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Some Study on Bacterial Infection in some Cultured Marine Fish

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ABSTRACT

Bacterial diseases affecting marine fishes are numerous and cause high economic losses in marine culture sector in Egypt, therefore; this study was conducted for two main goals; Isolation and identification of the bacterial isolates affecting cultured marine fishes in Egypt as well as incidence of these diseases. In our study, A total number of (200) marine fishes (100 fish from Seabass, Seabream,) were collected from cages in Wadi-mariut region at Borg -El Arab city at Alexandria governorate After culture on media and biochemical reactions, (160 isolates) from all naturally examined fish species were obtained as following: (70 isolates) from Seabass, (90 isolates) from sea bream . Then Serotyping of these isolates into 5 bacterial groups; Photobacterium species, Streptococcus species, Tenacibaculum species, Vibrio species and Pseudomonas species and we found that the highest percentage from all bacterial isolates was Vibrio species (41.2 %) and *Vibrio alginolyticus* (*V. alginolyticus*) was the most prevalent bacterial serotype. After that, the percentage of the bacterial serotypes for each fish species was identified. The incidence of bacterial isolates in the internal organs of all naturally examined fish species (Liver, Spleen, Heart and Kidney) has been studied and the results revealed that : (a) The total number of bacterial isolates from liver was (100 isolates), (49 isolates) from heart, (62 isolates) from kidney and (39 isolates) from spleen and this means that the highest percentage of isolates were from Liver (about 40 %) from all bacterial isolates. (b) It was found that highest percentage from all bacterial isolates was from liver of Seabass (14 %), heart of Seabream (10 %), kidney of Seabream (8%) and spleen of Seabream (6%) in comparison with all naturally examined diseased marine fishes. PCR method used for detection of the most prevalent bacterial serotype among all bacterial serotypes (*V. alginolyticus*) which is used after that for experimental infection.

Keywords:

INTRODUCTION

FAO (2003) stated that the world aquaculture production in 2001 was approximately of 37.9 million tons, which represents around 41% of that obtained from extensive captures for human consumption. Also, Marine fish culture is dominated by Atlantic salmon (*Salmo salar*) led by Norway, then Chile, United Kingdom, Canada and Ireland. Other economically important marine fish are Gilthead Seabream (*Sparus aurata*), Seabass (*Dicentrarchus labrax*) and Turbot (*Scophthalmus maximus*) in countries such as Greece, Italy, France, Spain and Portugal, and Yellowtail (*Seriola quinqueradiata*), Ayu (*Plecoglossus altivelis*), Flounder (*Paralichthys olivaceus*) and Seabream (*Pagrus major*) in Japan.

In 2008, the world aquaculture production in major species groups showed that freshwater fishes continued to dominate with a production of 28.8 million tonnes (54.7 percent) valued at US$40.5 billion (41.2 percent), followed by mollusks (13.1 million tonnes), crustaceans (5 million tonnes), diadromous fishes (3.3 million tonnes), marine fishes (1.8 million tonnes) and other aquatic animals (0.62 million tonnes) *(FAO, 2010).*

The present work aimed to study the following items :

1. Isolation and identification of the bacterial isolates encountered in some cage-cultured marine fishes as Seabass *(Dicentrarchus labrax L.*), Seabream *(Sparus aurata L.)* native to private fish farms at Wadi-mariout - Borg El-Arab city at Alexandria governorate.
2. Recording the clinical signs and P.M lesions associated with such bacterial diseases in naturally infected fishes.
3. Serotyping of these bacterial isolates from naturally infected fishes.
4. Application of PCR for accurate identification of the isolated *Vibrio alginolyticus*.

Listing the prevalence of the identified bacterial isolates among the above mentioned diseased fishes.,

MATERIALS AND METHODS

A- MATERIAL
1. Fish
1.1. Naturally infected fishes

In our investigation, a total number of 200 cage-cultured marine fishes of different body weight range (50 g to 3 Kg) of two different species were collected; 100 Seabass (***Dicentrarchus labrax L***.), 100 of Seabream (***Sparus aurata L***.), fishes were collected showing clinical signs from private fish farm at Wadi-Mariut region at west Alexandria governorate, Egypt. The sources of water in Wadi-Mariut are numerous; underground water, drainage of canal originated from El-Banger area as well as from rainfall water downstreams from Borg-El-Arab city. Fishes were transferred as soon as possible to the laboratory located in the same farm where sampling occurs. The freshly dead fish specimens were subjected to full clinical, postmortem (PM) lesions, parasitic and mycological as well as bacteriological examinations.

2. Culture media

2.1. Media used for the isolation of the bacteria

2.1.a. Liquid media

Tryptic Soya broth (TSB) (Difco, Detroit, MI, USA) supplemented with 3% NaCl which was used for the growth of some suspected isolates prior to plating.

