

Studies on Bacterial Infection in Marine Fish**Saad, T. T^{1*} and Atallah S. T. ²**¹ Poultry and fish diseases Dept., Fac. of Vet. Med. Alex. University² Animal Husb. And Anim. Wealth. Dev. Dept. Fac. of Vet. Med. Alexandria

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ABSTRACT

In our study, total number of (400) cage and pen cultured diseased sea bass and sea bream (200 fish from each) were collected from Wadi Mariut at Borg El-Arab City at Alexandria Governorate, Egypt . The collected fishes were examined for parasitological and fungal infection firstly to ensure the real cause of signs and mortalities, the results showed no parasitic infection or fungal infection . After that the fishes subject to bacteriological examination as the following : Culturing on different ordinary and specific laboratory media Morphological identification of bacterial isolates, biochemical characterization of these isolates . API 20E system for serotyping of Pseudomonas species isolates and slide-agglutination test for serotyping of all bacterial isolates. The obtained results showed that the total number of bacterial isolates (290) divided as (136) from sea bass and (154) isolates from sea bream . The prevalence of bacterial species isolates in sea bass were (47%) vibrio, (27.3%) Pseudomonas and (25.7%) T. maritimum, while in sea bass were (53%) vibrio, (28%) Pseudomonas and (19%) T. maritimum . The most highly incidence bacterial isolates serotypes in sea bass and sea bream were *V. anguillarum*, *T. maritimum*, *V. alginolyticus* and *Ps. fluorescens*. NaCl tolerance test : Different concentration of NaCl used on pleat count agar media 2, 3, 4, 5, 6, 8% for different bacterial serotypes, incubate for 24 hours after that count the total bacterial colonies, the results revealed that the best NaCl concentration for Pseudomonas species growth was 2%, but *T. maritimum* grow well in 3% NaCl and best growth recorded at 4% NaCl concentration . Antimicrobial susceptibility test : For control and treatment of these bacterial infections, applying Antimicrobial susceptibility test by using disc diffusion technique and reading the zone of inhibition . The results revealed that most Pseudomonas species serotypes were sensitive to Ciprofloxacin and intermediate sensitive to flumequine enrofloxacin and gentamycin, but in *T. maritimum* isolates were resist to all antimicrobial except Ciprofloxacin, enrofloxacin and doxycycline . In regards to vibrio species susceptibility, this study noticed that all serotypes of vibrio were sensitive to all antimicrobial except amoxicilline, sulphamethoxale/Trimethoprim were resist and intermediate sensitive to erythromycin and oxytetracycline in some serotypes. The results indicated that, the higher return losses observed in seabass higher than seabream fish as the losses 460LE/100 fish examined and 185.50LE/100 fish examined in seabream, and the Vibrio Spp, Pseudomonas and Tenabaculum Spp.

Keywords:**INTRODUCTION**

The marine fishes represent the major investment choices for the national fishermen (Ellis, 1999) and also represent the major investment choices for the Egyptian fishermen

due to its high economic value (Moustafa et al., 2010).

The appearance and development of a fish disease is a result of the interaction among pathogen, host and environment.

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Therefore, multidisciplinary studies involving the characteristics of potential pathogenic microorganisms for fish, aspects of the biology of the fish hosts as well as a better understanding of the environmental factors affecting such cultures, will allow the application of adequate measures to prevent and control the main diseases limiting the production of marine fishes.

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of these microorganisms may vary from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other disease process (Richards and Roberts, 1978).

Surveys have involved the marine fish species diseases outbreaks (Toranzo *et al.*, 2005) Therefore, the present study was aimed to:

Isolation of the bacterial isolates encountered, API-20E system for identification of *Pseudomonas* isolates.

Serotyping of the bacterial isolates from naturally infected fishes .

NaCl tolerance for all bacterial serotypes.

Recording the most effective antimicrobial drugs used in treatment of these bacterial diseases.

MATERIALS AND METHODS

A- Material

Fish

Naturally infected fishes

In this study, a total number of 400 cage cultured marine fishes with different body weights ranged 50 to 200g whereas 200 fish of Seabream (*Sparus aurata L.*) and (200) fish of Seabass (*Dicentrarchus labrax L.*) were collected showing clinical signs from

private fish farm in Wadi-Mariut region at west Alexandria governorate, Egypt.

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. Identification of bacterial isolates

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

4. Biochemical characters using conventional tests

The biochemical tests used in our study were : Cytochrome oxidase, Citrate utilization, Indole production, Voges–Proskauer, H₂S production, Hydrolysis of urea, Sugar fermentation, Vibrostatic agent test . Amlacher (1970).

5. API 20E system for identification of *Pseudomonas* isolates

6. Serotyping of the bacterial isolates

Bacterial strains

All bacterial isolates were used as antigens in the agglutination tests. The strains were isolated from epizootics occurring in the Wadi-Mariut area at Alexandria governorate. Stock cultures of the isolates were maintained by periodic transfer on tryptic soy agar (Oxoid Manual, 1982) slants with the appropriate salt concentration. For long term storage, cultures were kept at - 80 C⁰ in medium with 15% glycerol.

