Isolation of *Staphylococcus epidermidis, Bacillus cereus* and *Pseudomonas stutzeri* from diseased European sea bass (*Dicentrarchus labrax*) for the first time in Egypt

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ABSTRACT

During this study, 20 % mortality was recorded in *Dicentrarchus labrax* brood stock in marine hatchery of National Institute of Oceanography and Fishery at Alexandria province. Observed clinical signs were decrease appetite, off-food, skin ulcerations and tail erosions. The main post mortem lesion was enlarged liver with presence of hemorrhagic foci. Seven bacterial isolates were recovered by initial isolation on typtic soy agar media from topical lesions (skin, musculature and tail) and from internal organs (heart, liver, spleen and posterior kidney). No growth was detected on specific media including Rimler-Shotts, Pseudomonas selective agar, Thiosulfate Citrate Bile Salts Sucrose agar and Edwards media that indicate absence of major fish pathogens. Identification of the causative agents was performed using VITEK 2 automated biochemical identification system. Four *Staphylococcus epidermidis*, two *Bacillus cereus* and one *Pseudomonas stutzeri* isolates were identified as the causative agents responsible for mortalities in diseased *Dicentrarchus labrax*. Stress factors induced by hatchery conditions could be the predisposing caused of such infection.

Keywords: European sea bass, *Dicentrarchus labrax*, VITEK 2, *Staphylococcus epidermidis*, *Pseudomonas stutzeri*, *Bacillus cereus*.

INTRODUCTION

European sea bass is considered one of the important marine cultured fish, especially with declining fisheries production. Egypt produces about 15167 ton of cultured European sea bass (GFARD, 2016) and has been ranked as the fifth producer that represent 4 % of total Mediterranean aquaculture of this species (Vázquez and Muñoz-Cueto, 2015).

The most important problem facing expansion in marine aquaculture in Egypt is severe shortage of seed production, decreased hatchery numbers and brood stock mortalities that make the problem more prominent.

Bacterial diseases are considered one of the major causes of economic losses affecting marine culture (Anderson and Conroy, 1970), and are the biggest challenge regarding the European sea bass (*Dicentrarchus labrax*) Mediterranean aquaculture (Toranzo *et al.*, 2005). *Aeromonas* spp., *Bacillus* spp., *Flavobacterium* spp., Photobacterium, *Pseudomonas* spp., *Staph. epidermidis* and *Vibrio* spp. are the most common bacteria pathogens affecting farmed European sea bass in Greece (Yiagnisis and Athanassopoulou, 2011).Anorexia, lethargies, disorientation, abdominal swelling and external haemorrhages in the head, eyes, skin, gills and at the bases of the fins as

well as skin ulcers are the most common clinical signs. The main post-mortem lesions include visceral petechiation, pale liver, kidneys, and enlarged spleen (Yiagnisis and Athanassopoulou, 2011).

The VITEK 2 is an automated microbial identification system that provides highly accurate and reproducible results. VITEK 2 system is also a rapid and reliable method for pathogens identification. It has advantage over PCR, PCR need to suspect the tested bacterial strain and use its specific primer and so its results are either positive or negative, while VITEK 2 system is only need to know the tested bacteria is either gram positive or negative.

This study has been conducted to determine the direct cause of mortalities affecting the European sea bass brood stock.

MATERIALS AND METHODS

Study area:

Samples were taken from the Marine Hatchery of National Institute of Oceanography and Fishery atAlexandria governorate north Egypt. The affected hatchery complain brood stock mortality reached 20 % between January and March 2016.

Samples:

Five live moribund fish suffered fromskin ulceration and haemorrhages were collected. Each fish was ranged between 1100 and 1500 g in body weight. Each fish sample was packed alive in a separate stile labeled plastic bag and transported in ice box to Fish Disease Lab, for isolation of the causative agents.

Clinical examination:

The clinical examination was performed according to the method described by Noga (2010).

Post mortem examination:

The post mortem examination was performed according to the method described by Heil (2009).

