 16 | 1 | 2 |
| S. vulgaris | 100 | 41 | 6 | 14.63 | 0 | 0 | 5 | 12.19 | 8 | 19.51 | 7 | 17.07 | 4 | 9.75 | 9 | 21.95 | 2 | 4.87 |
| M capito. | 100 | 34 | 3 | 8.82 | 2 | 5.88 | 4 | 11.76 | 5 | 14.7 | 3 | 8.82 | 9 | 26.47 | 6 | 17.64 | 2 | 5.88 |
| M. sahlae | 100 | 38 | 7 | 18.42 | 1 | 2.63 | 8 | 21.05 | 6 | 15.78 | 10 | 26.31 | 3 | 7.89 | 3 | 7.89 | 0 | 0 |
| Tilapia zilli | 100 | 40 | 5 | 12.5 | 3 | 7.5 | 9 | 22.5 | 7 | 17.5 | 4 | 10 | 6 | 15 | 5 | 12.5 | 1 | 2.5 |
| Total | 600 | 245 | 27 | 11.02 | 15 | 6.12 | 39 | 15.91 | 43 | 17.55 | 41 | 16.73 | 38 | 15.51 | 33 | 13.46 | 9 | 3.67 |

[•]Percentage was calculated according to the total number of infected fish.

Table (4) Prevalence of bacterial infections in Lake Qarun and Suez Gulf.

| Locality | Lake Qarun | Prevalence % | Suez Gulf | Prevalence % |
|------------------|------------|--------------|-----------|--------------|
| M.O | - | | | |
| A. hydrophila | 14 | 5.71 | 13 | 5.30 |
| A. sobria | 5 | 2.04 | 10 | 4.08 |
| Ps. fluorescens | 18 | 7.34 | 21 | 8.57 |
| V. anguillarum | 20 | 8.16 | 23 | 9.38 |
| V. alginolyticus | 14 | 5.71 | 27 | 11.02 |
| P. piscicida | 19 | 7.75 | 19 | 7.75 |
| S. fecalis | 20 | 8.16 | 13 | 5.30 |
| Staph. aureas | 5 | 2.04 | 4 | 1.63 |
| Total | 115 | 46.93 | 130 | 53.06 |

Table (5): Seasonal prevalence of bacterial infections in the examined marine fishes.

| Table (3). Seasonal prevalence of bacterial infections in the examined marine fishes. | | | | | | | | | | | | | | | | | |
|---|-----------|----------|-------------------------|------|---------------|-------|-----------------|-------|-------------|-------|-----------|-------|--------------|-------|-----------|------|-------|
| Type. | A.hya | lrophila | A.sobria Ps.fluorescens | | V.anguillarum | | V.alginolyticus | | P.piscicida | | S.fecalis | | Staph.aureus | | Total | | |
| M.o season | No inf | % | No inf | % | No inf | % | No inf | % | No inf | % | No inf | % | No inf | % | No inf | % | Total |
| Winter | 14 | 5.71 | 4 | 1.63 | 19 | 7.75 | 1 | 0.40 | 2 | 0.81 | 0 | 0 | 0 | 0 | 0 | 0 | 15.91 |
| Spring | 7 | 2.85 | 9 | 4.08 | 3 | 1.22 | 9 | 3.67 | 5 | 2.04 | 6 | 2.44 | 3 | 1.22 | 0 | 0 | 17.55 |
| Summer | 3 | 1.22 | 1 | 0.40 | 6 | 2.44 | 23 | 9.38 | 21 | 8.57 | 19 | 7.75 | 20 | 6.16 | 7 | 2.85 | 40.81 |
| Autumn | 3 | 1.22 | 1 | 0.40 | 11 | 4.48 | 10 | 4.08 | 13 | 5.30 | 13 | 5.30 | 10 | 4.08 | 2 | 0.81 | 25.71 |
| Total | 27 | 11.02 | 15 | 6.12 | 39 | 15.91 | 43 | 17.55 | 41 | 16.73 | 38 | 15.51 | 33 | 13.46 | 9 | 3.67 | |



Fig. 1: Naturally infected *S. vulgaris* with *V. anguillarum* showing haemorhages on the ventral surface of the body.



Fig. 3: Naturally infected *M. capito* with *A. hydrophila* showing severe congestion of gills.



