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Survey on the most common bacterial pathogens of the Nile tilapia fries in Kafr El sheikh governorate, Egypt

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ABSTRACT

Recently, aquaculture in Egypt has faced multiple records of mass mortality resulting in high economic losses. The mass mortality among tilapia fries is mostly attributed to bacterial pathogens either with or without other pathogens. This study is designed to investigate the etiological factors implicated in mortality of the Nile tilapia fries. Bacteriological examination of the Nile tilapia fries including isolation and identification using a range of techniques (API NE20, Vitec, PCR and 16SrRNA Sequencing) revealed an array of typical pathogens known to cause lethal infections in tilapia. The most frequently isolated strains were Aeromonads 33.3% (A. veronii, A. sobria, A. hydrophila), Staphylococcus epidermidis, 11.1 % Pseudomonas aeruginosa, 11.1% and Shigella sonnei 11.1%. Furthermore, three emerging freshwater aquaculture pathogens which have zoonotic significance, namely Providencia rettgeri (11.1%), Shewanella putrefaciens (11.1%) and Acinetobacter lwoffii (11.1%) were isolated from fish fries. Despite known pathogenicity, inoculation of fish fingerlings with the different bacterial isolates resulted in only mild mortality rates under non-stress conditions, emphasizing the role of additional environmental stressors in triggering mass mortality of juvenile fish. Conclusively, these pathogens have a significant negative impact on tilapia fries, particularly in combination with environmental stress.

INTRODUCTION

Fish is considered as food resource that could help to meet food security needs across the globe, particularly in developing and populous countries. Fish currently represents around 16 percent of all animal protein consumed globally **(FAO, 2018)**. Moreover, fish constitutes about 30 percent of all animal protein intake in the developing countries **(Wang *et al.*, 2015)**. Aquaculture, in particular, has played a major role over the past decades by increasing the production of fish efficiently and at an affordable cost. It currently accounts for more than half of worldwide fish production **(Subasinghe *et al.*, 2009)**. Recently, Egypt ranks the tenth in fish farming production and the first among African countries, the industry employs more than

580,000 workers and contributes to around 77 percent of the total fish production in Egypt **(GAFRD, 2014; FAO, 2016)**. The majority of fish farms are located close to the Nile in the northern part of Egypt and the Delta region **(FAO, 2018)**. Kafr El-Sheikh, Damietta, Port Said, Fayoum, Behira, and Sharkia are the leading governorates in aquaculture production **(GAFRD, 2014)**. Tilapia is the most predominant species in fish farms, representing about 55 percent of the total production of aquaculture, **(Sadek, 2013)**. However, aquaculture in Egypt suffers from elevated mortality due to parasites, bacteria, fungi, and viruses, resulting in high economic losses. These pathogens have a significant negative impact on feed conversion rates and post-infection total weight of fish that could not recover **(Shaalan *et al.*, 2018)**. Besides, environmental issues as abrupt thermal changes, extreme reduction in dissolved oxygen and high ammonia levels, are major reasons for mass mortalities among fish populations **(Harris *et al.*, 1998)**. In fish farms, high mortality rates are attributed to bacterial rather than other infections. In Egyptian fish farms, infections with *Aeromonas hydrophila*, *Flavobacterium columnaris*, *Pseudomonas fluorescens*, *Yersinia ruckeri*, *Edwarsiella tarda*, *Edwardsiella ictaluri*, *Vibrio* spp. Mycoplasma and *Streptococcus* spp. are prevalent **(Moustafa *et al.*, 2010; Zhang *et al.*, 2014; Abdelsalam *et al.*, 2017; El-jakee *et al.*, 2020)**. Pseudomonas is reported to be an important cause for Egyptian aquaculture outbreaks in the last decade **(Khalil *et al.*, 2010)**. Aeromonas species have also been isolated from some fish farms in Egypt **(Khairul Afizi *et al.*, 2013; Noor El-Deen *et al.*, 2014)**. Lactococcosis, Enterococcosis and Streptococcosis are found in tilapia farms in Egypt. Additionally, ammonia, high water temperature, organic matter, fish density, low oxygen level and pH are regarded as predisposing factors. Thus, a proper management is the first preventive measure needed to avoid outbreaks in fish farms (**Abu-elala *et al.*, 2020a)**.

Hence,this study was designed to investigate the most common bacterial causes of mortality rates among tilapia fries.