Isolation and identification of the causative agent:

Under complete aseptic condition a small pieces of tissues from heart, liver, spleen and posterior kidney were taken from each fish to a test tube containing 10 ml sterile peptone water, after that the sample was homogenized at 3000 rpm for 1 min using homogenizer pro® USA. Test tubes were centrifuged at 1000 rpm for 30 sec and one ml from supernatant was added to another test tube containing sterile tryptic soy broth and incubated for 24 h at 33℃.

Another sample from the external lesions in the skin, musculature and or tail, were taken using sterile swabs after disinfection of affected area with 70 % ethyl alcohol to avoid contamination from bond water.After that swabs from skin lesion was taken to sterile tryptic soy broth tubesand incubated for 24 h at 33℃.

Rimler-Shottsmedia with Ampicillin selective supplement 5 mg / litter (HiMedia), Pseudomonas selective agar with CFC selective supplement (LabM), Thiosulfate CitrateBile Salts Sucrose **(**TCBS) agar media (Oxoid), Edwards media (Oxoid) and Tryptic soy agar (Oxoid) were streaked from each sample then incubated at 33℃ for 24 h.

VITEK 2 system identification for recovered isolates:

Gram staining procedures:

Gram stain procedures were performed according to the method described by Collins *et al.* (2004).

VITEK 2 system protocol:

For Gram positive and Gram negative recovered isolates:

Few morphology similar colonies were picked up from tryptic soy agar plate, then dissolved in sterile test tube contain 3 ml of 0.5 % NaCl saline. The optical density of the solution was tested using Densi Chek Pluscalibrator and adjusted to 0.6 McFarland standards. In the VITEK 2 apparatus Gram positive ID card (GP) was set in the cassette with the suspension test tube. Cards were then inoculated automatically with microorganism suspensions using an integrated vacuum apparatus. The software of the system was logged for data entry and starting the automated pathogen identification process according to manufactures instructions.

For Bacillus isolates:

The same as in Gram positive isolate except that the optical density of the solution was adjusted to 2 McFarland standard and using Gram Positive Bacillaceae ID card (PCL).

RESULTS AND DISCUSSION

Bacterial fish diseases represent the major danger facing aquaculture causing severe economic losses, either direct including fish mortality or indirect including costs of treatment (Aboyadak, 2016).

Clinical examination of the diseased *Dicentrarchus labrax* revealed loss of appetite and off-food, sluggish movement with absence of scape reflex, fish present near bond bottom and before death float in inverted position. Skin ulcerations with presence of hemorrhages, pelvic fin and tail hemorrhage and erosions were also observed (Plates A 1 & 2& 3). The main gross internal lesions of diseased fish were enlarged congested liver with the presence of hemorrhagic foci (Plate A4). Similar clinical signs and post mortem lesions were recorded by Kusuda and Sugiyama (1981), Yiagnisis and Athanassopoulou (2011) and Varvarigos (2016) in Red Sea bream *(Chrysophrys major)* and in *Dicentrarchus labrax* fryandadult fish naturally infected with *Staphylococcus epidermidis.* Parallel to our recorded clinical signs Goodwin *et al.* (1994) and Chandra *et al.* (2015) also, recorded presence of skin ulcers and dermatitis in channel catfish and stinging catfish, *Heteropneustes fossilis* naturally infected with *Bacillus mycoides and Bacillus cereus* respectively. Sariati, *et al.* (2015) recorded the presence of petechial hemorrhage on the skin of the catfish and detached scales with hemorrhagic ulcers of tilapia naturally infected with *Pseudomonas stutzeri* in Indonesia. The recorded clinical signs and post mortem lesions are mainly attributed to both colonization and multiplication of isolated bacteria and the defense mechanism of fish against it by induction of inflammatory response.

All recovered bacterial isolates did not grow on any of specific media including Rimler-Shotts media with Ampicillin selective supplement, Pseudomonas selective agar and Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar media and Edwards media, except *Staphylococcus epidermidis* that grown on Rimler-Shotts media giving green colonies (Plat A5).*Staphylococcus epidermidis* may be resistant to Ampicillin that explain its growth on Rimler-Shotts. The isolated bacteria did not grow on mentioned media because they are selective media contain inhibitor for other bacterial species.This indicating absence of major famous fish pathogens including *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp. and *Streptococcus* spp.