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7. Evaluation of the NaCl tolerance for all bacterial serotypes

Different concentration of NaCl used on plate count agar media by rate 2, 3, 4, 5, 6, 7 and 8% for different bacterial isolates. Each isolates of bacteria adjusted at Macfarland's No. (3) then dilute this suspension until 10^2 CFU/ml by ten fold serial dilution after that take 1 ml from final dilution for each concentration of NaCl, after that incubated at 25 C for 24-48 hours. *Austin and Austin (1999)*.

8. Antimicrobial sensitivity test

Susceptibility to several antimicrobials was determined using the disc diffusion technique (*Barry and Thornsberry, 1991*).

9 - Economic losses

The economic losses of the fish due to infection with bacteria were determined from dead infected fish, weight of dead infected fish and the losses in return due to dead infected fish/100 fish according to the following equations (*Atallah and El-Banna, 2005 and Saad et al., 2006*):

a-Weight of dead fish = Number of dead infected fish X Average weight of the fish (gm).

b-Losses in returns (L.E) = Weight of the fish (Kg) X Price of Kg fish (L.E).

10- Statistical analysis

The statistical analysis was made according to (SAS, 2004), where we used the χ^2 - test for detection the significance of the incidence of the different parameters studied among different types of fish of either seabass and seabream.

RESULTS

Results of naturally collected fishes

1- a. Clinical signs of naturally examined Seabass

Some of naturally examined Seabass showed severe hemorrhage at the base of anal and pelvic fins and around the operculum (**Photo 1**) while others showing different degrees of abdominal swelling (Dropsy). (**Photo 2**).

1- b. PM lesions of naturally examined Seabass

Some of naturally examined Seabass showing serous abdominal ascetic fluid with congestion in the gills (Photo 3). While others showing severe congestion in the liver (Photo 4).

2- a. Clinical signs of naturally examined Seabream

Some of naturally examined Seabream showed darkening of skin color, complete removal of the eye ball with necrosis of the tail fin (Photo 5); while other naturally examined Seabream showing hemorrhage and complete removal of the eye ball (Photo 6).

2-b. PM lesions of naturally examined Seabream

Some naturally examined Seabream showed different degrees of liver congestion (Photo 7) while others showing severe congestion in the gills and hemorrhages on the surface of the liver (Photo 8).

3. Results of bacteriological examinations *Pseudomonas* species:

There are many isolates of *Pseudomonas* species were identified such as *Ps. fluorescense*, *Ps. aeruginosa* and *Ps. anguilliseptica*.

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For further identification of *Pseudomonas* species, the API20E system used.

Tenacibaculum maritimum :

T. maritimum isolates were difficult in classical ager media due to slow growth. So we used tryptone yeast extract salt media at 25 C for 48-72 hours.

Morphological characters:

Colonies of *T. maritimum* are flat, pale yellow with uneven edges and strongly adherent to the medium.

T. maritimum is a Gram negative, aerobic bacillus, 0.5 mm wide and up to 12 mm long. It is motile by a peculiar flexing movement. When placed on a moist slide the microorganisms move into columns or hay stacks which give it the specific name *T. maritimum*.



Photo (1) : Naturally examined Seabass showed severe hemorrhage at the base of anal and pelvic fins and around the operculum.



Photo (2) : Naturally examined Seabass showing different degrees of abdominal swelling (Dropsy).



Photo (3) : Naturally examined Seabass showing serous abdominal ascetic fluid with congestion in the gills.



Photo (4) : Naturally examined Seabass showing severe congestion in the liver and in the gills.

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Photo (5) : Naturally infected Seabream showed different degrees of liver congestion.



Photo (6) : Naturally examined Seabream showed darkening of skin color, complete removal of the eye ball with necrosis of the tail fin.



Photo (7) : Naturally examined Seabream showed hemorrhage and complete removal of the eye ball.



Photo (8) : Naturally examined Seabream showed severe congestion in the gills and hemorrhages on the surface of the liver.

***Vibrio (Listonella) anguillarum* :**

During this study, the isolation was made on TCBS media as a selective media for *V. species* .

Morphological characters:

Staining of a film prepared from pure cultures of the suspected colonies, and microscopical examination revealed the presence of Gram negative short rods or coccobacilli, arranged singly, in pairs or groups, non spore forming, and non capsulated.

The two methods used for detection of motility of suspected strains revealed the following :

Hanging drop technique:

The microscopical examination of the slide indicated that the isolated strains were highly motile bacteria.

On Trypticase Soya agar, Colonies appeared large, 1-4 mm in diameter, white, circular, convex with raised center and glistening appearance and yellow colonies were observed on TCBS agar after 24 hrs from culturing .

***Vibrio alginolyticus* :**

Morphological characters:

On TSA media the colonies appeared grayish white in color after 24 hours incubation at 25C.

On TCBS agar media colonies appeared small 2-3mm in diameter yellowish in color after 24 hours.

