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Monitoring of bacterial mass mortalities in farmed pre-growing stage Gilthead seabream (*Sparus aurata*) with control trail

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Abstract

This study aimed to investigate the bacterial diseases causing mortalities among pre-growing stage Gilthead seabream (*Sparus aurata*) of private farm in Diba triangles- Damietta Governorate, Egypt . The isolated bacteria was identified as *Vibrio alginolyticus* and *Pseudomonas fluorescens.* Bacteriological phenotypic and biochemical identification of bacterial isolates were performed by API*20NE system and isolated with incidence rate (62.5%) and (25%) respectively. Also, water parameters were measured to find the relation between unfavorable values and mortality causes. Experimentally infected pre-growing stage seabream with *V. alginolyticus* and *P. fluorescens* through I/P route showed clinical signs mostly similar with naturally infected pre-growing stage seabream with similarity of pathological changes. The susceptibility of treatment with selected antibiotic upon antibiogram sensitivity proved that the isolates were sensitive to Ciprofloxacin and sulfamethoxazole, fish treated with antibiotics in concentration 3g/kg ration for 5 days . Survival rate of fish after treatment with the two antibiotics were 80% and 60% respectively.

Keywords: *Seabream, marine fish, Vibrio alginolyticus,. Pseudomonas fluorescens, API 20 NE, pathological changes, Ciprofloxacin, sulfamethoxazole*.

Introduction

Egyptian fresh and marine aquaculture expanded rapidly and greatly. Marine fish species such as European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (**Eissa, 2012**), contributes to about 2.8% of the total fish farm production (**GAFRD, 2014**). Production value of Gilthead seabream cultured equal 90.558 Production value (USD 1000 /year in Egypt at 2014, Data calculated by **FishStatJ, (2016)**. (Fish species Production value, USD 1000) Mariculture of Gilthead sea bream was introduced to Egypt for the first time in 1976 **(Eisawy and Wassef, 1984).**

Salwany *et al*., (2019) increase growth in aquaculture production is parallel with the increasing number of disease outbreaks, which could be affect the production, gains, and continuity of the global aquaculture industry.

Bacterial fish diseases are the main causes of disease problems that has direct colossal impacts on Egyptian mariculture **(Grisez and Ollevier, 1995).** Vibrios are one of the most challenging pathogens among mariculture development causing high mortalities between fish farms **(Austin and Austin, 2012 and Fadel, 2014).** While outbreak is accompanied with strongly stress factors affected to fish **(Austin and Austin 2012)** *V. alginolyticus* causes many epizootic outbreaks among the Gilthead seabream and European seabass populations, which possess high economic value at marine aquarium **Bakhrouf *et al*., (1995)** and **Zorrilla *et al*., (2003)**

Also, in Egypt, the genus Pseudomonas have been described as causative agents of diseases in fish, where *P. fluorescens, P. angulliseptica, P. aeruginosa and P. putida* were identified in various species of fish as etiological agents of pseudomonas septicemia **(Eissa *et al*., 2010** and **EL-Nagar, 2010).** Psudomonas spp. are abundant in aquatic environment. When fish exposed to stressors as extreme changes in temperature, poor water quality, overcrowding and poor nutrition .

Water quality is important for growth marine

aquaculture organisms that thrive in water **MADEP, (2007).** Which giving the chance for opportunistic pathogens to invade and cause disease **(Robert, 2012).** Also poor water quality and low hygienic condition and stress **(Austin and Austin, 2012)** lead to high mortalities in fish farms **(Mellergaard and Nielsen, 1995).** In spite of that antimicrobial chemotherapy has side effects, but it remains vitally important for treating bacterial diseases through appropriate diagnosis, antibiotic selection at suitable administration route and dose **(Mahmoud *et al*., 2018).** Knowledge of the complete life history of disease causing organisms will improve understanding and approach to disease surveillance, prevention and control **(Afonso *et al*., 2005).**

This study worked on:1- isolation and identification of some of bacterial agents infecting pre -growing stage seabream farm in Diba triangles- Damietta Governorate, fish suffered from re-current appearance of the same symptoms as eye obesity then unilateral /bilateral eye drop, off food, abnormal swimming then accompanied by high mortality. 2- trail to control by using suitable antibiotics in experimentally infected fish by infecting agents.

Materials and Methods

2.1. Fish sampling

Eighty moribund, freshly dead and alive pre-growing seabream that weighted 60 ±5 g. were transported as soon as possible to Fish Diseases Department, AHRI, Dokki. from private farm in Diba triangle- Damietta Governorate, Egypt. Alive seabream samples were put in a plastic tank filled with aereated water Moribund and freshly dead fish transported in ice-box . Lesions and postmortem of naturally infected fish were recorded according to **Conroy and Herman (1981) Austin and Austin (2012).**

2-2 .Water sampling

Water samples were collected, from fish farm, according to standard **Boyd (1990)** and **APHA (1998)** and **Canadian Council on Animal Care (2005).**Water parameters pH, temperature, dissolved oxygen, salinity, Un-ionized ammonia, Nitrate(NO_3), Nitrite(NO_2) and sulphate were measured. On spot using pH meter, oxygen meter, salinometer and kites (HANNA Instruments, Romania) respectively.