On tryptic soy agar *Staphylococcus epidermidis* has grown producing white pin headed colonies about 1 mm in diameter (Plat B1), which is identical to that described

by Huang *et al.* (1999). *Bacillus cereus* haslarge, white spherical colonies with dull surface and undulate margins (4-5 mm in diameter, Plate B2), which is agree with Buller (2004). *Pseudomonas stutzeri* grows producing white to creamy colonies; about 2 - 3 mm in diameter (Plate B3) that partially agrees with Sariati *et al.* (2015).

Gram stain of *Staphylococcus epidermidis* revealed the presence of gram positive cocci, found as single, pairs, short chain and irregular clusters of cells (Plat B4), that is nearly similar to that recorded by Austin and Austin (2012).*Bacillus cereus*is present as gram positive long bacilli; single, pairs and chains. It has centrally located endospore (Plate B5) that was recorded also by PHE (2015). *Pseudomonas stutzeri* appeared as gram negative short bacilli, present single or in pairs (Plate B 6), and this agrees with Buller (2004) description.

VITEK2 is considered advanced automated biochemical identification system. Four *Staphylococcus epidermidis* isolates were identified by VITEK2 system with98 % probability; analysis time was 7 hours. The biochemical details are mentioned in (Table 1).

Table 1: Biochemical characters of *Staphylococcus epidermidis* identified by **VITEK 2** system, isolated from *Dicentrarchus labrax*.

Well N.	Biochemical reaction	Appreciation	Results
2	D-AMYGDALIN	AMY	-
4	PHOSPHATIDYLINOSITOL HOSPHOLIPASE C	PIPLC	-
5	D-XYLOSE	dXYL	-
8	ARGININE DIHYDROLASE 1	ADHl	+
9	BETA-GALACTOSIDASE	BGAL	-
11	ALPHA-GLUCOSIDASE	AGLU	-
13	Ala-Phe-Pro ARYLAMIDASE	APPA	-
14	CYCLODEXTRIN	CDEX	-
15	L-Aspartate ARYLAMIDASE	AspA	-
16	BETA GALACTOPYRANOSIDASE	BGAR	-
17	ALPHA-MANNOSIDASE	AMAN	-
19	PHOSPHATASE	PHOS	-
20	Leucine ARYI-AMIDASE	LeuA	-
23	L-Proline ARYLAMIDASE	ProA	-
24	BETA GLUCURONIDASE	BGURr	-
25	ALPHA-GALACTOSIDASE	AGAL	-
26	L-Pyrrolidonyl-ARYLAMIDASE	PyrA	-
27	BETA-GLUCURONIDASE	BGUR	-
28	Alanine ARYLAMIDASE	AlaA	-
29	Tyrosine ARYLAMIDASE	TyrA	-
30	D-SORBITOL	dSOR	-
31	UREASE	URE	+
32	POLYMYXIN B RESISTANCE	POLYB	-
37	D-GALACTOSE	dGAL	-
38	D-RIBOSE	dRIB	-
39	L-LACTATE alkalinization	ILATK	+
42	LACTOSE	LAC	+
44	N-ACETYL-D-GLUCOSAMINE	NAG	-
45	D-MALTOSE	dMAL	+
46	BACITRACIN RESISTANCE	BACI	+
47	NOVOBIOCIN RESISTANCE	NOVO	-
50	GROWTH lN 6.5 % NaCI	NC6.5	+
52	D-MANNITOL	dMAN	-
53	D-MANNOSE	dMNE	-
54	METHYL-B-D-GLUCOPYRANOSIDE	MBdG	-
56	PULLULAN	PUL	-
57	D-RAFFINOSE	dRAF	-
58	O/129 RESISTANCE (comp.vibrio.)	o129R	+
59	SALICIN	SAL	-
60	SACCHAROSE/SUCROSE	SAC	+
62	D-TREHALOSE	dTRE	-
63	ARGININE DIHYDROLASE 2	ADH2s	-
64	OPTOCHIN RESISTANCE	OPTO	+