Prevalence of bacterial isolates in naturally infected marine fishes

The results in Table (1) showed that (136) isolates from naturally infected seabass out of (200) were obtained by the prevalent rate (68%) and (154) isolates from naturally infected seabream out of (200) were isolated by prevalent rate (77%) . Results revealed that Seabream was the most infected species than Seabass.

5. Prevalence of bacterial species in naturally infected fish species :

The results in Table (2) Indicated that, the Prevalence of different bacterial species in naturally infected Seabass and Seabream where as Pseudomonas species reached to (27.5 %), *Tenacibaculum maritimum* (22 %), and Vibrio species (50.50 %) of the total examined fish.

Table (1): Showing the prevalence of bacterial isolates in the different examined naturally infected marine fishes :

Fish species	No. of examined fish	No. of isolates	Prevalence %
Seabass	200	136	68
Seabream	200	154	77
Total	400	290	72.5

$Chi^2 = 3.37^{**}$

** = Significant at ($P < 0.01$)

Table (2): Prevalence of species in naturally infected fish species.

Bacterial species	Number of isolates	Prevalence %
<i>Vibrio species</i>	146	50.5
<i>Pseudomonas species</i>	80	27.5
<i>Tenacibaculum species</i>	64	22
Total	290	100

$Chi^2 = 7.40^{**}$

** = Significant at ($P < 0.01$)

6. Prevalence of bacterial species in naturally infected Seabass

Table (3) Illustrated that; the results showed that, the prevalence of bacterial species among Seabass were Pseudomonas species (27.3 %), *Tenacibaculum species* (25.7 %), and Vibrio species (47 %) of the total examined Seabass.

7. Prevalence of bacterial species in naturally infected Seabream

The results cleared in Table (4) Indicated that, the prevalence of different bacterial species differed among Seabream were pseudomonas species (28 %), *T. maritimum* (19 %), Vibrio (53 %) of the total examined Seabream.

Table (3): Prevalence of bacterial species in naturally infected Seabass.

Bacterial species	Number of isolates	Prevalence %
<i>Vibrio species</i>	64	47
<i>Pseudomonas species</i>	37	27.3
<i>Tenacibaculum species</i>	35	25.7
Total	136	100

$Chi^2 = 6.33^{**}$

** = Significant at ($P < 0.01$).

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Table (4): Prevalence of bacterial species in naturally infected Seabream.

Bacterial species	Number of isolates	Prevalence %
<i>Vibrio</i> species	82	53
<i>Pseudomonas</i> species	43	28
<i>T. maritimum</i>	29	19
Total	154	100

$Chi^2 = 7.24^{**}$

**= Significant at ($P < 0.01$)

8. Results of prevalence of the bacterial serotypes in naturally infected marine fishes

The incidence of bacterial serotypes in naturally infected marine fishes was illustrated in Table (5) Whereas *Ps. fluorescence* (9%), *Ps. aeruginosa* (4.8%), *Ps. anguilliseptica* (7.6%), *Ps. plecoglossicida* (3.8%), *Ps. chlororaphis* (2.5%), *T. maritimum* (22%), *V. anguillarum* (23.5%), *V. alginolyticus* (13.8%), *V. ordalii* (4%), *V. harveyi* (4.8%), *V. viscosus* (2.5%) and *V. vulnificus* was (1.7%).

9. Results of Prevalence of the bacterial serotypes in naturally infected Seabass

Table (6) revealed that, the prevalence of bacterial serotypes differed among naturally infected Seabass.

10. Results of Prevalence of the bacterial serotypes in naturally infected Seabream

Table (7) revealed that, the prevalence of bacterial serotypes differed significantly ($P < 0.01$) among naturally infected Seabream.

Table (5): Prevalence of the bacterial serotypes among naturally examined seabass and sea bream.

Bacterial serotypes	Number of isolates	%
<i>Ps. Fluorescence</i>	26	9
<i>Ps. Aeruginosa</i>	14	4.8
<i>Ps. anguilliseptica</i>	22	7.6
<i>Ps. plecoglossicida</i>	11	3.8
<i>Ps. chlororaphis</i>	7	2.5
<i>T. maritimum</i>	64	22
<i>V. anguillarum</i>	68	23.5
<i>V. alginolyticus</i>	40	13.8
<i>V. ordalii</i>	12	4
<i>V. harveyi</i>	14	4.8
<i>V. viscosus</i>	7	2.5
<i>V. vulnificus</i>	5	1.7
Total	290	100

$Chi^2 = 45.35^{**}$

** = Significant at ($P < 0.01$)

Table (6) : The prevalence of the bacterial serotypes in naturally infected Seabass :