Fig. 5: Naturally infected *E. tuvina* with *P. piscicida* showing enlargement of the liver with numerous scattered whitish nodules.



Fig. 7: *O. niloticus* I/P inoculated with *A. hydrophila* showing skin ulcers.



Fig. 2: Naturally infected *E. tuvina* with *P.piscicida* showing bilateral exophthalmia and corneal opacity.

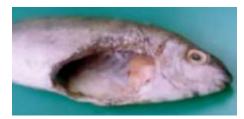


Fig. 4: Naturally infected *S. rivulatus* with *S. fecals* showing paleness of liver.



Fig. 6: *O.niloticus* I/P inoculated with *Ps. fluorescence* showing tail and fin rot.



Fig. 8: *O.niloticus* I/P inoculated with *S. fecalis* showing inflammation of the vent.

| Table (b). Mortality patterns of O. mioricus experimentary infected with different bacterial isolat | | | | | | | | | | | | | |
|---|----------------------|---|---|---|---|---|---|---|---|----|------------|-------|-----|
| Bacterial | No of mortality /day | | | | | | | | | | Mortality. | | |
| isolates | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12-15 | (%) |
| A.hydrophila | - | 1 | - | 2 | - | - | 2 | 1 | - | 1 | 1 | - | 80 |
| Ps. fluorescens | - | 2 | - | - | 1 | 1 | 2 | 1 | 1 | - | 1 | 1 | 100 |
| V. anguillarum | 1 | 1 | 2 | 1 | 2 | - | - | 1 | - | 1 | - | 1 | 100 |
| P. piscicida | 2 | 0 | - | 1 | 1 | - | - | 2 | - | - | 1 | 1 | 80 |
| S. fecalis | 1 | - | 1 | - | - | 1 | 1 | 1 | 1 | 2 | - | 1 | 90 |
| Staph. aureas | - | 1 | - | 2 | - | - | 1 | 1 | - | - | 2 | - | 70 |
| Control | - | - | - | - | - | - | - | - | - | - | - | - | 0 |

Table (6): Mortality patterns of *O. niloticus* experimentally infected with different bacterial isolates.

N.B. The dose of bacteria inoculated per fish were 0.2 ml of $3 \times 10^7 \text{ CFU}$ Number of I/P inoculated fishes per each group were 10.

4. DISCUSSION

Septicemic bacterial infections such as vibrios, aeromonads, pseudomonads, photobacteria, streptococci and staphylococci were recorded in several fingerlings, juveniles, adults and brood stocks of some marine fish species (Alicia *et al.*, 2005 and Samuelsson *et al.*, 2006).

In regards to bacterial pathogens that have been isolated, results came in this study revealed that Gram-negative bacteria prevailed the Gram-positive Vibrio isolates with anguillarum, Vibrio alginolyticus, Pasteurella piscicida (photobacterium damsella subspp piscicida), Pseudomonas fluorescens, Streptococcus fecalis, Aeromonas hydrophila, Aeromonas sobria and Staphylococcus aureus were the most common isolated spp. Results are supported by those reported by Zorrilla et al. (2003) who declared that the main pathogenic microorganisms isolated from diseased gilt-head seabream in the marine water at south western Spain were; Vibrio spp, Pseudomonas spp, P. piscicida, Flavobacteria maritimus, Aeromonas spp and Gram positive bacteria were also isolated but in low prevalence.

In concern to the total prevalence of bacterial infections in the naturally infected marine fishes (40.83 %) which may appear to be lower than those reported by some authors for freshwater fishes as Soliman (1999) who noticed that the total bacterial prevalence was (65%). This difference could be due to the unfavorable effect of the salinity of marine water on the viability of bacterial pathogens.

In regard to the localities of isolation, results revealed that the prevalence of bacterial infections was higher (53.06%) in fishes collected from the Gulf of Suez than that (46.93%) recorded for Lake Qarun. This may be explained by the fact that Lake Qarun is the largest reservoir of agricultural and sewage drainage of Fayoum province as well as the

drainage from fish farms established around the lake (Mansour & Sidky, 2003).

The high prevalence of isolation recorded from the Gulf of Suez may in part be attributed to the stress induced by high crude oil pollution at the Gulf water which is maintained by the low water flow, low water exchange rates and daily crowded ship traffic crossing the gulf as well as industrial effluents from oil refineries. All these factors are compromising to the fish immune system ending up with marked increase in the magnitude of bacterial infections.