Another two Gram positive isolates were identified biochemically by VITEK2 system as *Bacillus cereus*with85 % probability. Analysis time was 14.25 hours; the biochemical details of *Bacillus cereus* isolates are mentioned in (Table 2). Only one gram negative isolates was identified by VITEK2 system as *Pseudomonas stutzeri*, with91 % probability; analysis time was 10.25 hours. The biochemical details are mentioned in (Table 3).

Table 2: Biochemical characters of *Bacillus cereus* identified by VITEK 2 system, isolated from *Dicentrarchus labrax*.

Well N.	Biochemical reaction	Appreciation	Results
1	BETA-XYLOSIDASE	BXYL	-
3	L-Lysine-ARYLAMIDASE	LysA	-
4	L-Aspartate ARYLAMIDASE	AspA	-
5	LeucineARYLAMIDASE	LeuA	+
7	Phenylalanine ARYLAMIDASE	PheA	-
8	L-Proline ARYLAMIDASE	ProA	-
9	BETA-GALACTOSIDASE	BGAL	-
10	L-Pyrrolidonyl-ARYLAMIDAS E	PyrA	+
11	ALPHA.GALACTOSIDASE	AGAL	-
12	Alanine ARYLAMIDASE	AlaA	-
13	Tyrosine ARYLAMIDASE	TyrA	-
14	BETA-N-AC ETYL-GLUCOSAMINIDASE	BNAG	-
15	Ala-Phe-Pro ARYLAMIDASE	APPA	+
18	CYCLODEXTRINE	CDEX	-
19	D-GALACTOSE	dGAL	-
21	GLYCOGENE	GLYG	-
22	myo-INOSITOL	INO	-
24	METHYL-A-D-GLUCOPYRANOSIDEacidification	MdG	-
25	ELLMAN	ELLM	-
26	METHYL-D-XYLOSIDE	MdX	-
27	ALPHA.MANNOSIDASE	AMAN	-
29	MALTOTRIOSE	MTE	+
30	Glycine ARYLAMIDASE	GlyA	-
31	D-MANNITOL	dMAN	-
32	D-MANNOSE	dMNE	-
34	D-MELEZITOSE	dMLZ	-
36	N-ACETYL-D-GLUCOSAMI N E	NAG	+
37	PALATINOSE	PLE	-
39	L-RHAMNOSE	IRHA	-
41	BETA-GLUCOSIDASE	BGLU	-
43	BETA-MANNOSIDASE	BMAN	-
44	PHOSPHORYL CHOLINE	PHC	-
45	PYRUVATE	PVATE	+
46	ALPHA.GLUCOSIDASE	AGLU	+
47	D-TAGATOSE	dTAG	-
48	D-TREHALOSE	dTRE	-
50	INULIN	INU	-
53	D-GLUCOSE	dGLU	+
54	D-RIBOSE	dRIB	+
56	PUTRESCINE assimilation	PSCNa	-
58	GROWTH lN 6.5% NaCl	NaCI 6.5%	+
59	KANAMYCIN RESISTANCE	KAN	+
60	OLEANDOMYCIN RESISTANCE	OLD	-
61	ESCULIN hydrolyse	ESC	+
62	TETRAZOLIUM RED	TTZ	-
63	POLYMYXIN B RESISTANCE	POLYB_R	+

Table 3: Biochemical characters of *Pseudomonas stutzeri* identified by VITEK 2 system, isolated from *Dicentrarchus labrax*.