MATERIALS AND METHODS

1- Sample collection:

Six thousand tilapia fries (*Oreochromis niloticus*) were collected from Kafr El Sheikh hatcheries with average body weight of 0.65 g and average length 0.5 cm.Fish samples were transported in clean plastic bags to the laboratory of Aquatic animal's medicine and management, faculty of Veterinary Medicine, Cairo University for bacteriological examination. Water samples were collected in clean sterile glass bottle for water analysis.

2- Water analysis:

Water samples were used to analyze BOD, COD, NH_3, NH_4, NO_2, Fe, Cu, Cd, and Pb in the central laboratory for water analysis, faculty of Agriculture, Cairo University

according to the international standard methods for examination of water and wastewater **(APHA, 2005)**.

3- Bacterial isolation and identification:

Under complete aseptic conditions, swab from grinded pooling samples of fries was streaked on Nutrient agar, Todd Hewitt agar, tryptone soya agar, blood agar and pseudomonas agar media after external sterilization by 70% ethyl alcohol and incubated at 28°C for 24-48 hrs. according to **Whitman and MacNair (2004)**.

A single colony from isolate was picked up, re-streaked again on the aforementioned media, and re-incubated at the same conditions for purification of bacterial isolates. When pure colonies were identified by gram stain, a loopful of each pure culture was streaked onto trypticase soya agar media to be used for further identification. Identification of all isolates were done by cultural, morphological and biochemical characters **(Austin & Austin, 2007; Panangala *et al.*, 2007; Quinn *et al.*, 2011)**.

3-1- Biochemical identification:

Biochemical identification was performed using API20NE following manufacturer's instructions (bioMerieux), and Vitek Analysis was done according to the manufacture's instruction **(Biomeriux, 2006)** in the department of fish diseases and management, animal health research institute, Dokki, Egypt.

3-2-Molecular identification (PCR and sequencing):

Total bacterial DNA was extracted from pure colonies of four isolates using GeneJet genomic DNA purification Kit (ThermoFisher, USA) according to the manufacturer's instructions. PCR for 16SrRNA was done using universal primers (Forward: 5'- AGA GTT TGA TCC TGG CTC AG-3') (Reverse: 5'-GGT TAC CTT GTT ACG ACT T-3') reported elsewhere **(Weisburg *et al.*, 1991)**by Maxima Hot Start PCR Master Mix (ThermoFisher, USA) as per manufacturer's instructions. Reaction conditions were as follows: Initial denaturation step at 95 °C for 10 min; thirty-five cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 1 min, and extension at 72 °C for 1 min30s; A final extension was done for 10 min at 72 °C. The reaction volume was 50 µl. The amplified products were analyzed by electrophoresis on 1 % agarose gel stained with ethidium bromide.

16SrRNA was sequenced in both directions using ABI 3730xl DNA sequencer (Applied BiosystemsTM, USA) at Sigma Scientific Services Laboratory (Cairo, Egypt). Sequences were compared to reference sequences using BLAST® of NCBI (**Altschul *et al.*, 1990)**.

4- Pathogenicity Test:

A total number of 50 healthy *Oreochromis niloticus* fingerlings, with average body weight of 20 gm, were held in aerated freshwater aquaria supplied with dechlorinated

tap water at 25˚C ± 2 for ten days before challenge for acclimatization. Tilapia under the experiment were divided into four groups (Ten fishes for each group). Each group was injected intraperitoneal with 0.2 ml of bacterial isolates (Table 1). The first, second, third and fourth group (control) was injected intaperitoneal with 6×10^8CFU/ml of *Aeromonas Veronii*, 3×10^8 CFU/ml of *Staphylococcus Epidermidis*, 6×10^8 CFU/ml of *Providencia Rettgeri* and saline–respectively. After the inoculation, the fish were observed for three weeks; during which the clinical signs, morbidity, and mortality percent were recorded **(Huang *et al.*, 1999; Hassan *et al.*, 2017; Ramesh & Souissi, 2018)**.

Table 1: Doses of inoculated bacterial isolates.