Study declared that marine fish can succumb MAS, as supported by Larsen & Jensen (1977) who isolated A. hydrophila from ulcer disease in Cod, Gadus morhua L., a strictly marine fish. Authors added that motile aeromonas group especially A. hydrophila is considered as one of the most important pathogen responsible for haemorrhagic septicemia in a wide variety of marine water fish. Moreover, Vethaak (1992) isolated A. hydrophila from ulcers, lesions, and blood of ulcerated European flounder

Results pointed out that the highest prevalence of *A. hydrophila* was recorded in winter season (5.71%) followed by spring (2.85%), while in summer and autumn the results were the same (1.22%). These results were supported by Pathak *et al.* (1988) who suggested that the highest isolation rates of *A. hydrophila* occurred during late winter followed by a progressive decline in density during summer and monsoon seasons. Moreover, Popovic *et al.* (2000) mentioned that there was clear seasonality in the prevalence of *A. hydrophila* as there were no isolates recovered in the summer months. On contrast, Meyer (1970) reported that the most epizootics of motile aeromonads were generally reported in spring and early summer.

In regards to the seasonal prevalence of *A. sobria*, our study recorded that the highest prevalence e of *A. sobria* septicemia was recorded during the spring (3.67%) followed by winter (1.63%) while the minimal prevalence e of infection (0.40%) was recorded during the summer and autumn. Results are in accordance with those obtained by Wahli *et al.* (2005) who noticed that mortalities due to *A. sobria* peaked during the low water temperatures of winter time and reached levels. On contrary, the results are not in concordance with those obtained by Kooj *et al.* (1988) who demonstrated that the highest prevalence of Aeromonads in marine water have been obtained in the warmer months.

The pathogenicty of *A. hydrophila* for experimentally infected *Oeorchromis niloticus* fishes may be attributed to toxins and extracellular enzymes produced by *A. hydrophila* (Saavedra *et al.*, 2004).

In regards to the total prevalence of pseudomonas septicemia, the study recorded that (15.91%) of infected fish were positive for pseudomonas infection, These results are in concordance with those obtained by Hussain (2002) who reported that (15.27 %) of naturally infected marine fishes were positive for *Ps. flourescens* septicemia. on contrast, results are lower than those reported by Khan *et al.* (1981) who reported that *Pseudomonas* spp accounted for (72 %) of the mortalities recorded in captive Atlantic cod, *Gadus morhus* L associated with fin rot disease.

The highest prevalence of Ps. fluorescens septicemia was recorded during the winter season (7.75%) followed by autumn (4.48%) summer (2.44%) and spring (1.22%), this reveals that Ps. fluorescens has certain affinity to low temperature for propagation and wide spreading infection (El-Moghazy, 2004). Results were supported by Golomazou et al. (2006) who demonstrated that the Pseudomonads were isolated mainly in cold months of winter. On the contrary, results are not in accordance with those obtained by Hoda et al. (1999) who revealed that the prevalence of pseudomonads was lower in winter than summer. This may also be attributed to amplified activity of proteinases produced by pseudomonads at the low temperature (10-25°C) that play significant role in the pathogenesis of pseudomonas septicemia (Hoshino et al., 1997).

The pathogenicty of *Ps. fluorescence* for experimentally infected *Oeorchromis niloticus* may be attributed to the production of extracellular enzymes and lethal toxins El-Attar & Moustaf (1996).

In regards to the total prevalence of vibriosis recorded (34.28%), result are in accordance with those reported by Khan *et al.* (1981) who recorded that vibrios accounted for (28%) of mortalities in captive Atlantic cod, *Gadus morhus* L associated with fin rot disease. the results are lower than those recorded by Zorrilla *et al.* (2003) who recorded that the prevalence of vibrios among diseased gilthead sea bream, *Sparus aurata* L in southwestern Spain was (69.90%).

V. anguillarum in this study was the Vibrio spp most frequently isolated as (17.55%) of the infected cases were positive for V. anguillarum. this is in accordance with Zorrilla et al. (2003). On the other hand V. alginolyticus was the cause of (16.73%) of the recorded cases. Such high prevalence of V. alginolyticus could explain its importance in mariculture as supported by Zhu et al. (2000) who suggested that V. alginolyticus causes great harm to a wide variety of marine fishes.