2.1.b. Semi-Solid media

0.5 % Nutrient agar medium (*Oxoid Manual, 1982*) which is supplemented with 3% NaCl which was used for the preservation of all isolated strains as well as for the detection of bacterial motility.

2.1.c. Solid media

Nutrient slope agar (*Oxoid Manual, 1982*) which is used for preservation of bacterial isolates and for detection of pigment production by bacterial isolates.

Trypticas Soya agar (TSA) (*Oxoid Manual, 1982*) which is a general purpose medium for isolation and cultivation of a variety of fastidious microorganisms and used supplemented with NaCl 3%.

Blood agar medium (Whitman, 2004).

Thiosulphate citrate bile salts sucrose agar (TCBS): (TCBS, Biolife, Milan, Italy) supplemented with 3% NaCl which was used as selective medium for the isolation of Vibrio species (*Whitman, 2004*).

Brain-heart infusion agar (BHI) (Difco) : for isolating and cultivating a variety of (fastidious) microorganisms.

B- METHODS

1. Gross clinical examination :

According to the method described by *Amlacher (1970*).

2. Postmortem (PM) examination

According to Conroy and Herman (1981).

3. Bacteriological examination

3.1. Identification of bacterial isolates

According to the methods described by Austin and Austin (1999).

4. Serotyping of the bacterial isolates

5. PCR for detection of virulent strain of V. alginolyticus

5.a. Detection of V. alginolyticus DNA by PCR (Cai et al., 2009)

5.a.1. Extraction of DNA of V. alginolyticus

5.a.2. Oligonucleotide primers

Primers were dissolved in nuclease-free water to obtain 50 - 1000 pmol concentration (Sambrook et al., 1989).

The oligonucleotide sequence for toxR gene of *V. alginolyticus* :

Product name	**Sequence (5' to 3')**	**Product size (bp)**
toxR gene		
Forward primer	CAGAAGAAT CGGAAGAACA	173
Reverse primer	TAGAATGACGCACAAAGG	

5.a.3. Polymerase chain reaction protocol

The PCR thermal cycler was adjusted as shown in Table (1).

Table (1) : Programming of thermal cycler for detection of ***V. alginolyticus*** *toxR* gene:

No. of cycles	**Temperature (0C)**	**Time**	**Target**
1 Cycle	94	5 min	Initial denaturation
30 cycles	a) 94 b) 65 c) 72	1 min 1 min 1.5 min	Denaturation Annealing Extension
1 cycle	72	10 min	Final extension
1 cycle	- 20	Until used	Preservation

6. Detection of PCR products (Sambrook et al., 1989)

6.1. Placement of combs and gel pouring
6.2. Loading buffer
6.3. Electrophoresis time and voltage
6.4. Gel examination

RESULTS

1. Results of naturally collected fishes

1.a. Results of Clinical examination (Clinical signs and Postmortem (PM) lesions)

1.b. Clinical signs of naturally examined Seabass

Some of naturally examined Seabass showing erythema in the mouth and severe exophthalmia (Fig. 1); severe hemorrhagic patches at the body and severe hemorrhages at base of pectoral fins (Fig. 2); severe hemorrhagic swollen protruded anal opening While others show hemorrhages at pelvic & anal fins moreover showing petechial hemorrhages at the operculum and around the eye .

1.c. PM lesions of naturally examined Seabass

Some of naturally examined Seabass showing hemorrhages at gills and at heart ; others show serous ascetic fluid tinged with blood dropping on behind paper upon opening the fish (Fig. 3) while the others show congestion of different degrees on the liver of examined fish (Fig. 4).

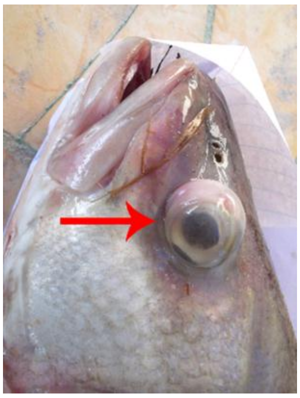

Fig. (1) : ***A naturally examined Seabass showing erythema in the mouth and severe exophthalmia***

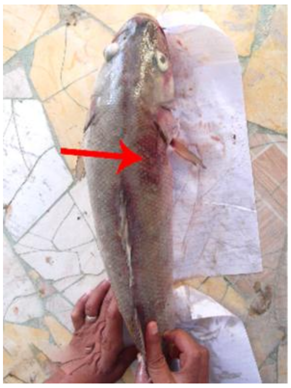

Fig. (2) : ***A naturally examined Seabass showing severe hemorrhagic patches on the body and severe hemorrhages at base of pectoral fins***

Fig. (3) : ***A naturally examined Seabass showing serous ascetic fluid tinged with blood dropping on behind paper upon opening the fish.***

Fig. (4) : ***A naturally examined sea bass showing serous ascetic fluid with different degrees congestion on the liver of infected fish.***

1.d. Clinical signs of naturally examined Seabream

Some of naturally examined Seabream showing hemorrhages at anal opening and at pelvic & anal fins (Fig. 5); darkness of the body of the fish and complete loss of the eye with erosions of the tail fins (Fig. 6); severe hemorrhagic ulcers on the skin and penetrate to the underlying musculature and others showing swollen hemorrhagic anus .