First group	6×108CFU/ml of Aeromonas Veronii
Second group	3×108 CFU/ml of Staphylococcus Epidermidis
Third group	6×108 CFU/ml of Providencia Rettgeri
Fourth group	Control group (saline only)

RESULTS

1- Water Analysis:

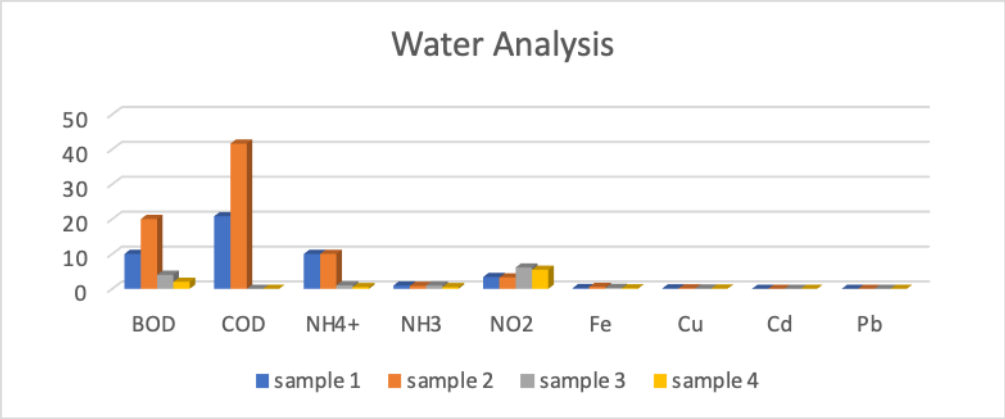

Fig.1: Results of examination of water samples from hatcheries.

BOD (Biological Oxygen Demand) ranged from 2 - 20 mg/l. The highest value (20 mg/l) is observed at the second sample (spring), while the lowest value of 2 mg/l is observed at the fourth sample (summer). COD (Chemical Oxygen Demand) range from ND (Not Detected) to 41.6 mg/l. The highest value (41.6 mg/l) is observed at the second sample (spring), while the lowest value of ND is observed at the third and fourth samples (summer). NH_4+ ranged from 0.5 - 10 mg/l. The highest value (10 mg/l) is observed at the first and second samples(spring), while the lowest value of 0.5 mg /l is observed at

the fourth sample(summer). NH_3 ranged from 0.47-0.95 mg/l. The highest valueof 0.95 mg/l is observed at the first samples(spring), while the lowest value of 0.47 mg /l is observed at the fourth sample(summer). Fe (Iron) ranged from 0.13 - 0.53 mg/l. The highest value of 0.53 mg/l is observed at the second sample (spring), while the lowest value of 0.13 mg /l is observed at the fourth sample (summer). Cu (Copper) ranged from 0.05 to 0.13 mg/l. The highest value of 0.13 mg/l is observed at the first sample (spring), while the lowest value of 0.05 mg /l is observed at the third and fourth samples (summer). Cd (Cadmium) and Pb (Lead) are ND (Not Detected) in all water samples.

2- Isolation and Identification of bacterial isolates:

2.1- Cell morphology:

The isolated species of bacteria are categorized according to Gram's stain into Gram-negative rods and Gram-positive cocci.

2.2- Biochemical identification:

2.2.1- API20-NE Analysis:

According to the API 20-NE identification, there was a strain from genus Shewanella (*Shewanella puterfaciens*) **(Fig.2).**

Fig. 2: API20- NE Strip used for identification of *Shewanella puterfaciens*.

2.2.2- Vitek analysis:

Identification by Vitek2 compact system revealed the following **(Tables 2, 3, 4, 5 and 6):**

1- Aeromonas *sobria* (86% probability), Bio number (5675613150544271)

Table 2: Biochemical details of Aeromonas *sobria*																	
1	-		2	APPA	+	3	ADO	-	4	PyrA	+	5	IARL	-	6	-	
7	dCEL	+	8	-		9	BGAL	+	10	H2S	+	11	BNAG	+	12	AGLTP	+
13	dGLU	+	14	GGT	-	15	OFF	+	16	-		17	BGLU	-	18	dMAL	+
19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-	23	proA	+	24	-	
25	-		26	LIP	+	27	PLE	-	28	-		29	TyrA	+	30	-	
31	URE	-	32	dSOR	-	33	SAC	+	34	dTAG	-	35	dTER	+	36	CIT	-
37	MNT	-	38	-		39	5KG	-	40	ILATK	+	41	AGLU	-	42	SUCT	+
43	NAGA	-	44	AGAL	-	45	PHOS	+	46	GlyA	-	47	ODC	-	48	LDC	+
49	-		50	-		51	-		52	-		53	IHISa	-	54	-	
55	-		56	CMT	+	57	BGUR	-	58	O129R	+	59	GGAA	+	60	-	
61	IMLTa	+	62	ELLM	+	63	-		64	ILATa	-						