Well N.	Biochemical reaction	Appreciation	Results
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	-
3	ADONITOL	ADO	-
4	L- Pyrrolydonyl-ARYLAMIDASE	PyrA	-
5	L-ARABITOL	IARL	-
7	D-CELLOBIOSE	dCEL	-
9	BETA-GALACTOSIDASE	BGAL	-
10	H2S PRODUCTION	H25	-
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	-
12	GlutamylArylamidasepNA	AGLTP	-
13	D-GLUCOSE	dGLU	+
14	GAMMA-GLUTAMYL-TRANSFE RASE	GGT	-
15	FERMENTATION/ GLUCOSE	OFF	-
17	BETA-GLUCOSIDASE	BGLU	-
18	D-MALTOSt	dMAL	+
19	D-MANNITOL	dMAN	+
20	D-MANNOSE	dMNE	-
21	BETA-XYLOSIDASE	BXYL	-
22	BETA-Alanine arylamidasepNA	BAlap	-
23	L-Proline ARYLAMIDASE	ProA	+
26	LIPASE	LIP	-
27	PALATINOSE	PLE	-
29	Tyrosine ARYLAMIDASE	TyrA	+
31	UREASE	URE	-
32	D-SORBITOL	dSOR	-
33	SACCHAROSE/SUCROSE	SAC	-
34	D-TAGATOSE	dTAG	-
35	D-TREHALOSE	dTRE	-
36	CITRATE(SODTUM)	CIT	+
37	MALONATE	MNT	+
39	5-KETO-D-GLUCONATE	5KG	-
40	L-LACTATE alkalinization	ILATK	+
41	ALPHA-GLUCOSIDASE	AGLU	-
42	SUCCINATE alkalinization	SUCT	+
43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	-
44	ALPHA-GALACTOSIDASE	AGAL	-
45	PHOSPHATASE	PHOS	-
46	Glycine ARYLAMIDASE	GIyA	-
47	ORNITHINE DECARBOXYLASE	ODC	-
48	LYSINE DECARBOXYLASE	LDC	-
52	DECARBOXYLASE BASE	ODEC	-
53	L-HISTIDINE assimilation	IHISa	+
56	COURMARATE	CMT	-
57	BETA-GLUCURONIDASE	BGUR	-
58	O/129 RESISTANCE (comp.vibrio.)	o129R	-
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	-
61	L-MALATE assimilation	IMLTA	-
62	ELLMAN	ELLM	+
64	L-LACTATE assimilation	ILATA	-

Two *Staphylococcus epidermidis* isolates were recovered from internal organs of systemically affected fish. From topical lesions other five isolates (two *Staphylococcus epidermidis*, two *Bacillus cereus* and one *Pseudomonas stutzeri*) were isolated from swabs taken from skin, tail and musculature (Table 4). To the best of our knowledge edge, there is no any report for isolation of *Staphylococcus*

epidermidis, Bacillus cereus and *Pseudomonas stutzeri* from diseased European sea bass (*Dicentrarchus labrax*) in Egypt and this article is considered the first report documented the presence of these isolates.

Table 4: Identified bacteria from internal organs and topical lesions of *Dicentrarchus labrax*.

Bacterial Isolates	From internal organs	From topical lesions
Staphylococcus epidermidis	2	2
Bacillus cereus	-	2
Pseudomonas stutzeri	-	1

Brood stock fish during spawning season are suffered from variety of stress factors including continuous handling and excessive gonads production, which may affect its immune status negatively, inducing immunosuppression that subsequently make it more susceptible to pathogens. Furthermore, continuous handling may induce skin abrasions and lacerations that consider a portal of entry of pathogenic bacteria. Some of these bacteria can induce systemic disease after replication and propagation in local lesions (as skin and musclulature) and this can be an explanation for systemic infection with *Staphylococcus epidermidis*. Moreover, *Staphylococcus epidermidis* was isolated from a variety of diseased fresh and marine fished causing systemic infection as recorded by Kusuda and Sugiyama (1981), Yiagnisis and Athanassopoulou, (2011) and Varvarigos, (2016). *Bacillus cereus* and *Pseudomonas stutzeri*are considered environmental bacteria (not considered a potential pathogen but cause disease in immunosuppressed host) more than obligate pathogen, although others, including Goodwin *et al.* (1994), Chandra *et al.* (2015) and Sariati *et al.* (2015) recorded their isolation from systemically affected fish. The present results revealed the presence of this two species only in local lesions (skin, musculature and tail) and this does not decrease their role in the disease occurrence, but to give best understanding to current research results, and to overcome this argue about their role and potency to induce such disease, further detailed studied are needed.