Bacterial serotypes	No. of prevalence	
	No. of isolates	Prevalence %
<i>Pseudomonas fluorescence</i>	12	8.8
<i>Pseudomonas aeruginosa</i>	5	3.7
<i>Pseudomonas anguilliseptica</i>	10	7.3
<i>Pseudomonas pleoglossicida</i>	7	5.1
<i>Pseudomonas chlororaphis</i>	3	2.2
<i>Tenacibaculum maritimum</i>	35	25.7
<i>Vibrio (Listonella) anguillarum</i>	37	27.2
<i>Vibrio alginolyticus</i>	14	10.3
<i>Vibrio ordalii</i>	5	3.7
<i>Vibrio harveyi</i>	5	3.7
<i>Vibrio viscosus</i>	2	1.5
<i>Vibrio vulnificus</i>	1	0.7
Total	136	

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Table (7) : *incidence of bacterial serotypes of naturally infected Seabream :*

Bacterial serotypes	No. of prevalence	
	No. of isolates	Prevalence %
<i>Pseudomonas fluorescens</i>	14	9
<i>Pseudomonas aeruginosa</i>	9	5.6
<i>Pseudomonas anguilliseptica</i>	12	7.8
<i>Pseudomonas pleogloissida</i>	4	2.6
<i>Pseudomonas chlororaphis</i>	4	2.6
<i>Tenacibaculum maritimum</i>	29	19
<i>Vibrio (Listonella) anguillarum</i>	31	20
<i>Vibrio alginolyticus</i>	26	17
<i>Vibrio ordalii</i>	7	4.5
<i>Vibrio harveyi</i>	9	5.90
<i>Vibrio viscosus</i>	5	3
<i>Vibrio vulnificus</i>	4	2.6
Total	154	

$\chi^2 = 20.22^{**}$

** = Significant at ($P < 0.01$).

11. Sodium Chloride tolerance test

The different bacterial serotypes were cultivated on plate count agar with different concentrations of NaCl and the results were illustrated in Table (8), whereas, most of *Pseudomonas* serotypes were grown at 2, 3 and 4% NaCl and the best growth were observed at 2% concentration. While *T. maritimum* well grown at 3 and 4 NaCl and stopped growth at 5 concentration. Also, In *Vibrio* species, all serotypes well grown at 4% NaCl and some serotypes as *L. anguillarum*, *V. alginolyticus* and *V. harveyi* can grow until 8 % NaCl.

12. Results of antimicrobial sensitivity test

All different serotypes of bacterial isolates tested for sensitivity of different antimicrobial discs whereas; the results in Table (9 and 10) Revealed that, all serotypes of *Pseudomonas* species were sensitive to ciprofloxacin and flumequine, and resistant to Erythromycin, Oxytetracycline, Doxycycline, Amoxicillin and Sulphamethoxazole with Trimethoprim with variable results with gentamycin and Enrofloxacin.

Also, the results indicated that, the *T. maritimum* more sensitive to Ciprofloxacin and Enrofloxacin, similarly intermediate sensitive to, Doxycycline and Amoxicillin while resistant to other antimicrobial discs.

On the other hand, *Vibrio* species more sensitive to Ciprofloxacin, gentamycin, Flumequine, Enrofloxacin and Doxycycline with variable results in other antimicrobial discs.

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Table (8): Total bacterial count of different bacterial serotypes in different NaCl concentration (CFU / ml x 10²)

Bacterial serotype	NaCl concentrations							
	2%	3%	4%	5%	6%	7%	8%	
<i>Ps. fluorescence</i>	1171	841	251	-ve	-ve	-ve	-ve	
<i>Ps. aeruginosa</i>	987	697	287	-ve	-ve	-ve	-ve	
<i>Ps. anguilliseptica</i>	1708	968	318	-ve	-ve	-ve	-ve	
<i>Ps. plecoglossicida</i>	1047	751	362	-ve	-ve	-ve	-ve	
<i>Ps. chlororaphis</i>	1612	824	434	32	-ve	-ve	-ve	
<i>T. maritimum</i>	322	601	287	102	49	-	-	
<i>V. anguillarum</i>	494	533	1735	617	467	430	340	
<i>V. alginolyticus</i>	458	715	1378	730	535	366	289	
<i>V. ordalii</i>	386	680	837	157	77	-ve	-ve	
<i>V. harveyi</i>	503	827	1240	494	390	357	266	
<i>V. viscosus</i>	347	576	936	-ve	-ve	-ve	-ve	
<i>V. vulnificus</i>	421	800	1090	41	-ve	-ve	-ve	

Chi² = 78.23**

** = Significant at (P < 0.01)

Table (9): The diameters of inhibition zone of antimicrobial discs were read and it summarized in the following table mm.