2- Aeromonas *hydrophila* (97% probability), Bio number (050002003231231)

Table 3: Biochemical details of Aeromonas *hydrophila*																	
1	-		2	APPA	+	3	ADO	-	4	PyrA	+	5	IARL	-	6	-	
7	dCEL	+	8	-		9	BGAL	+	10	H2S	+	11	BNAG	+	12	AGLTP	+
13	dGLU	+	14	GGT	-	15	OFF	+	16	-		17	BGLU	-	18	dMAL	+
19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-	23	proA	+	24	-	
25	-		26	LIP	+	27	PLE	-	28	-		29	TyrA	+	30	-	
31	URE	-	32	dSOR	-	33	SAC	+	34	dTAG	-	35	dTER	+	36	CIT	-
37	MNT	-	38	-		39	5KG	-	40	ILATK	+	41	AGLU	-	42	SUCT	+
43	NAGA	-	44	AGAL	-	45	PHOS	+	46	GlyA	-	47	ODC	-	48	LDC	+
49	-		50	-		51	-		52	-		53	IHISa	-	54	-	
55	-		56	CMT	+	57	BGUR	-	58	O129R	+	59	GGAA	+	60	-	
61	IMLTa	+	62	ELLM	+	63	-		64	ILATa	-						

3- Providencia *rettgeri* (98% probability), Bio number (0694212003231231)

Table 4: Biochemical details of Providencia *rettgeri*

1	-		2	APPA	-	3	ADO	+	4	PyrA	-	5	IARL	+	6	-	
7	dCEL	-	8	-		9	BGAL	-	10	H2S	-	11	BNAG	-	12	AGLTP	-
13	dGLU	+	14	GGT	-	15	OFF	+	16	-		17	BGLU	+	18	dMAL	-
19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-	23	proA	-	24	-	
25	-		26	LIP	-	27	PLE	-	28	-		29	TyrA	+	30	-	
31	URE	+	32	dSOR	-	33	SAC	-	34	dTAG	-	35	dTER	-	36	CIT	+
37	MNT	-	38	-		39	5KG	-	40	ILATK	-	41	AGLU	-	42	SUCT	+
43	NAGA	-	44	AGAL	-	45	PHOS	+	46	GlyA	-	47	ODC	-	48	LDC	-
49	-		50	-		51	-		52	-		53	IHISa	-	54	-	
55	-		56	CMT	+	57	BGUR	-	58	O129R	-	59	GGAA	-	60	-	
61	IMLTa	-	62	ELLM	+	63	-		64	ILATa	-						

4- Shigella *sonnei* (98% probability), Bio number (0694212003231231)

Table 5: Biochemical details of Shigella *sonnei*

1	-		2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	6	-	
7	dCEL	-	8	-		9	BGAL	+	10	H2S	-	11	BNAG	-	12	AGLTP	-
13	dGLU	+	14	GGT	-	15	OFF	+	16	-		17	BGLU	-	18	dMAL	+
19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-	23	proA	+	24	-	
25	-		26	LIP	-	27	PLE	-	28	-		29	TyrA	+	30	-	
31	URE	-	32	dSOR	+	33	SAC	-	34	dTAG	-	35	dTER	+	36	CIT	-
37	MNT	-	38	-		39	5KG	+	40	ILATK	+	41	AGLU	-	42	SUCT	+
43	NAGA	-	44	AGAL	+	45	PHOS	-	46	GlyA	+	47	ODC	-	48	LDC	-
49	-		50	-		51	-		52	-		53	IHISa	-	54	-	
55	-		56	CMT	+	57	BGUR	+	58	O129R	+	59	GGAA	-	60	-	
61	IMLTa	+	62	ELLM	-	63	-		64	ILATa	+						

5- Pseudomonas *aeruginosa* (98% probability), Bio number (0694212003231231)