CONCLUSIONS

In conclusion *Staphylococcus epidermidis, Bacillus cereus* and *Pseudomonas stutzeri* were isolated from diseased European sea bass (*Dicentrarchus labrax*) for the first time in Egypt. They are considered the main causative agents. Further studies are needed to determine their virulence factors and disease pathogenesis.

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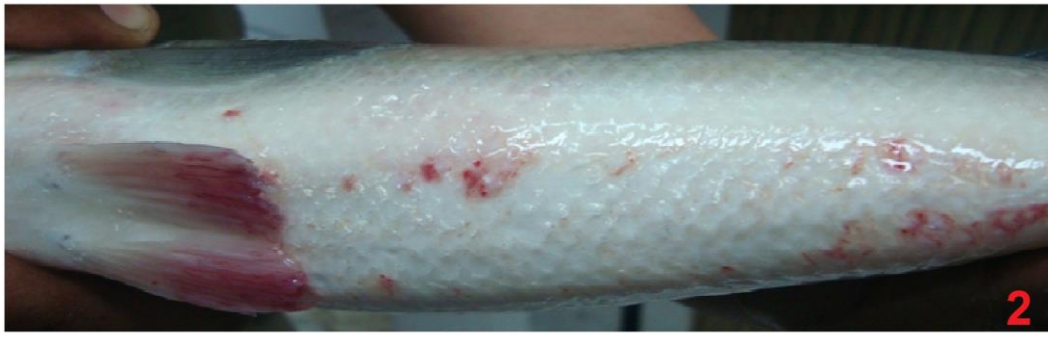
Plate A

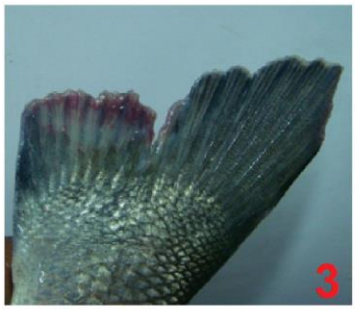
1	Naturally infected European sea bass (*Dicentrarchus labrax*) showing scale desquamation and skin ulceration (arrow).
2	Naturally infected European sea bass (*Dicentrarchus labrax*) with multiple hemorrhagic spots in ventral surface and hemorrhagic eroded pelvic fin.
3	Naturally infected European sea bass (*Dicentrarchus labrax*) with severely eroded hemorrhagic tail.
4	Naturally infected European sea bass (*Dicentrarchus labrax*) with enlarged congested liver with presence of hemorrhagic foci.
5	*Staphylococcus epidermidis* grown on Rimler-Shottsmedia giving green colonies.