2.3. Bacteriological examination

It was performed according to **Austin and Austin (2012)** and **Lajnef *et al*., (2012)** for bacterial isolation, samples were aseptically taken from the brain, eye, kidney, liver and then directly streaked on the different selective media as TCBS agar and Pseudomonas Agar Base and incubated at 25°C for 24h., the similar and dominant bacterial colonies are examined microscopically and biochemically according to Bergey's Manual of Systematic Bacteriology **(Holt, *et al*., 1994)**, **(Whitman 2004)** and API*20NE (BIO-Merieux) for identification. Pure colonies were transferred to glycerol broth 20% at -80°C **(Pujalte *et al*., 2003).**

2.4 Antibiogram sensitivity discs:

Sensitivity was determined by the agar diffusion method **(Quinn *et al*., 2002)** using 6 mm diameter commercial discs (Oxoid) included the following antibiotics,: Amoxicillin 25 µg (AX25) Ciprofloxacin 5 µg (CIP 5), Erythromycin 15 µg (E15) Lincomycin 2 µg (L2), Gentamycin 10 µg (GN 10), Norfloxacin 30 µg (NOR30) Nalidixic Acid 30 µg (NA30) Oxolinic acid 2 µg (OA 2), Oxytetracycline 30 µg (TE30), Trimethoprime /Sulfamethoxazole 25µg (SXT25), Tetracycline 30 µg (T 30) and Vibriostate O/129 (150 µg). Antibiotic sensitivity was tested on Mueller-Hinton agar supplemented with 1.5% NaCl (for *V, alginolyticus* isolate), inhibition zones diameters were interpreted as sensitive, intermediate and resistant according to **CLSI (2010).**

2.5 Experimental fish

Inside private marine fish farm 190 apparently healthy pre-growing seabream 60 ±5 g were obtained with marine water supplied for the experimental fish. Acclimatization for two weeks prior to experimental infection **(Innes, 1966),** 10 seabream were examined for free from natural pathogenic infection (bacterial, fungal and parasitic). **(Austin and Austin, 2007)**.

2.6. Experimental design:

The total of 180 apparently healthy pre-growing seabream were divided into 3 groups: group (1) infected with *V. alginolyticus*, group 2) infected with *P. fluorescens* and group (3) kept as control, as in table (1). All groups were

kept under observation to detect symptoms and recorded the mortality: once symptoms and mortalities appeared, protocol of treatment with antibiotic started: 40 moribund experimentally infected seabream with V. alginolyticus

Table (1). Experimental pre-growing stage seabream design.

Fish groups	No. of fish	Bacterial strain challenge	Dose and route	Protocol of Treatment with antibiotic	
				Ciprofloxacin 3g/kg ration	Trimethoprime/ Sulfamethoxazole 3g/kg ration
				No of treated fish	No of treated fish
Group 1	80	*V. alginolyticus*	0.1ml (1.5×10^8 CFU/ml) / IP **(Zorrilla *et al.*, 2003)**	20	20
				40 experimentally infected un treated fish	
Group 2	80	*P. fluorescens*	0.2ml ($1X10^7$ CFU/ml /IP**(Abo El-atta and El-Tantawy2008**	20	20
				40 experimentally infected un treated fish	
Group 3 as control	20	PBS	0.1 ml	-	-

cus were used to treated with Ciprofloxacin and Trimethoprime/Sulfamethoxazole, 20 fish for each drug , and other 40 moribund experimentally (infected seabream with *P. fluorescens* were treated with Ciprofloxacin and Trimethoprime/Sulfamethoxazole 20 fish for each drug, as in table (1). Treated fish feeding were 3% from fish weight divided twice /day for 5 successive days and clinical signs and mortality were recorded.

2.7 Histopathological examination:

Tissue samples from naturally infected (eye, brain ,kidney and liver) and experimentally injected fish were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed by conventional method and finally stained by Heamatoxylene and Eosin **(Suvarna *et al*., 2013).**

Results and Discussion

This research planned to investigate the main causes of mortalities in pre-growing stage seabream. Clinical signs of infected fish showed, uni or bilateral corneal opacity, exophthalmia, unilateral eye cataract, others suffer from completely eyes dropped, abnormal swimming of alive fish, infected fish swimmed on one side and lost balance (fig:1-3), off food, abdominal dropsy. The postmortem examination showed severe congestion and heamorrhages of the internal organs fig: 4 & 5). **Rameshkumar *et al*., (2014)** focused on *V. alginolyticus* caused sluggish swimming and bilateral exophthalmia leading to death. The picture of clinical signs were nearly similar to **(Abualreesh, 2017 and Salwany *et al*., 2019)** affected fish with *V. alginolyticus* show blindness, muscle opacity, and mortality also **Rao *et al*., (2005)** proved that Psoudomonas caused severe heamorrhages of the internal organs, ulceration, corneal opacity.

