Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 25(3): 171 – 185 (2021) www.ejabf.journals.ekb.eg



Isolation, Identification and Antimicrobial profile of *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp. from the Nile Tilapia, *Oreochromis niloticus* in fish farms

Hala F. Ayoub^{*1}. Eman Y. Tohamy². Hediat M. salama². Sara S. Mohamed²

1- Department of Fish Health and Management, Central Laboratory for Aquaculture Research, Agricultural Research Center, Abbassa, Abo-Hammad, Sharkia, Egypt

2- Department of Botany and Microbiology, Faculty of science, Zagazig university, Egypt *Corresponding author: Dr.Halakg@hotmail.com

ARTICLE INFO

Article History: Received: Feb. 3, 2021 Accepted: Feb. 25, 2021 Online: May 25, 2021

Keywords:

Nile tilapia; Oreochromus niloticus, pathogenic bacteria, Antimicrobial profile; Aeromonas spp.

ABSTRACT

Nile tilapia (Oreochromis niloticus) aquaculture represents one of the most important cultivation species in Egypt. However, Tilapia farming encounters some problems that result in high fish mortalities and huge economic losses due to bacterial pathogens. Thus, the current study aimed to isolate, identify, and characterize the pathogenic bacteria affecting Nile tilapia farming in El-Abbassa, Abo-Hammad, Sharkia. Besides, antibiotic susceptibility to pathogens was determined as a primary step for controlling diseases. Hence, a collection of 252 fish samples was gathered from 200 fish farms. Upon microbiological and biochemical analysis, the presence of only 3 bacterial genera was detected, including Gram-negative bacteria of Aeromonas spp. (52.80 %), Pseudomonas spp. (27.9 %), and Vibrio spp. (19.3 %). Aeromonas sobria showed the highest prevalence in the infected Oreochromis niloticus. Antibiogram test revealed high levels of resistance expressed by all bacterial isolates to Ampicillin, Amoxicillin, and Erythromycin. On the other hand, Norfloxacin was effective against all isolated bacteria followed by Ciprofloxacin. Establishing effective control methods for pathogenic bacteria would greatly enhance fish production.

INTRODUCTION

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Fish is globally a major source of food and income (**Tacon & Metian, 2013**). Many countries are currently dependant on capture fisheries as the major source of fish. Despite the worldwide increase in fish production, a decline has been observed in several cases (**Noakes** *et al.*, 2000; **Burkhart-Holm** *et al.*, 2002), that has been majorly attributed to over-fishing and climate-related threats.

Tilapia aquaculture is one of the most important aquacultures in fish production in Egypt because of its tolerance to poor water quality and potentiality to feed on a wide range of natural food organisms (**Shaheen** *et al.*, **2013**). Tilapia fish farming is challenged by some problems, among which is the presence of bacterial pathogens that

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leads to high fish mortalities and huge economic losses. Nile tilapia (*Oreochromis niloticus*) aquaculture represents one of the most important cultivation species in Egypt because of its high nutritional qualities, fast growth rate and resistance to diseases (**Mapenzi & Mmochi, 2016**). Nile tilapia production constitutes about 65.15% of Egyptian fish production (**Shalloof, 2020**). However, under intensive farming, tilapia becomes sensitive to several aquatic pathogens such as bacteria, parasites, fungi, viruses and water molds (**Plumb & Hanson, 2010**).

Bacterial pathogens caused by *Aeromonas* species might show high prevalence in farmed Nile tilapia (*Oreochromis niloticus*),compromising its production and causing economic losses (**Subasinghe** *et al.*, **2001; Bondad-Reantaso** *et al.*, **2005**). These bacteria can cause diseases when the fish immunity decreases with the effect of different stressors (**Briede, 2010**). A number of bacterial pathogens have been reported to cause fish diseases worldwide. Bacteria of particular importance in fresh water include *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. (**Zahran** *et al.*, **2016**).