Table 6: Biochemical details of Pseudomonas *aeruginosa*																	
1	-		2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	6	-	
7	dCEL	-	8	-		9	BGAL	-	10	H2S	-	11	BNAG	-	12	AGLTP	-
13	dGLU	+	14	GGT	-	15	OFF	-	16	-		17	BGLU	-	18	dMAL	-
19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-	23	proA	-	24	-	
25	-		26	LIP	-	27	PLE	-	28	-		29	TyrA	+	30	-	
31	URE	-	32	dSOR	-	33	SAC	+	34	dTAG	-	35	dTER	+	36	CIT	-
37	MNT	-	38	-		39	5KG	-	40	ILATK	-	41	AGLU	-	42	SUCT	-
43	NAGA	-	44	AGAL	-	45	PHOS	+	46	GlyA	-	47	ODC	-	48	LDC	-
49	-		50	-		51	-		52	-		53	IHISa	-	54	-	
55	-		56	CMT	+	57	BGUR	-	58	O129R	-	59	GGAA	-	60	-	
61	IMLTa	-	62	ELLM	+	63	-		64	ILATa	-						

2.3- Molecular identification (PCR and sequencing):

The sequencing of four bacterial 16S rRNA gene were identified, two isolates were identified as *Aeromonas veronii* and the other two isolates were identified as *Staphylococcus epidermidis* and *Acinetobacter lwoffii*. The nucleotide sequences of the 16S rRNA gene of *Aeromonas veronii* (two isolates), *Staphylococcus epidermidis* and *Acinetobacter lwoffii* were submitted to the GenBank sequence database under the accession numbers MT293773, MT293858, MT293804 and MW393763 respectively.

3- Pathogenicity Test Result:

Tilapia fingerlings exposed to *Aeromonas veronii*, *Staphylococcus epidermidis* and *Providencia rettgeri* revealed 20 % mortality rate showing slight hemorrhagic changes all over the body. Whereas with PM examination mortality rate showed slight hemorrhagic changes along the intestinal tract, while control group did not exhibit any mortality.

Table 7: Mortality rates of inoculated bacterial isolates.

Bacterial isolate	Mortality rate
Aeromonas *veronii*	20%
Staphylococcus *epidermidis*	20%
Providencia *rettgeri*	20%
Control Group	0%

DISCUSSION

Mass mortality of fish fries following transportation stress to larger farming ponds is a common phenomenon in aquaculture. Understanding the different etiological factors behind it is critical to boost the productivity and efficiency of fish farms. In this study, the role of prevalent pathogens in this vulnerable fish population in Egypt was investigated. A total of 9 different bacterial pathogens were isolated from the Nile tilapia fries in Kafr El Sheikh which are known for causing mortalities in adult fish.

Water quality was also investigated as a probable cause for mass mortality. In Kafr El Sheikh, fish farming relies on earthen ponds supplied by runoff water from agricultural fields. Four water samples taken over the year revealed high levels of ammonia in sample 1 (spring) and 2 (summer), reflecting the impact of varying fertilizing cycles on the quality of wastewater from the field. The high degree of biochemical contamination is indicated by the respective BOD and COD scores for these samples. Notwithstanding, sampled fish fries did not exhibit asphyxia typical signs of respiratory distress associated with ammonia toxicity. This could be attributed to tilapia's high tolerance of impaired water quality, elevated concentrations of ammonia, and low levels of dissolved oxygen and the dietary inclusion of prebiotic mix- ture (bG & MOS) and fully fermented yeast S. cerevisiae which enhance immune response and disease resistance in the Nile tilapia (**Boyd, 2004; Abu-elala *et al.*, 2018; Abu-elala *et al.*, 2020b)**. However, chronic exposure to high levels of ammonia is known to severely affect fish physiological parameters and growth performance, which, left unchecked, would inevitably impact survival rate of fish fries **(Al Kobaby & Hassanien, 2007; Hegazi, 2011)**.

Compared to tank-based aquaculture and wild water, direct contamination by bacteria from surrounding soils contribute to the high prevalence and diversity of bacteria in earthen ponds.In this study, Aeromonas *sobria*, Aeromonas *hydrophila*, Aeromonas *veronii*, Shigella *sonnei*, Pseudomonas *aeruginosa* and Staphylococcus *epidermidis* were isolated. These have been repeatedly isolated in adult fish farms in Egypt **(David *et al.*, 2009; El-Hady & Samy, 2011; Pridgeon & Klesius, 2012; Zg *et al.*, 2016; El-Barbary & Hal, 2016; Zg, 2017; Atteia *et al.*, 2018).**

In adult tilapia fish, infection by Pseudomonas and Aeromonas bacteria typically causes hemorrhage, detachment of scales, and ulceration **(Lamiaa *et al.*, 2016)**. A. *hydrophila* and Staphylococcus strains are also known to result in high mortality rates in freshwater aquaculture **(Shalaan *et al.*, 2018)**. **Elsheshtawy *et al.* (2019)** have recently linked A. *hydrophila* to mass mortality in the Nile tilapia farms in Kafr El Sheikh.