Other naturally examined Seabream showing opaqueness on the eye and exophthalmia and other showing swollen abdomen due to ascitis with erythematic hemorrhages on the abdomen and at the operculum .

1.e. PM lesions of naturally examined Seabream :

Some naturally examined Seabream showing congestion at liver and at the intestine severe hemorrhages and congestion at gills and live (arrow) (Fig. 7) and an other group of fish showing grayish whitish nodules appear in the brain (Fig. 9); the spleen, liver and the entire kidney (Fig. 10, 11, 12) .

Fig. (5) ***: A naturally examined Seabream showing hemorrhages at anal opening and at pelvic & anal fins.***

Fig. (6) ***: spleen A naturally examined Seabream showing darkness of the body of the fish and complete loss of the eye with erosions of the tail fins.***

Fig. (7) : ***A naturally examined Seabream showing severe congestion at gills and liver.***

Fig. (8) : A brain of naturally examined Seabream showing nodular formation.

Fig. (11) : A naturally examined Seabream showing severe nodular formation fill the entire kidney (arrow).

Fig. (12) : ***A naturally examined Seabream showing large whitish nodules on the kidney (arrow).***

2. Results of bacteriological examinations

2.a. Streptococcus iniae (S. iniae)

Table (2) : ***Comparison of phenotypic characteristics of the isolates to the reference strains of S. iniae according to (Whitman, 2004)***

Tests	Isolated strain	Reference strain
Gram staining reaction	+	+
Cell morphology	Cocci in chain	Cocci in chain
Motility	-	-
Swarming on TSA (2.0% NaCl)	+	+
Growth on TCBS agar	-	-
NaCl tolerance:-		
TSA + 1 % NaCl	+	+
TSA + 8 % NaCl	-	-
TSA + 10 % NaCl	-	-
Catalase test	-	-
Cytochrome oxidase test	-	-
Voges Proskauer test	-	-
Production of:-		
H2S production	-	-
Indole (peptone H2O)	-	-
Urease	-	-
Utilization of:-		
Lactose	-	-
Maltose	+	+
Mannitol	+	+
Sorbitol	-	-
Sucrose	+	+
Haemolysis (5% sheep RBCs)	β haemolysis	β haemolysis

2.b. Vibrio alginolyticus (V. alginolyticus)

Table (3) : *Colony and biochemical characteristics of the isolated and reference strain of* ***V. alginolyticus (Liu et al., 2004)*** .

Tests	Isolated strain	Reference strain
Gram staining reaction	-ve	-ve
Cell morphology	Rod shaped bacilli	Rod shaped bacilli
Motility	+ ve	+ ve
Swarming on TSA (2.0% NaCl)	+ ve	+ ve
Growth on TCBS agar	Yes	Yes
NaCl tolerance:-		
TSA + 1 % NaCl	+	+
TSA + 8 % NaCl	+	+
TSA + 10 % NaCl	-	-
Catalase test	+	+
Cytochrome oxidase test	+	+
Voges Proskauer test	+	+
Production of:-		
H2S production	-	-
Indole (peptone H2O)	+	+
Urease	-	-
Utilization of:-		
Citrate	-	-
Glucose	+	+
Lactose	-	-
Maltose	+	+
Mannitol	+	+
Sorbitol	+	+
Sucrose	+	+
Haemolysis (5% sheep RBCs)	Non hemolytic	Non hemolytic

2.c. Photobacterium damselae (Ph. damselae)

Table (4) : *Colony and biochemical characteristics of the isolated and reference strain of Ph. damselae (Whitman, 2004) .*

Tests	Isolated strain	Reference strain
Gram staining reaction	+	+
Cell morphology	Short rods	Short rods
Motility	-	-
Growth on TCBS agar	+	+
Grow at:-		
10 0C	-	-
25 0C	+	+
37 0C	-	-
Catalase test	+	+
Cytochrome oxidase test	+	+
Voges Proskauer test	-ve	-ve
Production of:-		
H2S production	-	-
Indole (peptone H2O)	-	-
Urease	-	-
Acid production from:-		
Glucose	+	+
Lactose	+	+
Maltose	-	-
Mannitol	+	+
Sorbitol	+	+
Sucrose	-	-
Haemolysis (5% sheep RBCs)	+ with non hemolytic zone	+ with non hemolytic zone