Bacterial infections are often sub-clinical leaving no apparent signs. Under predisposing factors such as; poor water quality, high ammonia as a result of high stocking density and feeding, ectoparasites, inadequate handling and stressful conditions, microorganisms find a portal of entry into the fish host (Moraes & Martins, 2004). There are several studies on disease resistance or experimental infection and fish bacteria identification (Al-Harbi& Uddin, 2004; Cai et al., 2004). Among Pathogenic bacteria that can cause infection in tilapia fish include *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp., Streptococcus spp., Micrococcus spp., Enterococcus spp., Plesiomonas spp., Staphylococcus spp., Moraxellaceae, and Enterobacteriaceae (Zahran et al., 2016). Aeromonas spp. are opportunistic pathogens that can infect both healthy and infected fish (as second invaderfor the latter). They are divided into two fundamental subgroups: the non-motile psychrophilic species (A. salmonicida) and the motile and mesophilic species; namely, A.hydrophila, A.veronii, A.sobria, A.caviae and others. Frequently, several species of the genus Aeromonas causes problems in both feral and cultured fish, causing most financial losses due to high mortality. A profound feedback conserning antibiotic susceptibility of bacteria is crucial for the proper management of the diseases they may cause. The use of antibiotics worldwide in aquaculture and the potential transmission of resistant bacteria between terrestrial and aquatic environments have been reported (Cabello, 2006).

Therefore, the aim of the current study is to isolate and identify some bacterial pathogens from naturally infected Nile tilapia (*Oreochromis niloticus*) with special reference to the best effective antibiotics to control the infection.

MATERIALS AND METHODS

Fish

Two hundred of naturally infected Nile tilapia of different body weights and lengths were randomly collected from El-Abbassa fish farm, Sharkia Governorate. Fish were transferred alive in tanks containing pond water to the microbiological laboratory in the department of fish health and management, central lab for aquaculture research in Abbassa, Sharkia. Fish samples were subjected to clinical, postmortem and bacteriological examinations as described by Noga (2010) and Austin et al. (2012). **Bacteriological examination**

Under aseptic conditions, samples from kidney, liver, spleen, tail and gills were taken from diseased fish. They were inoculated into Tryptic soy broth (Difco) and incubated at 27°C for 24 hours, then, a loop full of broth was streaked onto Tryptic soy agar (TSA) plates and TSA plates with 2% NaCl, and incubated at 27±1°C for 48 hours. For purification purposes, pure colonies were further sub-cultured into Tryptic soy agar. The suspected purified colonies were picked up and streaked over Thiosulphate citrate bile salt agar (TCBS, Oxoid) as a selective diagnostic media. Aeromonas base agar media were supplemented with ampicillin (5 mg/L) and Pseudomonas base agar at 27°C for 48 hours. A loop full of pure culture inoculated on nutrient agar slant was kept for further identification, while another loopful inoculated on semisolid nutrient agar was preserved for testing the motility and preservation. Identification of the isolates was carried out according to Quinn et al. (2002) and Austin & Austin (2012) using the routine study of the morphological character and biochemical reactions as shown in table 1, 2,3 and 4.

Antibiotic sensitivity test

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Oxoid) using the disc diffusion method as described by Hudzicki (2009) Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. Antimicrobial agents tested included the following: Norfloxacin (NOR), Ciprofloxacin (CIP), Amoxicillin (AX), Amikacin (AK), Tetracycline (TE), Novobiocin(NV), Cefixime Nitrofuron (F), Amoxicillin+Clavulanic (AM) and Ampicillin (AM). (CFM), Susceptibility was defined as absence of growth on solid medium containing any of these antimicrobial agents. Presence of growth indicated nonsusceptibility. The diameters of the inhibition zone appearing in the agar plate were measured and interpreted as susceptible (S), intermediate (I) or resistant (R).

RESULTS

Clinical examination

The clinical examination of the collected naturally infected Nile tilapia (Oreochromis niloticus) showed body depigmentation, corneal opacity, frayed fins and tail,

exophthalmia, detachment of scales, body ulceration, and hemorrhages all over the fish body especially at fins and tails. In some cases, the infected fish showed erythema around the mouth and swelling in the abdomen (Fig. 1).



Fig. 1: Naturally infected Nile tilapia showing hemorrhage, scales- loss and fin and tail rot (A), skin- ulceration (B) Corneal opacity, Scales- detachment and Exophthalmia (C) Frayed tail and scales- loss (D). Abdominal swelling (E).