The highest prevalence of *V. anguillarum* infection was recorded during the summer (9.38%), followed by autumn (4.08%), spring (3.67%), and only (0.4%) were recorded in winter. On the other hand the highest prevalence e of *V. alginolyticus* infection was recorded in summer (8.57%), autumn (5.30%), spring (2.04%) and (0.81%) in winter. The results of the seasonal prevalence of *Vibrio* spp are in concordance with those reported by Roberts (2001) who demonstrated that in wild, vibriosis normally occurs in fish in late summer when the temperatures are high. On the other hand, (Golomazou *et al.* (2006) reported that *V. alginolyticus* were not associated with a particular season.

The pathogenicty of *V. anguillarum* for experimentally infected *O. niloticus* may be attributed to extracellular toxins and enzymes produced by the bacterium (Nottage & Birkbeck, 1987).

In respect to the total prevalence of *P. piscicida* in this study recorded that (15.51%) of diseased fish were positive for *P. piscicida*. The results are higher than those recorded by Balebona *et al.* (1998) who declared that (6.7%) of diseased gilt-head sea bream, *Sparus aurata* L. in southwestern Spain were infected with *P. piscicida*. On the other hand results are lower than those recorded by Athanassopoulou *et al.* (1999) who recorded that the prevalence of *P. piscicida* in diseased Cuvier, *Puntazzo puntazzo* L. collected from marine aquaculture systems in Greece was (80%).

Seasonally, the highest prevalence e of P. piscicida was recorded during summer season (7.75%), autumn (5.30%) followed by the spring

(2.44%) on the other hand, it was not recorded in winter. Results are in concordance with those reported by Magarinos *et al.* (1996) who declared that *P. piscicida* causes high fish mortality only when the water is warm. On the other hand, Mladineo *et al.* (2006) suggested that temperature has no strong influence on the course of pasteurellosis.

The pathogenicty of *P. piscicida* for experimentally injected *O. niloticus* may be attributed to extracellular products of the bacterium (Nakai *et al.*, 1992).

In regards to the total prevalence of streptococcal septicemia, results indicated that (13.46 %) of infected fishes were positive for streptococcal infection. Results were higher than that recorded by Zorrilla *et al.* (2003) who recorded (7%) of bacterial infection affecting cultured gilthead sea bream, *Sparus aurata* L was attributed to Grampositive bacteria. Hussain (2002) recorded that (6.25%) of naturally infected marine fish were positive for streptococcal septicemia.

In regards to the seasonal prevalence of streptococcal septicemia, the highest prevalence of the streptococcal infection was recorded in summer season (6.16%) followed by autumn (4.08%) and spring (1.22%) on the other hand it was not recorded during the winter. These results are in accordance with those obtained by Varvarigos (1997) who revealed that *Streptococcus* spp cause septicemia to all farmed species mainly during late spring and early summer when sea water temperatures are high.

In concern to the experimental infection of O. niloticus with S. fecalis, the pathogenicty of streptococci may be attributed to the effect of exotoxins produced by the bacterium (Kimura & kusuda, 1979 and Woo, 1999).

The total prevalence of *Staph. aureus* infections was (3.67%). Results were lower than those recorded by Athanassopoulou *et al.* (1999), who recoded that the total prevalence of *Staph. epidermidis* among diseased *Puntazzo puntazzo* in marine aquaculture systems in Greece was (10 %). Moreover, Zorrilla *et al.* (2003) recorded that (7%) of bacterial infections affecting gilthead sea bream *Sparus aurata* L. were attributed to Gram-positive bacteria.

Seasonally the highest recorded prevalence of Staphylococcal infection was recorded in the summer season (2.85%) followed by autumn (0.81%) with no records in spring or winter. Results are supported by Varvarigos (2001) who declared that *Staphylococcus* spp causing septicemia to all farmed species during high temperature of sea water in late spring and early summer.

Results of experimental infection of *O. niloticus* with *Staph. aureus* were in accordance with Huang (2000) who indicates that staphylococci can be a possible cause of mortalities and losses among fishes

From the present study it was concluded that bacterial pathogens are the most significant microbial agents affecting marine fishes and climatic changes may plays a great role in modulating the occurrence of bacterial fish diseases.

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