Providencia *rettegri*, an opportunistic gram-negative bacterium, has been rarely isolated in fish farms in Egypt in recent years. In the literature, **Faisal *et al.* (1987)** were able to first isolate *P. rettegri* in Edfina, Egypt using the API-20E system following summer mass mortality of the Nile tilapia in the mid-1980s. In this study, success in isolating *P. rettegri* is related, in part, to using the Vitek2 system which offers an extensive identification menu and significantly reduces time for correct microbiological identification, including gram-negative bacteria, with a probability score to gauge reliability **(Nonhoff *et al.*, 2005)**.

P. rettegri affects both man and fish which gives it zoonotic significance. In man, it is an emerging urinary tract pathogen, mostly in older catheter-using patients, but it has also been implicated as the causative agent of gastrointestinal illness, traveler's diarrhea, ocular infection; and it is often resistant to multiple antibiotics **(Muller, 1986; Koreishi *et al.*, 2006; Wie, 2015; Sagar *et al.*, 2017)**. In the Nile tilapia, the clinical picture included severe dyspnea and exophthalmia, extremely pale and swollen gills, complete loss of the flight reflex, darkening of the skin color, reddening of the anal region, and lepidarthosis **(Faisal *et al.*, 1987)**. Recently, **De Freitas Souza *et al.* (2019)** demonstrated that *P. rettegri* causes severe oxidative stress in liver and kidney tissues of the Nile tilapia which contributes to pathogenic manifestations. However, it remains unclear whether concomitant stressors and/or immune suppression are necessary for a *P. rettegri* infection to overpower the host and result in full-blown pathogenesis.

In this study, examiners succeeded to isolate another emerging freshwater pathogen, *Shewanella putrefaciens*, an opportunistic gram-negative bacterium that is more commonly found in marine fish and has only been recently described as pathogen of several freshwater fish by **Pezkala *et al.* (2015)**. Diseased fish were reported to suffer from lethargy, swollen abdomen, skin discoloration, and degenerative changes in the kidney, liver, spleen, and intestine **(Altun *et al.*, 2013; Pezkala *et al.*, 2015)**.

El-Barbary (2017) isolated *S. putrefaciens* for the first time in Egypt from cultured adult Nile tilapia near El Manzala lake using API20- NE. In addition, a recent report implicated it in mass mortality of cultured Nile tilapia in India **(Sood *et al.*, 2020)**. This study successfully isolated *S. putrefaciens* from non-diseased tilapia fries in Egypt also using API20NE, suggesting it is more commonly present in freshwater ecosystems as postulated by **Lu and Levin (2010)**. Like *P. rettegri*, *S. putrefaciens* has possible zoonotic potential, having been incidentally implicated in some secondary infections of immunocompromised patients **(Holt *et al.*, 2005)**.

In this study, researchers succeeded to isolate *Acinetobacter lwoffii*, an opportunistic gram-negative bacterium, and diseased fish were reported to suffer from listless, loss of appetite, emergence to the water surface, lethargy ,skin ulcers, haemorrhage around the mouth, congested fins, exophthalmia, opaque lenses and bleeding of internal organs **(Cao *et al.*, 2018)**. It also has a zoonotic effect as it causes

meningitis, bacteremia, peritonitis, pneumonia, endocarditis, gastritis and urinary tract infection **(Rathinavelu *et al.*, 2003; Debarry *et al.*, 2007; Elsayyed *et al.*, 2010)**.

In spite of the presence of the above pathogens, no pathological abnormalities were observed upon clinical examination of sampled fish fries. Lethargy, skin lesions, or signs of sepsis were not present. In addition, inoculation of fish fingerlings with *A. veronii*, *S. epidermidis*, and *P. rettegri* resulted in a maximal mortality rate of only 20 percent after 3 weeks despite the confirmed high pathogenicity of these strains in adult fish.