2.d. Pseudomonas fluorescence (*Ps. fluorescence*) :

Table (5) : *Colony and biochemical characteristics of the isolated and reference strain of Ps. fluorescence (Whitman, 2004)*

Tests	Isolated strain	Reference strain
Gram staining reaction	G –ve	G –ve
Cell morphology	Rod shaped bacilli	Rod shaped bacilli
Motility	-	-
Catalase test	+	+
Cytochrome oxidase test	+	+
Voges Proskauer test	-	-
Production of:-		
H2S production	-	-
Indole (peptone H2O)	-	-
Urease	-	-
Utilization of:-		
Citrate	+	+
Glucose	-ve	-ve
Lactose	-	-
Maltose	-ve	-ve
Mannitol	-ve	-ve
Sorbitol	-ve	-ve
Sucrose	-ve	-ve
Haemolysis (5% sheep RBCs)	Non hemolytic	Non hemolytic

3. Incidence of bacterial isolates in naturally infected marine fish species

The prevalence of bacterial isolates in the different examined naturally infected marine fishes is illustrated in table (6) Results indicated that (160) naturally examined fishes out of (200) were found to be infected with different types of bacteria. Moreover, the results revealed that Seabream was the most infected fish species (36 %), followed by Seabass (28%) .

4. Prevalence percentages of different bacterial groups from some diseased marine fishes

Incidence percentages of different bacterial groups from all cage cultured naturally infected marine fishes were illustrated in table (7); whereas the Vibrio species were the most prevalent among all bacterial groups where as they were (41.2%) while other bacterial groups were; (10%) for Photobacterium species, (22%) for Streptococcus species, (8.4%) for Tenacibaculum species and (18.4%) for Pseudomonas species.

5. Results of Incidence of the bacterial serotypes in naturally infected marine fishes :

Results indicated that (160) naturally examined fishes out of (200) were found to be infected with different types of bacteria. Moreover, the culture results demonstrated that the percentage of Gram negative bacteria was (78%) while the percentage of Gram positive bacteria was (22%).

Table (6) : ***Showing the prevalence of bacterial isolates in the different examined naturally infected marine fishes :***

Fish species	No. of examined fish	No. of isolates	Percentage (%)
Seabass	100	70	28
Seabream	100	90	36
Total	200	160 isolates	

Table (7) : ***Prevalence percentages of different bacterial groups from diseased marine fishes cultured during the period of the survey.***

Bacterial isolates	Number of serotypes	Incidence
Photobacterium species	15	10
Streptococcus species	45	22
Tenacibaculum species	21	8.4
Vibrio species	63	41.2
Pseudomonas species	16	18.4
Total	160 isolates	

The total prevalence of bacterial serotypes was illustrated in table (8) . Where as found that (5.2 %) of the infected fishes were positive for Ph. damselae subsp. piscicida, (4.8 %) for Ph. damselae subsp. damselae, (3.2 %) for *Vagococcus salmoninarum*, (4 %) for *Strept. parauberis*, (3.2 %) for *Strept. agalactiae*, (3.6%) for *Strept. iniae*, (4.8 %) for *Lactococcus garvieae*, (3.2%) for *Lactococcus piscium*, (8.4 %) for *Tenacibaculum maritimum*, (3.6%) for *V. anguillarum* serotype 01, (14 %) for V. alginolyticus, (2.8%) for *V. vulnificus* biotype 1, (2.4%) for *V. viscosus*, (6%) for *V. harveyi*, (3.2%) for *V. vulnificus* biotype 2, (4%) for *V. ordalii*, (5.2%) for *V. anguillarum* serotype 023, (3.6%) for *Ps. aeruginosa*, (4%) for *Ps. pleoglossicida*, (4%) for Ps. fluorescence, (2.8 %) for *Ps. chlororaphis* and (4 %) of the surveyed fishes were positive for *Ps. anguilliseptica*.

6. Incidence of bacterial serotypes among naturally infected Seabass

According to Table (9) the highly bacterial serotype in naturally infected Seabass was *V. alginolyticus* (17.14%), *T. maritimum* was (11.43%), *V. harveyi* was (10%) followed by *V. anguillarum* serotype 023 that was (8.57 %).