Bacterial serotype	Antimicrobial disc									
	CIP 5 μg	CN 10 μg	UB 30 μg	ENR 5 μg	DO 30 μg	SXT 25 μg	OT 30 μg	AMX 10 μg	E 15 μg	
<i>Pseudomonas fluorescence</i>	24	14	20	14	-	7	-	-	-	
<i>Pseudomonas aeruginosa</i>	18	-	18	12	-	6	-	-	-	
<i>Pseudomonas anguilliseptica</i>	20	15	18	14	-	7	-	-	-	
<i>Pseudomonas pleoglossicida</i>	20	14	20	16	-	8	-	-	-	
<i>Pseudomonas chlororaphis</i>	20	16	14	14	-	7	-	-	-	
<i>Tenacibaculum maritimum</i>	20	-	20	20	13	-	-	15	8	
<i>Vibrio (Listonella) anguillarum</i>	23	24	30	3	30	10	-	-	20	
<i>Vibrio alginolyticus</i>	34	22	22	22	26	8	-	-	-	
<i>Vibrio ordalii</i>	30	22	26	26	30	-	20	-	18	
<i>Vibrio harveyi</i>	26	27	32	32	18	-	-	-	-	
<i>Vibrio viscosus</i>	23	20	30	30	26	8	12	-	18	
<i>Vibrio vulnificus</i>	29	22	28	28	32	-	30	-	-	

Chi² = 98.55**

** = Significant at (P < 0.01)

CN : Gentamycin
DO : Doxycycline
AMX : Amoxicillin

UB : Flumequine
SXT : Sulphamethoxazole and Trimethoprim
E : Erythromycin

CIP : Ciprofloxacin
ENR : Enrofloxacin
OT : Oxytetracycline

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Table (11): Economic losses resulted from bacterial isolates among seabass and seabream fish.

Bacterial isolates	Seabass				Seabream			
	Number	Percentage	Weight losses* (Kg)	Return Losses**	Number	Percentage	Weight losses*	Return losses**
Total number of fish examined			200				200	
Vibrio species	100	50	20	500 LE (250LE/100 fish)	46	23	4.60	161 (80.5 LE/ 100 fish)
Pseudomonas species	50	25	10	250 (150 LE/100 fish)	30	15	3.00	105 (52.50 LE/ 100 fish)
Tenacibaculum species	34	17	6.80	170 (85 LE/100 fish)	30	15	3.00	105 (52.50 LE/ 100 fish)
Total	184	92	36.80	920 (460 LE/100 fis)	106	53	10.60	371 (185.5 LE/100 fish)

*calculated according to the average weight of seabass and seabream = 200 gm.

**Calculated on the base the price of Kg seabass = 25 LE and seabream = 35 LE

DISCUSSION

Heavy mortalities in both wild and cultured fish are due to bacterial fish pathogens and they are one of the most important factors of economic losses since the beginning of marine fish culture (Anderson and Conroy, 1970).

In our present work, we spot light on the clinical picture and PM lesions of the most prevailing bacterial pathogens affecting some marine fishes as cultured Seabass (*Dicentrarchus labrax*) and cultured Seabream (*Spaurus aurata*) native to Wadi-mariut region, Borg El-Arab

city, Alexandria governorate. Moreover, isolation and identification of these bacterial infections by both biochemical traditional methods and by API20E system, serological identification as well as by PCR, Afterthat studying the tolerance of the bacterial isolates to different concentrations of NaCl studying the sensitivity of isolated serotype to antimicrobial disc.

Concerning the clinical signs and Postmortem lesions of naturally examined marine fishes; our results were agreed to that

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obtained by *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. Also, *Austin and Austin (2007)* reported that the typical external clinical signs of *V. anguillarum* infection include red spots on the ventral and lateral areas of the fish and swollen and dark skin lesions that can ulcerate and bleed. The eyes were also infected, resulting in opacity at first, and later in ulceration and exophthalmia. Moreover; *Kumaran et al. (2010)* mentioned that clinical signs of Asian Seabass (*Lates calcarifer*) infected with *V. salmonicida*, *V. harveyi* and *V. anguillarum* respectively appeared as necrosis and hemorrhagic areas near the base of fins, exophthalmia and ulcers on the skin surface.

In regard to the disease caused by *V. alginolyticus* in Seabream included septicemia, hemorrhage, 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*). Moreover; *Korun (2007)* found that *V. alginolyticus* infect European Seabass (*Dicentrarchus labrax, L.*) and he found that all affected fish showed ulcers on the dorsal part of the body and hemorrhages on the operculum. Internal lesions included enlargement of the spleen, paleness of the liver and kidney, hemorrhages in the intestine. Furthermore; *Sharma et al. (2011)* illustrated that *V. alginolyticus* infected Asian Seabass and induced ulcers with exposed underlying muscle were also noticed in many cases and the PM lesions showed that brain, spleen and gill were extremely congested, liver showed mottled appearance with the presence of numerous necrotic foci.

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These pathological alterations may be due 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*). Furthermore, the results may be attributed to the virulence factors such as the adherence of *V. alginolyticus* which gave it the ability to adhere to skin, gill, and intestinal mucus of fish and the biological activities of extra-cellular products of *V. alginolyticus* were hydrolytic activities and cytotoxic for fish (*Balebona et al., 1998a*).

Moreover, there were other pathogenicity mechanisms such as hemolytic activity, hemoagglutination and protease production (*Zanetti et al., 2000*), the ability for growth in iron-limiting conditions, survival in fish serum and virulence genes of *V. alginolyticus* as *toxR* and *toxS* genes (*Ben Kahla-Nakbi et al., 2009*).

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* septicemia 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.