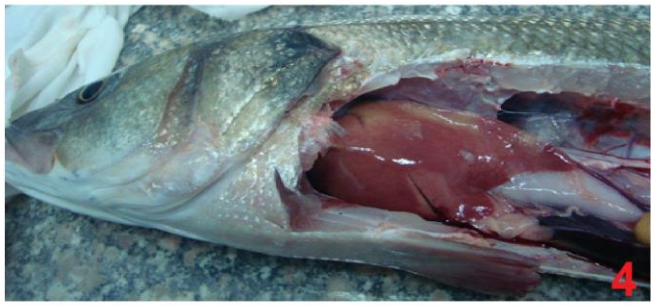
Plate B

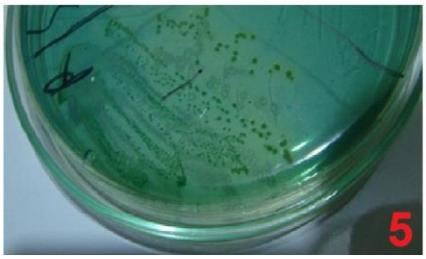
1	Pin headed size white colony of Staphylococcus *epidermidis* grown on Tryptic soy agar.
2	Large white spherical colonies with dull surface and undulate margins (4 – 5 mm in diameter) of Bacillus *cereus* grown on Tryptic soy agar.
3	Rounded creamy colonies (2 - 3 mm in diameter) of Pseudomonas *stutzeri* grown on Tryptic soy agar.
4	*Staphylococcus epidermidis*, gram positive cocci present as single, pairs, short chain and irregular clusters (oil immersion lens, X = 1000)**.**
5	*Bacillus cereus*, gram positive long bacilli present single, pairs, and chains, has centrally located endospore (oil immersion lens, X = 1000)**.**
6	*Pseudomonas stutzeri*, gram negative short bacilli present single or in pairs (oil immersion lens, X = 1000)**.**

Plate A









1- Naturally infected European sea bass (*Dicentrarchus labrax*) showing scale desquamation and skin ulceration (arrow).

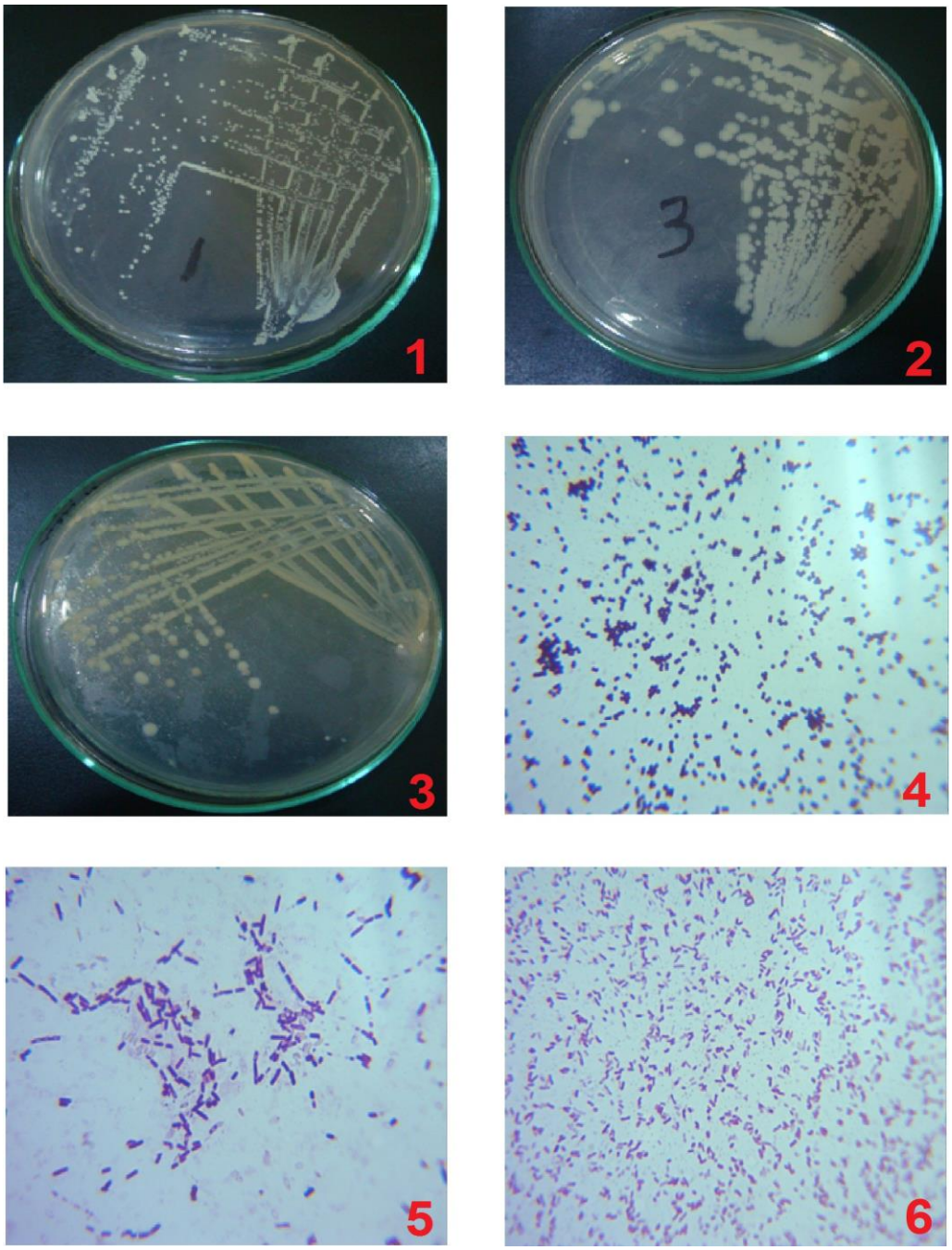
2- Naturally infected European sea bass (*Dicentrarchus labrax*) with multiple hemorrhagic spots in ventral surface and hemorrhagic eroded pelvic fin.