Table (8) : ***Prevalence of the bacterial serotypes among all species of naturally examined marine fishes :***

Bacterial isolates	Number of isolates	Prevalence %
Ph. damselae subsp. piscicida	13	5.2
Ph. damselae subsp. damselae	12	4.8
Vagococcus salmoninarum	8	3.2
Strept. parauberis	**10**	**4.0**
Strept. agalactiae (strept. difficile)	8	3.2
Strept. iniae	9	3.6
Lactococcus garvieae	12	4.8
Lactococcus piscium	8	3.2
Tenacibaculum maritimum	21	8.4
V. anguillarum serotype 01	**9**	**3.6**
V. alginolyticus	35	14.0
V. vulnificus biotype 1	7	2.8
V. viscosus *(Moritella viscosa)*	6	2.4
V. harveyi	15	6.0
V. vulnificus biotype 2	8	3.2
V. ordalii (V. anguillarum biotype 2)	10	4.0
V. anguillarum serotype 023	13	5.2
Ps. aeruginosa	9	3.6
Ps. pleoglossicida	10	4.0
Ps. fluorescence	10	4.0
Ps. chlororaphis	7	2.8
Ps. anguilliseptica	10	4.0
Total	**250 serotypes**	

Table (9) : ***Prevalence of different bacterial serotypes in Seabass .***

Bacterial isolates	Number of isolates	Incidence %
Ph. damselae subsp. piscicida	4	5.71
Ph. damselae subsp. damselae	5	7.14
Vagococcus salmoninarum	3	4.28
Strept. parauberis	4	5.71
Strept. agalactiae	2	2.86
Strept. iniae	3	4.29
Lactococcus garvieae	4	5.71
Lactococcus piscium	3	4.29
Tenacibaculum maritimum	8	11.43
V. anguillarum serotype 01	4	5.71
V. alginolyticus	12	17.14
V. vulnificus biotype 1	2	2.86
V. viscosus	3	4.29
V. harveyi	7	10
V. vulnificus biotype 2	3	4.29
V. ordalii	3	4.29
V. anguillarum serotype 023	6	8.57
Ps. aeruginosa	4	5.71
Ps. pleoglossicida	5	7.14
Ps. fluorescens	3	4.29
Ps. chlororaphis	4	5.71
Ps. anguilliseptica	3	4.29
Total number of isolates	**70 isolates**	

7. Incidence of bacterial serotypes among naturally infected Seabream

According to Table (10) the highly prevalent bacterial serotype in naturally infected Seabream was ***V. alginolyticus*** (16.66 %), ***T. maritimum*** was (7.77 %) followed by ***Ph. damselae subsp. piscicida*** that was (5.55 %).

8. The Incidence of bacterial isolates in the internal organs of the naturally infected marine fishes

The incidence of bacterial isolates retrieved from internal organs in each fish species of naturally infected marine fishes was illustrated in table (11) .

Table (10) : ***Incidence of different bacterial serotypes in Seabream.***

Bacterial isolates	Number of isolates	Incidence (%)
Ph. damselae subsp. piscicida	5	5.55
Ph. damselae subsp. damselae	3	3.33
Vagococcus salmoninarum	2	2.22
Strept. parauberis	3	3.33
Strept. agalactiae	3	3.33
Strept. iniae	2	2.22
Lactococcus garvieae	5	5.55
Lactococcus piscium	2	2.22
Tenacibaculum maritimum	7	7.77
V. anguillarum serotype 01	2	2.22
V. alginolyticus	15	16.66
V. vulnificus biotype 1	2	2.22
V. viscosus	2	2.22
V. harveyi	3	3.33
V. vulnificus biotype 2	2	2.22
V. ordalii	2	2.22
V. anguillarum serotype 023	4	4.44
Ps. aeruginosa	2	2.22
Ps. pleoglossicida	2	2.22
Ps. fluorescens	4	4.44
Ps. chlororaphis	0	0
Ps. anguilliseptica	4	4.44
Total number of isolates	**90 isolates**	

9. Total prevalence of bacterial serotypes retrieved from the internal organs of all naturally infected marine fishes :

The total prevalence of bacterial serotypes retrieved from the internal organs of all naturally infected marine fishes was illustrated in table (12) . Which showed that the high incidence of bacterial serotypes was in Liver (40 %), followed by the kidney (24.80 %), (19.60 %) in the heart and the lowest incidence occur in Spleen (15.60 %).

10. Results of PCR for detection of V. alginolyticus strain

The primer sequence showed maximum identity with the sequence of ***V. alginolyticus*** with 100% homology and the *toxR* gene - PCR amplified the correctly sized products for the isolates identified as virulent strains of *V. alginolyticus* (Fig. 13) .

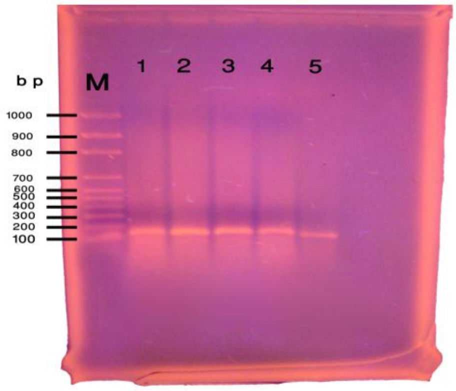

Fig. (13) : ***Showed amplified products when utilizing polymerase chain reaction (PCR); where as lane M is, 100-bp DNA ladder (Biotools; B&M laboratories S. A., Madrid, Spain) while lanes 1 to 5 are toxR gene (173 bp) of 5 isolates of V. alginolyticus strain***

DISCUSSION

In the present work, we spot light on the clinical picture and PM lesions of the most predominant bacterial pathogens affecting some marine fishes native to Wadi-mariut region, Borg El-Arab city, Alexandria governorate.