The clinicopathological picture of *Pseudomonas* septicemia may be attributed to the effect of extra-cellular proteinase which attacks the endothelial lining of the blood vessels (*Hoshino et al., 1997*).

In concern to results of signs and PM lesions of Tenacibaculosis in naturally infected fishes; our results agreed with that obtained by *Salati et al. (2005)* where they recorded *T.*

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maritimum caused a disease characterized by fin erosion and necrotic ulcers of skin and muscle in Seabass (*Dicentrarchus labrax*) and Gilthead Seabream (*Sparus aurata*).

These results may be attributed to the virulence factors of *T. maritimum* as the synergistic interaction of the toxins and enzymes present in the extra-cellular products (ECP) which have high proteolytic activity with an ability to degrade gelatin, amylase, casein and nucleases as well as a positive cytotoxic activity (Avendaño-Herrera *et al.*, 2005c).

In regards to biochemical characterization of *Ps. fluorescens* noticed in this study the results were concordance with those obtained by (Akayl and Timur, 2004).

In concern to the bacterial isolates prevalence among naturally infected marine cultured Seabass (*Dicentrarchus labrax*) and Seabream (*Sparus aurata*), this results revealed that Seabream was the most affected fish species (77%) than Seabass (68%) these results were in agreement with that obtained by Yiagnisis and Athanossopoulou, (2011) where they reported that Seabream was highest infected marine fish species in comparison with Seabass.

In regards to prevalence of bacterial species in naturally infected Seabass, our results revealed that vibrio species were the most prevalent (47%), followed by Pseudomonas species (27.3%) and tenacibaculum species (25.7%). This results agreed with these illustrated by (Toranzo *et al.*, 2005) who reviewed that the vibrio species is the main bacterial fish disease in mariculture system.

In concern to prevalence of bacterial species in naturally infected Seabream in this study the results showed that vibrio species

(53%) were the highest prevailing bacterial species, this results were concordance with those reported by (Zorrilla *et al.*, 2003b) who illustrated that the most frequently isolates microorganisms were identified as vibrio (69.9%) in 25 outbreaks affecting cultured Gilthead Seabream with size ranging between 2 and 25 gram.

Moreover, Moustafa *et al.*, (2010) studied that the highest prevalent bacterial infection that affecting marine fish in Egypt, Epinephelus Tuvina, Siganus rivulatus, Dedlechilus labiosus, Tilapia zilli, Mugil capito and Solea vulgaris were (34.28%) vibrio species.

In contrast, our results disagreed with that recorded by Nedoluho and West hoff (1997) where they cleared that most predominant bacterial pathogens isolated from diseased striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone chrysops*) collected from different aquaculture system were accounted for Aeromonas species (19%), Pseudomonas (6%).

Furthermore, Soliman *et al.* (2011) recorded that most prevalent isolates that may lead to mortalities of adult grouper fish at Libyan coast were pasteurella, vibrio and Aeromonas species were the most isolated bacteria with high incidence of *Pasteurella piscicida* (64%).

These difference may be due to species difference, season, locality and contamination .

Also our results agreement with that obtained by Moustafa *et al.* (2010) who illustrated that *V. anguillarum* is the most frequent isolates (17.55%) of infected cases of some marine fish species. Abd El-Latif (2013) recorded that *V. alginolyticus* was the most

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frequent vibrio species (21.35%) affecting cultured Seabass, Seabream, Grouper and Meagre.

This difference in the results may be attributed to size and age of variation also season and pollution of water by heavy metals.

In concern to prevalence of *T. maritimum* infection, this study showed that, *T. maritimum* was isolated from (22%) of the infected fishes, this results were supported by these results obtained by (Avendano-Herrera *et al.*, 2004 a, b 2005 b) they reported that out breaks of Tebaciubaculosis occurred in Spain and Portugal, mainly in turbot (*Scophthalmus maximus*), Atlantic Salmon (*Salmo Solar*), and Gilthead Seabream (*Sparus aurata*).

In concern to tolerance of different bacterial isolates to different concentration of NaCl; our study showed that all bacterial strains grow on 2%, 3% and 4% NaCl. This results supported by Korum and Timur (2008) where they maintained that the media used for isolation of bacterial pathogen from marine fishes should be supplemented with 2% NaCl.

In concern to growth of *Pseudomonas* species on different NaCl concentration, these results illustrated that all bacterial species of *Pseudomonas* species grow well on 2% NaCl at high level of growth and moderate growth at 3% and slow growth at 4% NaCl, the growth of these bacterial isolates of *Pseudomonas* species stopped at concentrate of NaCl above 4%. Our results were in concordance with those of (Fernandez *et al.*, 1990), where they compared the characters of *Pseudomonas* species were isolated from diseased rainbow trout with reference strain of *Ps. fluorescence*, *Ps.*

aeruginosa and *Ps. anguilliseptica* illustrated that they all grow poorly at 4% NaCl.