These findings suggest that, at least in non-stress conditions, pathogenic factors do not independently trigger bacterial mass mortality at this stage of development in juvenile fish. Instead, a combination of factors may contribute to heightened mortality. Overcrowding and transient hypoxia during the transportation process as well as sudden changes in the culture medium from hatcheries to farming ponds may challenge the homeostatic capacity of the tilapia fries, weaken their immune system, and threaten their survival. Transportation stress has clear adverse impacts on physiological parameters like liver enzymes and thyroid hormones (**El-Khaldi, 2010**), while mechanical injury from manipulation and collection also increases chances of fine erosions and lesions that raise susceptibility to secondary infections **(Tørud & Håstein, 2008)**. Further, loss of temperature control in larger farming ponds and shifts in pH of the culture water of each pond, being impacted by the fertilizing cycles of nearby fields, would increase the vulnerability of the tilapia that survive at optimal temperatures of 24-30C **(El Sayed & Kawanna, 2008)** and pH of 7 to 8 **(El-Sherif & EL-Feky, 2008)**.

CONCLUSION

The interplay of multiple co-stressors in the etiology of bacterial mass mortality in fish farms needs to be better recognized. Although the tilapia fish are typically resilient and disease resistant, making it globally the second most cultured fish species **(Ng & Romano, 2013)**, while in the presence of multiple environmental and pathogenic stressors, incidents of mass mortality can occur. This is most evident in the case of opportunistic pathogens. For example, **El-Barbary (2017)** and **Sood *et al.* (2020)** described that low temperatures (15-20 C) in fish farms experience outbreaks of *S. putrefaciens*, while **Faisal *et al.* (1987)** suggested that mature females stressed by countercurrents near the Edfina dam during spawning season were more affected by *P. rettegri* infection.

More generally, reducing environmental stressors could increase resilience to even typical pathogens. **Charo-Karisa *et al.* (2004)** highlighted the role of cold stress in mass mortality of juvenile tilapia and emphasized the importance of size in cold

tolerance. In this context, adequate acclimatization of fries to new culture medium and optimization of rearing pond conditions prior to fries' transfer could prove a key to reduce shock, preserve fish immunity and minimize vulnerability to both typical and opportunistic pathogens.

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ARABIC SUMMARY

دراسة حول مسببات الامراض البكتيرية الشائعة بزريعه اسماك البلطي بمحافظه كفر الشيخ – مصر

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في الآونة الأخيرة عانت تربيه الاحياء المائية في مصر من ارتفاع معدل الوفيات مما ادي الي خسائر اقتصاديه عالية. ترجع معدلات النفوق المرتفعة بأسماك البلطي في الغالب الي مسببات الامراض البكتيرية سواء مع او بدون مسببات الامراض الأخرى. بناءا على تحليل العديد من عينات الزريعة وعينات المياه من محافظه كفر الشيخ، حاولت هذه الدراسة التحقق من العوامل المتعددة المسببة للنفوق الشديد لزريعه اسماك البلطي. بدا العزل والفحص البكتريولوجي باستخدام مجموعه من التقنيات (API NE20, Vitec, PCR and 16SrRNA Sequencing) وكشف عن مجموعه من مسببات الامراض المعروفة بانها تسبب عدوي قاتله لاسماك البلطي البالغة. الأنواع التي تم عزلها بكثره كانت:

- *Aeromonads (A. veronii, A. sobria A. hydrophila)* ٣٣٪
- *Staphylococcus epidermidis* ١١.١٪
- *Pseudomonas aeruginosa* ١١.١٪
- *Shigella sonnei* ١١.١٪

علاوة على ذلك تم عزل ثلاث مسببات للأمراض البكتيرية والتي تنتقل الي الانسان وهي:

- *Providencia rettgeri* ١١.١٪
- *Shewanella putrefaciens* ١١.١٪
- *Acinetobacter lwoffii* ١١.١٪

علي الرغم من مسببات الامراض المعروفه فان حقن اصبعيات الأسماك بمختلف البكتيريا المعزولة ادي الي معدلات نفوق متواضعة في ظل ظروف خاليه من الاجهاد والمؤثرات الخارجية مما يؤكد دور الضغوط البيئية المختلفة والإضافية التي تسبب نفوق جماعي للأسماك الصغيرة.

وبالتأكيد فان هذه العوامل الممرضة لها تأثير سلبي كبير على زريعه اسماك البلطي خاصه عندما تتواجد مع عوامل الاجهاد البيئ.