Fig. (1-2): Naturally infected pre-growing stage seabream, showing abnormal swimming
Fig. (3): Naturally infected pre-growing stage seabream showing complete loss of eye.

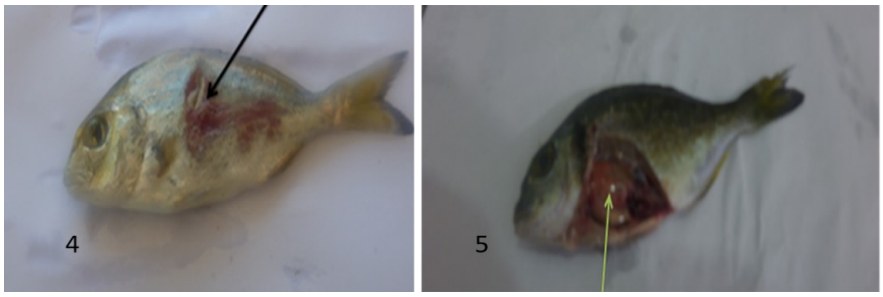

Fig. (4): Naturally infected pre-growing stage seabream with heamorrhages at body surface, with eye cataract.
Fig. (5): Naturally infected pre-growing stage seabream with general congestion of internal organs.

Bacteriological and morphological characterization of isolates from infected pre-growing stage seabream are shown in table (2), *V. alginolyticus* isolates appeared as yellow colony on TCBS agar (fig. 6) while *P. fluorescens* appeared yellowish green colonies on Pseudomonas agar base while bluish colour under UV light (fig. 7).

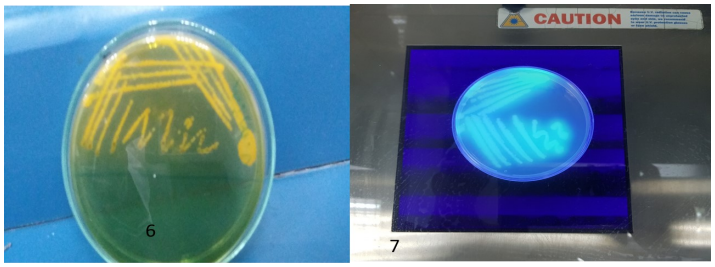

Fig. (6): *V. alginolyticus* showing large yellow coloring (sucrose-fermenting) colonies on TCBS agar,
Fig. (7): *P. fluorescens* colonies under UV light.

Phenotyic and biochemical characteristics of *V. alginolyticus* isolates were gram-negative slightly curved rods motile, oxidas, Indole production, gelatine hydrolysis and catalse positive, urease, simmon citrate were negative. Those findings agree with **Abou Okaada (2013)** and **Dalia, (2017)** who isolated *V. alginolyticus* from seabream, seabass and solea fish with the same phynotypic characters. While *P. fluorescens* isolates were Gram-negative short rods, motile, oxidas, and simmon citrate were positive. Indole production , urease and gelatin hydrolysis were negative. Growth at 4^0C. That agree with **Soraya (2009)** and **Manal and Hal (2016)** .

Table (2). Biochemical characteristics of isolated bacteria by API*20 NE

Biochemical tests		*V.alginolyticus*	*P. fluorescens*
NO3	Potassium nitrate	+	+
TRP	Trytophane production	+	-
GLU	Glucose fermentaion	+	-
ADH	Arginine Dihydrolase	-	+
URE	Urease	-	-
ESC	Esculin	+	-
GEL	Gelatin	+	-
PNG	Para Nitrophenyl D Galacto-pyranosidase B Glucosidase	-	-
GLU	Glucose assimilation	V	+
ARA	Arabinose assimilation	+	+
MNE	Mannose assimilation	-	+
MAN	Mannitol assimilation	+	+
NAG	N acetyl Glucosamine assimi-lation	-	+
MAL	Maltose assimilation	+	-
GNT	Potassium GlucoNate aasimi-lation	+	+
CAP	Capric acid assimilation	-	+
LDI	Adipic acid assimilation	-	-
MLT	Malate assimilation	+	+
CIT	Tri sodium Citrate assimila-tion	-	+
PAC	Phenyle acetic acid assimla-tion	-	-
OX	Oxidase	+	+