In regards to tolerance of vibrio strains isolates to NaCl concentration this study revealed that all serotypes of vibrio grow at 2, 3 and 4% NaCl, the best concentration of growth was 4% in all vibrio strain, the growth stopped to some strain at concentration 5% and above as *V. viscosus* and *V. vulnificus* while the others serotypes of vibrio decreased its growth at 5% NaCl and above, some vibrio species as *V. anguillarum*, *V. alginolyticus* and *V. harveyi* resist NaCl concentration up to 8%.

These our results complete agreement with that obtained by (Zorrilla *et al.*, 2003b) where they recorded that *V. anguillarum*, *V. alginolyticus* and *V. harveyi* can grow at 8% NaCl but another serotypes of vibrio can't grow at this concentration. In regards to growth and tolerance of *T. maritimum* to NaCl concentration, our results showed that *T. maritimum* moderately growth at 2%, 3% and 4% NaCl and slowly growth at 5% and 6% NaCl, above 6% the growth was stopped.

Moreover, Handlinger *et al.* (1997) modified Ancker and ordal ager media by prepared in 70% sea water. On the other hand, Avendano-Herrera *et al.* (2005 a) reported that *flexibacter maritimus* media (FMM) prepared with commercial sea salts instead of sea water.

In concern to the sensitivity of *Pseudomonas* species to different antimicrobial agent, this study revealed that all species were sensitive to Ciprofloxacin and resistant to doxycycline, oxytetracycline, erythromycin, and sulphamethoxazole with trimethoprim. Also all these serotyping were intermediate sensitive to enrofloxacin and flumequine except *Ps.*

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aeruginosa was resistant to enrofloxacin, among gentamycin *Ps. anguilliseptica* and *Ps. chororaphis* were sensitive but *Ps. fluorescens* was resistant. These results almost agreed with *El-Shorbagy et al., (2001)* where they illustrated that *Ps. aeruginosa* highly sensitive to Polymyxin R, enrofloxacin, ofloxacin, gentamycin and ciprofloxacin. However, high degree of resistant to chloramphenicol, erythromycin, amoxicillin and ampicillin.

All results recorded by different authors were variable and differ from one to others and with our results and in concordance of some results this differences may be attributed to antibiotic resistant, differences in fish species, locality, season and type of aquaculture.

In regards to susceptibility of vibrio species to antimicrobial, our results revealed that all vibrio species were sensitive to Ciprofloxacin, enrofloxacin, gentamycin, flumequine and doxycycline, resistant to Amoxicilline and sulphamethoxazole with trimethoprim except *V. anguillarum* was intermediate sensitive to Sulpha with trimethoprim and variable results with oxytetracycline and erythromycin . Our results were in agreement with *Korum and Timur (2008)* results, where they stated that vibrio species were sensitive to flumequine, kanamycin, novobiocin, oxalic acid, penicillin G and tetracycline.

In regards to antimicrobial susceptibility of *T. maritimum*; this study showed that *T. maritimum* was sensitive to ciprofloxacin and enrofloxacin, moderate sensitive to doxycycline and amoxicilline, and resistant to gentamycin, fluqueuine, erythromycin, oxytetracycline and trimethoprim with sulphamethoxazole. These results agreed with that obtained by (*Avendano-Herrera, 2005*) who recorded that enrofloxacin

proved to be the most useful compound for controlling *T. maritimum* outbreaks.

Also, *Zorrilla et al. (2003b)* recorded that low percentage of resistant of flumequine to *T. maritimum*.

On the other hand, *Avendano-Herrera et al. (2004 b, 2005 a)* reported that *T. maritimum* was susceptible to nitrofurans, penicillins, erythromycin, tetracycline, chloramphenicol, trimethoprim, and fluoroquinolones and resistant to colistin, kanamycin, neomycin, oxalic acid and flumequine.

The results of economic analysis cleared that, the seabass of higher economic losses than seabream due to the resistant of seabream to bacterial diseases higher than that of seabass which is of high susceptible to bacterial diseases (*Abd El-Latif, 2013*). And the most bacterial isolates causes severe economic losses among examined fish were *Vibrio Spp*, *Pseudomonas* and *Tenabaculum Spp*. This results agreed with those of (*Korum and Timur, 2008 and Moustafa et al., 2010*) where they reported that, the main bacterial isolates cause severe economic losses to marine fish were *Vibrio Spp*, *Pseudomonas* and *Tenabaculum Spp*.

From the results of this study, it can be concluded that :

1. The most prevailing bacterial diseases affecting cultured sea bass and sea bream were Vibriosis, Pseudomoniasis and Tenacibaculosis respectively .
2. The best concentration of NaCl for isolation of *Pseudomonas* species was 2%, while for *Tenacibaculum* was 3 and 4% for isolation of vibrio species .

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- The best antimicrobial used for treatment of Pseudomoniasis and Tenacibaculum infections were Ciproflexacin, while in Vibriosis can be treated by Ciproflexacin, Enrofloxacin, Gentamycin, Flumequine and Doxycyline .

The seabream resist bacterial infection than the seabass fish and the main bacterial isolates causes severe economic losses among examined fish were *Vibrio* Spp, *Pseudomonas* and *Tenacibaculum* Spp.