3- Naturally infected European sea bass (*Dicentrarchus labrax*) with severely eroded hemorrhagic tail.

4- Naturally infected European sea bass (*Dicentrarchus labrax*) with enlarged congested liver with presence of hemorrhagic foci.

5- *Staphylococcus epidermidis* grown on Rimler-Shottsmedia giving green colonies.

Plate B



1- Pin headed size white colony of Staphylococcus *epidermidis* grown on Tryptic soy agar.
2- Large white spherical colonies with dull surface and undulate margins (4 – 5 mm in diameter) of Bacillus *cereus* grown on Tryptic soy agar.
3- Rounded creamy colonies (2 - 3 mm in diameter) of Pseudomonas *stutzeri* grown on Tryptic soy agar.
4- *Staphylococcus epidermidis*, gram positive cocci present as single, pairs, short chain and irregular clusters (oil immersion lens, X = 1000).
5- *Bacillus cereus*, gram positive long bacilli present single, pairs, and chains, has centrally located endospore (oil immersion lens, X = 1000).
6- *Pseudomonas stutzeri*, gram negative short bacilli present single or in pairs (oil immersion lens, X = 1000).

ARABIC SUMMARY

عزل بعض انواع من البكتريا الممرضة لسمكة القاروص الاوروبي لأول مرة في مصر.

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1- معمل امراض الاسماك – المعهد القومي لعلوم البحار والمصايد ، مصر.

2- معمل تفريخ الاسماك (المفرخ البحري) – المعهد القومي لعلوم البحار والمصايد ، مصر.

خلال هذه الدراسة تم تسجيل حدوث وفيات بنسبة 20 % في قطيع امهات القاروص الاوروبي بالمفرخ البحري التابع للمعهد القومي لعلوم البحار والمصايد بالإسكندرية. كانت الاعراض الظاهرة على الاسماك المصابة هي فقد الشهية والامتناع عن الطعام ووجود تقرحات جلدية مع تآكل في الذيل. اوضحت الصفة التشريحية وجود تضخم في الكبد مع وجود نقط نزفية به. تم عزل سبعة معزولات بكتيرية من الجلد والعضلات والذيل ومن الاعضاء الداخلية (القلب والكبد والكلي والطحال) وذلك علي مستنبت الصويا. لم تنمو اي من المعزولات علي المستنبتات الخاصة مثل مستنبت الرملر شوت و السودوموناس اجار و الثيوسلفات سترات بيل سولت اجار او ادوارد ميديا والذي أوضح عدم وجود اي من مسببات امراض الاسماك البكتيرية التقليدية. تم تصنيف المعزولات بأستخدام تقنية التعريف البيوكميائي الاوتوماتيكي. تم التعرف علي 4 معزولات من نوع ستافيلوكوكسابديرميدس و معزولاتين من باسليس سيريس ومعزولة واحدة من سودوموناسستوتزري كمسبب مرضي لحدوث هذا النفوق في قطيع امهات القاروص الاوروبي, وذلك لأول مرة في مصر.كما استنتج ان الاجهاد الحادث اثناء عملية التفريخ يعد من العوامل الممهدة لحدوث هذا النفوق.