The prevalence of bacterial isolation *V. alginolyticus* and *P. fluorecsens* were 70/80 (87.5%), and 20/80(25%) respectively, among examined naturally infected pre-growing stage seabream. Results nearly similar as **Mustapha *et al*., (2012)** who recorded 47% and 71% prevalence rate of *V. alginolyticus* infection while the rate was lower in seabass (14.61%) **(Mahmoud *et al*., 2018).** Higher prevalence of *V. alginolyticus* was 82.19% and 46% in *Sparus auratus* **(Abdel-Aziz *et al*., 2013** and **Eisaa *et al*., 2013)** respectively. In case of *P. fluorescens*, **Manal and Hal (2016)** reported that prevalence of *P. fluorescens* were13.8% and 14.5% between examined fish.

The changes of global temperature and increase water pollution that changed environment around the pathogen which lead to mutation of its behavior. Analysis of water parameters of infected fish farm as in table (3) revealed that low dissolved oxygen, increase of un-ionized ammonia to 1 mg/l, Nitrate 8mg/l while sulphate 150mg/l within normal level. Poor water quality affected indirectly on immune system of fish, causing environmental stressor giving the chance for pathogen to invade and establish diseases with emergency of higher mortalities occurring **(Mellergaard** and **Nielsen, 1995** and **Robert, 2012).**

Table (3). Water quality of infected seabream farm.

Water prameters	Results	Permissible limits
pH	8.40	7.5-8.5
Tempreature ^{0}C	25	
Dissolved oxgyen	4	5-6mg/L
Salinity PPT	30	28-35ppt for marine
Un-ionized ammonia	1	0.0 -.0.0125mg/L
Nitrate(No3)	8	10 mg/L
Nitrite(NO2)	3	0.0-0.3 mg/L
Sulphate	150	<3000 mg/L

Antibiotic sensitivity

In our study *V. alginolyticus* and *P. fluorescens* strains were sensitive to ciprofloxacin and Trimethoprime /sulfamethoxazole and that might be effective antibiotics to eliminate the both pathogen. Other susceptibility results were recorded in table (4).

Table (4). Antibiotic sensitivities of identified isolates.

Antibiotics discs	*V. alginolyticus*	*P. fluorescens*
AX (10 µg)	R	R
CTP (5 µg)	S	S
E (15 µg)	I	R
L (2 µg)	R	R
GN (10 µg)	R	S
NOR (30 µg)	R	S
NA (30µg)	I	R
OA (2 µg)	I	S
TE (30µg)	R	I
SXA (25µg)	S	S
T (30 µg)	I	S
O/129 (150 µg)	S	-

S: Sensitive R: Resistant I: Intermediate

The pathogenicity assay revealed that *V. alginolyticus* and *P. fluorescens* were injected I/P seabream showed lesions and PM as naturally infected (fig:8-10) Once clinical signs appeared, antibiotic treatment applied with Ciprofloxacin or Trimethoprime / Sulfamethoxazole as in table (4) showed that survival rate in *P. fluorescens* experimental

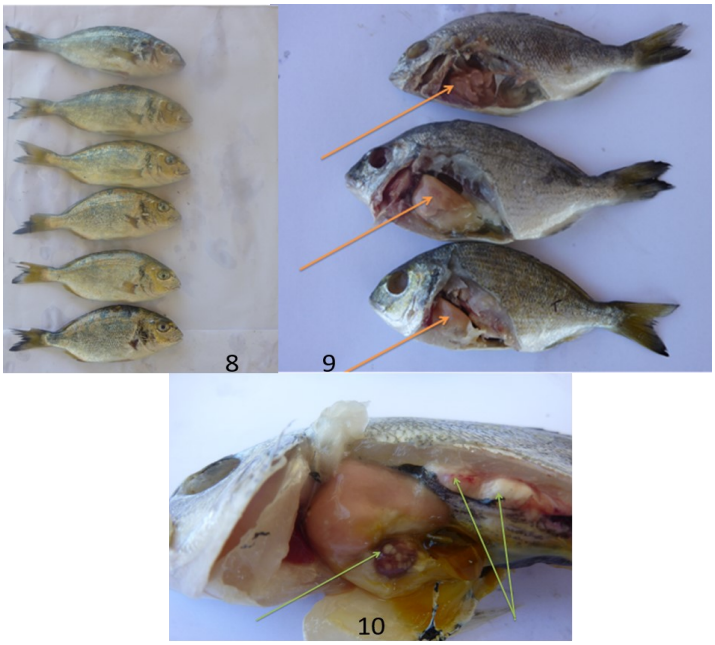

Fig. (8): Experimentally infected pre-growing stage seabream injected with *V. alginolyticus*, showed degree of eye cataract to blind,
Fig. (9): Experimental infected pre-growing stage seabream with *P. fluorescens* after one week showing different degree of congestion at liver and at the intestine with eye dropped.
Fig. (10): Experimental infected pre-growing stage seabream with *V. alginolyticus* showing large whitish nodular formation on spleen and kidney .