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دراسات على الأمراض البكتيرية السائدة فى أسماك القاروص والدينيس المستزرعة

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الأمراض البكتيرية فى أسماك القاروص والدينيس المستزرعة تسبب نفوق كبير وبالتالي تؤدي إلى خسارة اقتصادية كبيرة . ولذلك أجريت هذه الدراسة بغرض عزل وتصنيف مسببات الأمراض البكتيرية السائدة فى هذه الأسماك ومحاولة علاجها وكذلك معرفة أنسب ظروف لعزل مسببات هذه الأمراض .

أجريت هذه الدراسة على (٤٠٠) سمكة مريضة مستزرعة فى أقفاص ومسجلات تم تجميعها من منطقة برج العرب بمحافظة الإسكندرية بمصر بمعدل (٢٠٠) سمكة من أسماك القاروص و (٢٠٠) سمكة من أسماك الدينيس .

(١) تم فحص هذه الأسماك للتأكد من خلوها من المسببات الغير بكتيرية للأمراض مثل الطفيليات والفطريات وتأكد خلوها من مثل هذه المسببات .
(٢) بعد ذلك تم عمل التحاليل والاختبارات المعملية للعزل البكتري المعلمى وذلك عن طريق :

- الزرع البكتيرى على المنابت العامة والخاصة لكل نوع من أنواع البكتريا .
- الشكل الظاهرى لمستعمرات البكتيريا على المنابت وكذلك لون هذه المستعمرات وأيضاً عن طريق صبغ البكتيريا وفحصها تحت الميكروسكوب ورؤية حركتها .
- الاختبارات البيوكيميائية للمعزولات التى تم الحصول عليها .
- التصنيف السيرولوجى لهذه المعزولات .
- تصنيف أنواع بكتيريا السودوموناس عن طريق نظام API20E .

وأوضحت النتائج الآتى :

- أ- إجمالى عدد المعزولات فى أسماك القاروص والدينيس (٢٩٠) معزولة منهم (١٣٦) معزولة من أسماك القاروص (١٥٤) معزولة من أسماك الدينيس .
ب- أنواع البكتيريا التى تم عزلها فى أسماك القاروص هى قبيرو (٤٧%) ، (٢٧.٣%) السودوموناس و (٢٥.٧%) تنيسيكيو لم بينما فى اسماك الدينيس كانت أنواع

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البكتيريا هي فييرو (٥٣%) ، السودوموناس (٢٨%) وتنيسيبكولم ماريتيم (١٩%)

ج- أكثر العترات شيوعاً التي تم الحصول عليها في أسماك القاروص وأسماك الدنيس كانت عترة فييرو وانجيليريم ثم عترة التنيسيبكولم ماريتيم يليها عترة فييرو الجينوليتكس ثم عترة السودوموناس فلورسينس ولكن بنسب مختلفة .
(٣) اختبار مقاومة الملح (NaCl) وذلك عن طريق :

عمل تركيزات مختلفة من الملح (NaCl) ٢ ، ٣ ، ٤ ، ٥ ، ٦ ، ٧ ، ٨ % على أطباق بها pleat ceunt ager وذلك لكل عترة من العترات التي تم الحصول عليها وتم زرع هذه الأطياف وتحضينها لمدة ٢٤ ساعة وبعد ذلك تم تسجيل العدد الكلي للمستعمرات على كل طبق وأسفرت النتائج من الآتي :

أفضل تركيز لنمو العترات المختلفة من بكتيريا السودوموناس كان ٢% بينما التنيسيبكولم ماريتيم كان أفضل نموها عند ٣% وكانت معظم عترات بكتيريا الفييرو مقاومة للملح وكان أفضل نموها عند ٤% .

(٤) اختبار الحساسية للمضادات البكتيرية : محاولة التقليل من حدوث الأمراض البكتيرية وعلاجها وذلك من طريق عمل اختبار حساسية للمضادات البكتيرية لجميع العترات التي تم عزلها من الأسماك المريضة والنافة .

وذلك عن طريق استخدام أقراص من هذه المضادات وقياس المسافة المحيطة بالقرص التي لا تنمو فيها البكتيريا . وأوضحت النتائج أن معظم عترات بكتيريا السودوموناس حساسة للسيبروفلوكاسين ومتوسطة الحساسية لأقراص فلموكوين ، انروفلوكساسين وجينتاميسين ، ولكن في عترة التنيسيبكولم ماريتيم كانت مقاومة لمعظم المضادات البكتيرية فيما عدا سيبروفلوكاسين وانروفلوكساسين أما بالنسبة لمقاومة عترات الفييرو فقد تم ملاحظة أن معظم العترات حساسة لمعظم المضادات البكتيرية ما عدا الأمبيسللين والسلفاميزوكول مع تراى ميثوبريم كانت مقاومة لها وكانت هناك حساسية متوسطة لأقراص اريثروماسين ودوكسى تتراسيكلين في بعض العترات .