Moreover, isolation and identification of these bacterial infections by both biochemical traditional methods serological as well as by PCR,

Concerning the clinical signs and Postmortem (PM) lesions of some naturally examined marine fishes; the clinical picture and gross lesions of Vibriosis (Listonellosis) in naturally infected some marine fishes; our results were in concordance to that obtained by

Table (11) : ***The prevalence of bacterial isolates in different organs of naturally infected marine fishes :***

Fish / Organ	Seabass No.	Seabass %	Seabream No.	Seabream %
Liver	35	14	30	12
Heart	15	6	25	10
Kidney	10	4	20	8
Spleen	10	4	15	6
Total	70	28	90	36

**** = significant at (P < 0.05)*** ***** = Significant at (P < 0.01)***

Table (12) : The total Incidence of bacterial serotypes retrieved from the internal organs of all naturally infected marine fishes.

Organs / Strains	Liver No.	Liver %	Heart No.	Heart %	Kidney No.	Kidney %	Spleen No.	Spleen %
Ph. damselae subsp. Piscicida	4	1.6	2	0.8	5	2	2	0.8
Ph. damselae subsp. Damselae	2	0.8	4	1.6	4	1.6	2	0.8
Vago. Salmoninarum	4	1.6	2	0.8	1	0.4	1	0.4
Strept. Parauberis	5	2	2	0.8	2	0.8	1	0.4
Strept. Agalactiae	2	0.8	2	0.8	2	0.8	2	0.8
Strept. Iniae	3	1.2	1	0.4	2	0.8	3	1.2
Lactococcus garvieae	6	2.4	2	0.8	2	0.8	2	0.8
Lactococcus piscium	2	0.8	2	0.8	2	0.8	2	0.8
Tenacibaculum maritimum	9	3.6	6	2.4	4	1.6	2	0.8
V. anguillarum serotype 01	2	1.2	1	0.4	3	1.2	3	1.2
V. alginolyticus	17	6.8	12	4.8	4	1.6	2	0.8
V. vulnificus biotype 1	2	0.8	1	0.4	3	1.2	1	0.4
V. viscosus	2	0.8	1	0.4	2	0.8	1	0.4
V. harveyi	6	2.4	2	0.8	5	2	2	0.8
V. vulnificus biotype 2	3	1.2	1	0.4	2	0.8	2	0.8
V. ordalii	5	2	1	0.4	3	1.2	1	0.4
V. anguillarum serotype 023	8	3.2	1	0.4	3	1.2	1	0.4
Ps. Aeruginosa	3	1.2	2	0.8	2	0.8	2	0.8
Ps. Pleoglossicida	4	1.6	1	0.4	3	1.2	2	0.8
Ps. Fluorescens	4	1.6	1	0.4	3	1.2	2	0.8
Ps. Chlororaphis	3	1.2	1	0.4	2	0.8	1	1.2
Ps. Anguilliseptica	4	1.6	1	0.4	3	1.2	2	0.8
Total number of isolates	100	40 %	49	19.60 %	62	24.80 %	39	15.6 %

Actis et al. (1999); where they stated that fish affected by *Listonella anguillarum* (*L. anguillarum*) showed typical signs of a generalized septicemia with hemorrhage on the base of fins, exophthalmia and corneal opacity.

In regard to the disease caused by *V. alginolyticus* in Seabream included septicemia, hemorrhaging, dark skin, and ulcers on the skin surface in some cases. Internally, accumulated fluid in the peritoneal cavity and in some cases revealed hemorrhagic livers (*Balebona et al., 1998a*).

These pathological alterations may be attributed to the extra-cellular products (ECP) especially proteases and other toxins produced by Vibrio species that capable of causing tissue and cell damage *(Nottage and Birkbeck, 1987).*

In regards to the pathological picture of Photobacteriosis that were reported in our study may be attributed to stress exerted on the fish which was favored by overcrowdness that facilitated the transmission of the pathogens according to *Fouz et al. (2000*) who declared that the infection of fish with *P. piscicida* is exaggerated in overcrowdness, stressed aquaculture conditions that spread the disease is accelerated by direct contact of the fish.