Table (4). Experimental treatment by Ciprofloxacin or Trimethoprime /sulfamethoxazole to experimentally infected pre-growing stage seabream with *V. alginolyticus and P. fluorescens.*

	V. alginolyticus		***P. fluorescens***		**Total survival %**
	Mortality/ No. of fish	**Survival %**	**Mortality/ No. of fish**	**Survival %**	
Ciprofloxacin	5/20 25%	15/20 75%	3/20 15%	17/20 85%	32/40 80%
Trimethoprime / Sulfamethoxazole	10/20 50%	10/20 50%	6/20 30%	14/20 70%	24/40 60%

infected group after treated was higher than *V. alginolyticus* experimentally infected group after treated.

The results of sensitivity to antibiotic similar appeared to **Bekta and Yildirim (2013)** and **Darak and Barde (2015)** *P. fluorescens* isolates were sensitive to Trimethoprime / sulfamethoxazole, Oxolinic acid, Tetracycline and Oxytetracycline, but regarding to other antibiotics agents were not highly active against *V. alginolyticus*, *V, vulnificus*, and *V. paramaemolyticus* Vibrio spp were able to develop resistance mechanism to resist antibiotics **(Abualreesh, 2017).** Also some resistance to ampicillin, amoxycillin and lincomycin has been observed with *V. alginoloiyticus* and other isolates of vibrio spp. were resistance to tetracycline and streptomycin which could be related to highly uses of these antibiotics reaching to aquaculture and increase its concentration in water agriculture /municipal wastes **(Abdel-Aziz *et al*., 2013).**

Treatments with Ciprofloxacin or Trimethoprime /Sulfamethoxazole showed higher survival rate 80% and 60% respectively. It was observed that the treated groups had recovered the clinical signs appeared after pathogenicity test at fifth day post treatment in all groups. A seawater fish as seabream drink significant amounts of water and may absorb large amounts of a drug via the gastrointestinal tract **(Noga, 2010 and Reimschuessel *et al*., 2013)**

Antimicrobials only should be used only in restricted circumstances in fish farms **(Bekta and Yildirim 2013).** Extensive use of chemotherapeutic agents is not recommended in fish culture (**Sugita *et al*., 2002 and Sarter, *et al.*, 2007**).

At the end of experimental, remainder untreated experimentally infected fish, with mortality rate 29/40 (72.5%) and 17/40 (42.5%) respectively. The results nearly similar to **Mahmoud, *et al*., (2018)** the mortality rate 76.6% in seabass in experimentally injected I/P by *V. alginolyticus*. While in experimentally infected with *P. fluorescens* mortality 100% within 9 days **(Soraya, 2009**).

Histopathological examination of eye of control fish revealing normal lens layers with regular arranged lens fibers (Fig. 11), while microscopical examination of naturally infected pre-growing stage seabream by *V. alginolyticus* showed opaque eyes appeared as, clefts in between layers of lens and accumulation of pink staining esinophilic globules between cortical fibers indicating eye cataract (Fig. 12), Histological sections from sloughed eyes exhibited nearly sloughed eye lens, only presence of small retracted degenerated small part of eye lens with present of corneal stroma (Bowman's membrane) and completely absence of retina (Fig. 13). That exopthalmia supported also by **(Roberts, 1989, Shayo *et al*. 2012**) who explained that this sloughing occurs as a sequence of traumatic ulceration by septicemic bacteria which lead to osmotic changes in anterior synechia followed by degeneration and phthisis of the orbit. Examination of the brain from pre-growing stage seabream affected by *P. fluorescens* and *V. alginolyticus* exhibited vasculitis degeneration, necrosis of neurons (shrinked small pyknotic esinophilic cells) and vacuolations around neurons. The neuropils had a moth eaten appearance as holes and sparing cortex due to prominent status spongiosis, Also dark stained degenerated neurons were seen (Fig. 14). These findings supported by **(Roberts, 1989)** who stated that Pseudomonas make inflammatory meningitis. **Wange *et al*., (2016)** stated that, the blindness lead to malnutrition and starvation and deficient nutrition lead to deficient vitamin E cause brain denaturation. The liver in fishes suffered by completely sloughed eyes affected by *V. alginolyticus* and *P. fluorescens* exhibited completely degeneration, necrosis of hepatocytes and aggregation of inflammatory cells (Fig. 15) this supported by **Balebona (1998)** who concluded that *V. alginolyticus* produce proteolytic enzymes collagenolytic causing degenerative and necrotic changes in liver and **Rameshkumar, *et al.*, (2017)**. **Soraya (2009)** found that liver with Pseudomonas spp. showing heamorrhage in between hypatocytes, vacuolar degeneration and infiltration of chronic inflammatory cells. Kidneys, in fish with cataract and affected by *V. alginolyticus* exihibited atrophy of glomerular tuft expansion of space inside the glomeruli, degenerative changes in some renal tubules (Fig. 16), while complete loss of renal tubular epithelium, and the other renal tissues showed high degeneration and necrosis with complete absence of renal glomeruli was recorded in fishes with anopthalmia (Fig. 17) that supported by **Ben Kahla- Nakbi *et al*., (2007)** who explained that *V. alginolyticus* in sea bream cause focal necrosis of, kidneys hypoplasia of splenic ellipsoids, degeneration and necrosis of splenic tissues.

The intestinal mucosa of fishes suffered from eye cataract and infected by *V. alginolyticus* and *P. fluorescens* revealed high aggregation of inflammatory cells with intact intestinal villi, severe destructed intestinal villi with loss of all covering epithelial cells recorded in sloughed eye fishes (Fig. 18). That findings similar with (**Balebona *et al.*, 1998**) who stated that *V. alginolyticus* causing fish mortality and economic losses. The organism having the ability to adhere the intestinal mucus of seabream, secret extracellular products have hydrolytic activities (proteolytic, collagenolytic lipolytic phospholypolytic, amyloltic and heamolytic,). Ability of vibrio to adhere to mucus of intestine and degradation of intestinal mucosa was recorded. These results are more or less similar to those findings of **Faisal and Easa (1987)** and **Iman(2004).** These changes may be due to pseudomonas produce several extracellular products that after colonization can cause extensive tissue damage, bloodstream invasion, and dissemination.

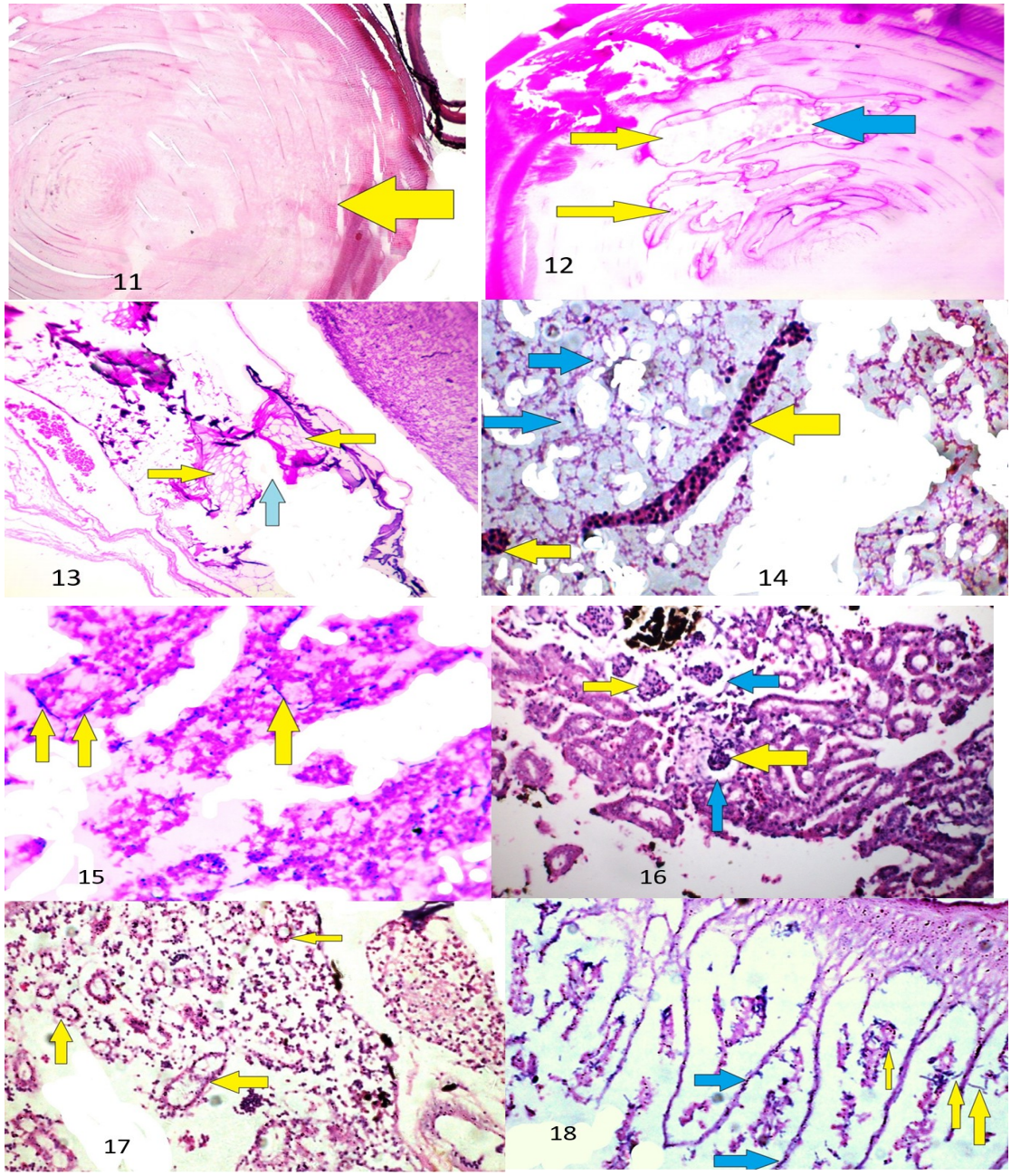

Fig. (11): Eye of normal pre-growing stage seabream showing normal lens structure normal lens layers with regular lens fibers (H&EX100).
Fig. (12): Transverse section in eye lens showing clefts in between layers of lens and accumulate of pink staining esinophilic globules (blue arrow) between cortical fibers indicating eye cataract (Stain H&EX100).
Fig. (13): Eye of fish infected with *V. alginolyticus* sagittal section with complete loss, sloughed eye lens, only present of corneal stroma (Bowman's membrane) (yellow arrows), with complete absence of retina (H&EX100).
Fig (14): Brain showing vasculitis, the neuropil has a moth eaten appearance due to prominent status spongiosis (blue arrows) ,dark stained degenerated neurons severe (H&EX200).
Fig. (15): Liver seabream showing severe degeneration and necrosis of hepatic cells. (yellow arrows) (H&EX200).
Fig. (16): Kidney of fish affected by cataract showing atrophy of glomerular tuft expansion of space inside the glomeruli ,degenerative changes in some renal tubules activation of melanomacrophage center (H&EX100).
Fig. (17): Kidneys of seabream showing complete loss of renal tubular epithelium (yellow arrows), the other renal tissues showing high degeneration and necrosis with complete absence of renal glomeruli (H&EX100).
Fig. (18): Seabream intestine with *V. alginolyticus* showing severe inflammation ,infiltrated inflammatory cells (yellow arrow) severe destructed intestinal villi with loss of all covering epithelial cells (blue arrows), (H&EX200)