In regard to the results of clinical signs and PM lesions of Streptococcosis *in naturally infected fishes; our results were in improvement with that obtained by (*Colorni et al., 2002)*; where they mentioned that* S. iniae *affecting* Dicentrarchus labrax *causing severe bilateral exophthalmia and internally, hemorrhages were conspicuous in the abdominal cavity.*

These results may be attributed to phosphoglucomutase enzyme as the virulence factor for S. iniae *which inter-converts glucose-6-phosphate and glucose-1-phosphate which play important role in the production of* S. iniae *polysaccharide capsules* (Buchanan et al., 2005).

In concern to the clinical picture and gross lesions of Pseudomoniasis in naturally infected some marine fishes were in similar manner to (*Toranzo et al., 2005*); where they illustrated that the main clinical signs of the fish affected by *Ps. anguilliseptica* septicaemia were abdominal distension and hemorrhagic petechiae in the skin and internal organs, but the lesions in eels are always more severe than those observed in Gilthead Seabream.

In concern to the culture, morphological and biochemical characteristics of the bacterial isolates; the culture and morphological characteristics of *S. iniae* noticed in this study, the results were in concordance with those reported by *(Colorni et al., 2002)*.

In regards to the biochemical characterization of *S. iniae* isolates in this study, the results of biochemical tests were in complete agreement with those obtained by (*Whitman, 2004*).

In concern to the culture and morphological characteristics of Photobacterium species noticed in this study, the results were in concordance with those reported by (*Mladineo et al., 2006*).

In concern to the culture and morphological characteristics of *Pseudomonas fluorescenc*e (*Ps. fluorescence*) noticed in this study, the results were in concordance with those reported by (*Megahed, 2000*) and (*El-Moghazy, 2004*).

The results of PCR for *V. alginolyticus* indicated that the primer sequence of the *toxR* gene showed maximum identity with the sequence of *V. alginolyticus* gene with 100% homology and the *toxR* gene - PCR amplified the correctly sized products for the isolates identified as virulent strains of *V. alginolyticus* and these results agreed with the results obtained by *(Cai et al., 2009)* who tested the bacterial strains obtained from various organs with the novel primers targeting the *Collagenase* gene, *OmpK* gene and *toxR* gene to establish a multiplex polymerase chain reaction (PCR) method and found that the multiplex PCR method was successfully developed to identify virulent strains of *V. alginolyticus*, and provides a rapid, sensitive, specific and reliable technology for diagnosing virulent strains of *V. alginolyticus* but the avirulent strains were not recognized by the multiplex PCR because of lack of the *Collagenase* and *toxR* genes.

The role of bacteria varies from their effect as primary pathogen to that of secondary invader in the presence of other disease agents; they may also serve as a stress factor and predispose fish to other diseases *(Badran and Eissa, 1991).*

In regards to the Incidence of the bacterial isolates among naturally examined marine fishes; Our results revealed that Seabream was the most infected fish species (36%), followed by Seabass (28%), and these results were in agreement with that obtained by *(Yiagnisis and Athanassopoulou, 2011)* where they stated that Seabream was highest infected marine fish species in comparison with Seabass.

In concern to the prevalence percentages of the isolated bacterial groups; our results revealed that Vibrio species were the most prevalent among all bacterial groups where as they were (41.2%) while other bacterial groups were; (10%) for Photobacterium species, (22%) for Streptococcus species, (8.4 %) for Tenacibaculum species and (18.4%) for Pseudomonas species.

These results agreed with the findings of (*Balebona et al., 1998b)* where they illustrated that the main pathogenic microorganisms isolated from gilthead Seabream (*Spaurus aurata*) were Vibrio species *(*67.8%) among all bacterial isolates.

In contrary, Our results disagreed with that published by (*Khan et al., 1981)* where they demonstrated that Pseudomonads, Aeromonads and Vibrio species were the most isolated bacterial agents from specimens collected from mortalities in captive Atlantic cod, *Gadus morhus L.* associated with fin rot disease and the results showed that Pseudomonads accounted for (72%) of the isolates while Vibrios and Aeromonads accounted for (28%).

In the same way, *Nedoluha and Westhoff (1997)* cleared that the most predominant bacterial pathogens randomly isolated from diseased Striped bass, *Morone saxatilis L.* and hybrid Striped bass, *Morone chrysops L.* and *Morone saxatilus L*. collected from different marine aquaculture systems were accounted for Aeromonas species (19%), Pseudomonas species (6%) and on the other hand, *Staphylococcus aureus* and Vibrio species were isolated from all systems but in low numbers.

Further more; *Athanassopoulou et al. (1999)* surveyed the bacterial and parasitic diseases of Cuvier, *Puntazzo puntazzo L.* collected from different marine aquaculture systems in Greece and they noticed that the prevalence of bacterial infections among diseased fishes were; *P. piscicida* (80 %), *V. alginolyticus* (29%), *V. vulnficus* (16%), *A. hydrophila* (15 %), *V. splendidus* (l0 %) and *Staph. epidermidis* (10%).