Pathological changes in experimental pre-growing stage seabream.
Eye of seabream experimentally infected with *V.alginoliticus* showed damage of lens capsule (Fig. 19) choroid edema, hyperemia and heamorrhage was appeared (Fig.20), and that agreed with **Xiao *et al*., (2009)** who said that endophthalmitis caused by *V. alginoliticus* **Rameskumar *et al.* (2014)** found that the eyes revealed congestion as well as infiltration of polymorphoneuclear cells in the choroid layer in fishes infected with *V. alginoliticus.* The examined brain showed massive vacuolation in brain tissues (Fig. 21). Focal gliosis and pyramidal cells. severe hyperemia in the blood vessels and capillaries of the cerebrum with perivascular and pericellular oedema hyperemic blood vessels and oedema in the meninges with dilatation of the cerebral blood capillaries. The Liver of seabream experimentally infected with *V. alginoliticus* exhibited swelling of hepatocytes and congested blood vessels multifocal hepatocyte necrosis (Fig. 22), **Martins (2010)** who concluded that hyperplasia, sinusoidal deformation and necrotic foci in the liver were observed in histopathological analysis. *V. alginolyticus* in fish. Kidneys showed vacuolar degeneration of tubular epithelial and atrophy of glomerular tuft, hemorrhages, (Fig. 23 & 24). **Miyashita (2009)** who found acute glomerulornephritis in the kidneys infected with *V. alginoliticus* **Martins (2010)** found leukocyte infiltration and necrotic foci in the kidney of fish infected by *V. alginolyticus.*

The histopathological findings of eye of pre-growing stage seabream experimentally infected with *P. fluorescens* revealed retinal hyperplasia, choroid heamorrhage and choroid edema with inflammatory cellular infiltration (Fig. 25), and that approved by **Miyazaki, *et al.* (1984)** who found that infected fish with *P. fluorescens* showed exophthalmia, nodular lesions in the liver and kidney. The histopathological aspects were focal necrosis in the liver, and kidney. Brain showed meningitis, congestion, edema with mononuclear leucocytic cells infiltration in the meninges (Fig. 26a); and Gliosis (Fig. 26b). Liver showed multifocal areas of necrosis in hepatic and pancreatic tissue with activation of melano-macrophage centers Kidney showed sever heamorrhage, damage and necrosis of renal tissue. (Fig. 27) **Miyazaki, *et al.* (1984)** who found that infected fish with *P. fluorescens* showed nodular lesions in the liver, kidney and the histopathological aspects were focal necrosis in the liver, and kidney.

Conclusion and Recommendations:
This should be taken into consideration when obtaining pre-growing stage, fingerlings or frys from external sources, the hatchery should be applying biosecurity program, and vaccination routing. Early diagnosis resulting in rapid and successful treatment. Usage of antibiotics are avoided or used under several restrictions and for short time. Water should be examined periodically.

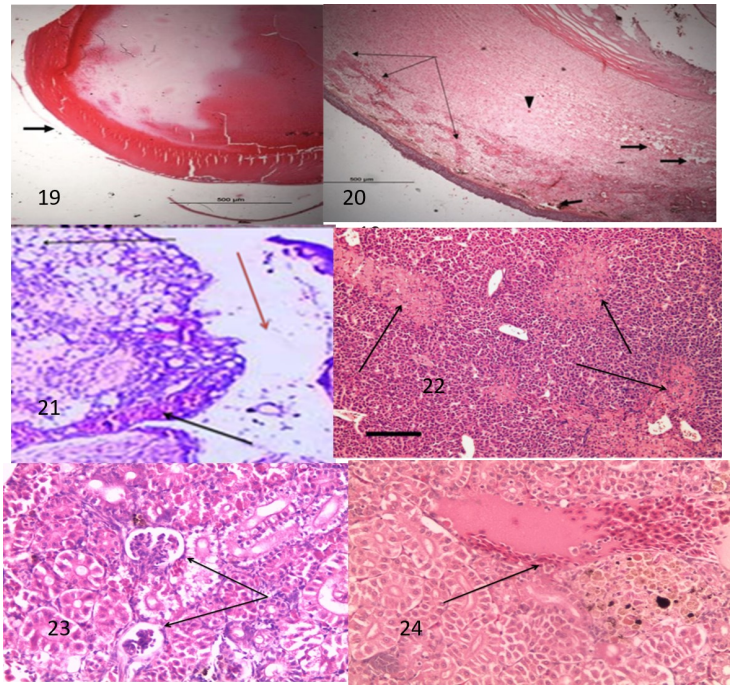

Fig. (19): Experimentally infected pre-growing stage seabream with *V. alginolyticus* eye showing damage of lens capsule (arrow) (H&E, X 40).
Fig. (20): Eye of seabream experimentally infected with *V. alginoliticus* showing choroid edema (thick arrow), hyperemic choroid (thin arrow) and heamorrhage (arrow head) (H&E, X 40).
Fig. (21): Brain showing massive vacuolation in brain tissues (Arrows). (H & E X 200).
Fig. (22): Liver of seabream experimentally infected with *V. alginoliticus* showing multifocal hepatocyte necrosis in seabream (Arrows) (H&E X100).
Fig. (23): Kidney of seabream experimentally infected with *V. alginoliticus* showed vacuolar degeneration of tubular epithelial and atrophy of glomerular tuft (H&E x200).
Fig. (24): Kidney of seabream experimentally infected with *V. alginoliticus* showed severe hemorrhage and hemolysis of RBCs with presence of large area of necrosis (H&E ×200).

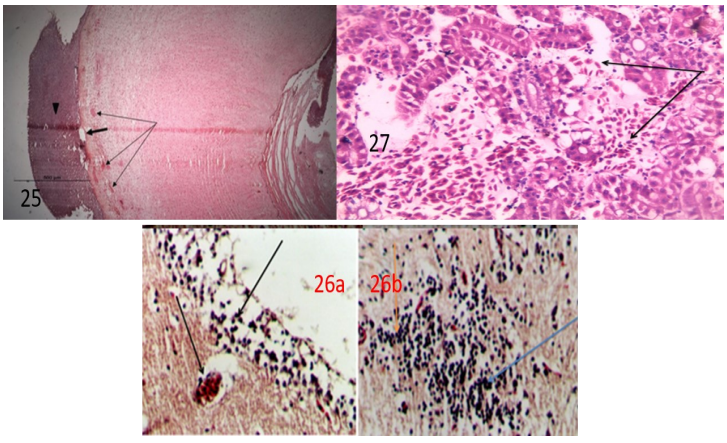

Fig. (25): Eye of seabream experimentally infected with *P. fluorescens* showing retina hyperplasia (arrow head), choroid hemorrhage (thin arrow), and choroid edema (thick arrow) (H&E, X 200) with inflammatory cellular infiltration (H&E, X 200).
Fig. (26a): Brain of seabream showing Meningitis "congestion, edema with mononuclear leucocytic cells infiltration in the meninges (H&E.X.400).
Fig. (26b): Brain of seabream showing: Gliosis (Arrows); (H&E.X.400).
Fig. (27): Kidney of seabream experimentally infected with *P. fluorescens* showed sever heamorrhage, damage and necrosis of renal tissue

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