These differences may be attributed to the differences in fish species, Seasons, water quality, locality and types of pisciculture used.

In regards to Serotyping of bacterial isolates; our results revealed that (78 %) fishes were found to be infected with Gram negative bacteria and only (22%) fishes were infected with Gram positive bacteria and *V. alginolyticus* was the most prevalent bacterial isolate (14 %) from the percentages of all isolates.

These results were in agreement with that obtained by (*Zorrilla et al., 2003b)* where they stated that most of the bacterial isolates were Gram-negative (93.19%) and 69.90% of these isolates were identified as species of Vibrio as well as *V. alginolyticus* being the most frequent Vibrio species (21.35%).

V. alginolyticus was not dominant in samples obtained from Common dentex (*D.*

dentex, L.) (*Company et al., 1999*), although its detection was noteworthy.

Furthermore, *Nedoluha and Westhoff (1997)* recorded that Pseudomonas species accounted (6%) from the bacterial isolates from diseased striped bass, *Morone saxatilis L.* and hybrid striped bass, *Morone chrysops L.* and *Morone saxatilus L*. collected from different marine aquaculture systems.

Moreover, *Balebona et al. (1998b)* recorded Pseudomonas species accounted (13.5%) from bacterial isolates from gilthead Seabream (*Spaurus aurata*).

On contrary, the results were lower than that recorded by (*Khan et al., 1981)* where they mentioned that Pseudomonads accounted for (72 %) of the isolates from mortalities in captive Atlantic cod, *Gadus morhus L.* associated with fin rot disease.

In concern with the results of the incidence of bacterial isolates retrieved from the internal organs of naturally infected Seabream were not in agreement with that obtained by (*Zorrilla et al., 2003b)* where they stated that the bacterial isolates from Seabream were isolated from spleen (49.51%), liver (29.12%), kidney (11.65%) and other organs such as ulcers and exophthalmic eyes.

Similarly, our results were not in concordance with that recorded by (*El-Gendy, 2007)* and *(Moustafa et al., 2010*) where they mentioned that the highest prevalence of *Ps. fluorescence* was recorded in Kidneys (40.32 %), Liver (27.41 %), spleen (20.96 %) and only (11.29 %) from the gills.

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بعض الدراسات على العدوى البكتيرية فى أسماك المياه المالحة المستزرعة

محمود الطنيخى

الأمراض البكتيرية التى تصيب أسماك المياه المالحة متعددة وتؤدى إلى العديد من الخسائر الاقتصادية فى مصر وبناءا على ذلك تم عمل هذه الدراسة والتى تهدف إلى عزل والتعرف على العديد من عترات البكتيريا التى تصيب أسماك المياه المالحة فى مصر بالإضافة إلى دراسة العلاقة بين المعادن الثقيلة وتركيزاتها فى الأنسجة والمياه والتى تؤدى إلى الإصابة بالأمراض البكتيرية .

فى هذه الدراسة تم استخدام عدد (200) سمكة من أسماك الأقفاص المستزرعة (100) من أسماك القاروص و (100) من أسماك الدنيس تم تجميعهم من مناطق وادى مريوط وبرج العرب بمحافظة الإسكندرية .

بعد استخدام الأوساط المختلفة لزراعة البكتيريا وعمل الاختبارات البيوكيميائية تم عزل (160) عترة من كل الأسماك المصابة طبيعيا (70) من أسماك الدنيس و (90) من أسماك القاروص وتم تصنيف عترات البكتيريا إلى (5) مجموعات وهى كالآتى : (الفوتوبكتيريا , الاستربتوكوكس , التينسبيكيولم , الفبريو ثم السودوموناس) وكان أهم أنواع البكتيريا المتواجدة بشكل كبير هى الفبريو بنسبة 41.2% وخصوصا نوع الفبريو الجينوليتكس وبعد ذلك تم تصنيف البكتيريا سيرولوجيا وتحديد نسبتها فى الأعضاء المختلفة (الكبد , الطحال , القلب , الكلى) **وكانت النتائج الآتى :**

1- تم عزل (100) كترة من الكبد , (49) من القلب , (62) من الكلى , (39) من الطحال فبالتالى كانت أعلى نسبة من الكبد بمعدل 40% من البكتيريا الكلية .

2- وجد أن أعلى نسبة من البكتيريا تم عزلها من الكبد فى أسماك الدنيس بنسبة 14% ومن القاروص 10% .

3- بالنسبة للكلى فكانت 8% من أسماك الدنيس و6% من القاروص .

4- تم إجراء اختبار تفاعل البلمرة المتسلسل لتحديد العترة الأصلية وتأكيدها من نوع فبريو الجينوليتكس.