Postmortem examination

Postmortem examination showed that the infected fish suffered from splenomegaly, congestion in gills, enlarged and dark congested kidney, congested liver with distended gall bladder and some cases showed a change in liver color (pale or green) and ascetic fluid which was yellowish in color and watery inconsistency (Fig. 2)



Fig. 2: Postmortem examination showing enlargement of gall bladder, yellowish liver, empty intestine and congestion in kidney, and G, Congested gills and yellowish ascetic fluid.

Isolation and identification of pathogenic bacteria

Two hundred and 252 bacterial isolates were isolated from the naturally diseased fish. These isolates were phenotypically identified following the standard protocol described bacterial isolation and identification. These isolates are identified as *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp.

Aeromonas spp. were identified biochemically as *A. hydrophila*, *A. sobria*, *A. veronei*, *A. cavae*, and *A. jandae*. All the isolated *Aeromonas* species were Gram-negative, short rod, positive for oxidase, motile in semisolid media, produce catalase, and fermentative. The data represented in Table 1 demonstrated the full identification scheme of isolated *Aeromonas* spp.

Pseudomonas spp. were identified biochemically as *P. fluorescens, P. Aeroginosa,* and *P. anguilliseptica*. All isolated *Pseudomonas* spp. were Gram-negative, long curved rods, motile and oxidase-positive as indicated in Table 1.

On the other hand, the *Vibrio* spp. were further identified as *V. harveyi*, *V. vulnificus* and *V. alginolyticus*. All isolated *Vibrio* spp. were Gram negative, curved rods, fermentative, and positive for oxidase, catalase, and methyl red. *V. harveyi and V. alginolyticus* produced yellow colonies on TCBS media while *V. vulnificus* produced green colonies. The complete characterization are represented in Table 1.

In addition to biochemical identification these bacteria were identified by using VITECK 2 system.

Prevalence of bacterial infections among the examined Nile tilapia fish

The prevalence of isolated bacteria are represented in Table 2. The highest prevalence rate of the isolated bacteria from naturally infected Nile tilapia, *Oreochromis niloticus* was Aeromonadaceae (52.80 %) including *A. sobria* (18.48%), *A. veronei* (6.59%), *A. hydrophila* (14.84%), *A. jandae* (8.14%) and *A. cavae* (4.75%) followed by the Pseudomonadaceae (27.9 %) as *P. Aeroginosa* (14.43%), *P. fluorescen* (8.75%), and *P. anguilliseptica* (4.72%). While Vibrionacea has prevalence rate (19.3%) as *V. harveyi* (9.33%), *V. vulnificus* (6.49%) and *V. alginolyticus* (3.48%).

Table (1): The morphological and biochemical characters of isolated Gram-negative bacteria(Aeromonas spp., Pseudomonas spp., and Vibrio spp.) from the examined Nile tilapia fish.

Identification test	A.hydrophila	A. veronü	A.sobria	A.Caviae	A. jandaei	P.aeroginosa	P.fluorescens	P.anguilliseptica	V.alginolyticus	V.harveyi	V.vulnificus
Gram-stain	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+
Shape	Short rod	Short rod	Short rod	Short rod	Short rod	Long curved rods	Long curved rods	Long curved rods	Curved rod	Curved rod	Curved rod
O/F	F	F	F	F	F	0	0	-	F	F	F
Cytochrom oxidase	+	+	+	+	+	+	+	+	+	+	+
Growth on 0%NaCl	+	+	+	+	+	+	+	+	+	-	+
Growth at 5oC	+	-	+	+	-	-	+	+			
6% NaCl	-	-	-	-	-	+	+	-	+	+	+
3.5% NaCl	+	+	-	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
Indol	+	+	+	+	+	-	-	-	+	+	-
H_2S (TSI)	+	-	-	-	-	-	-	-	+	-	-
Citrate	+	+	+	+	+	+	+	+	-	+	+
Methyl red	+	V	V	-	+	-	\mathbf{V}	+	+	+	+
Gelatin liquefaction	+	+	V	+	-	+	+	+	V	-	+
Vogaus proskauer	+	+	-	-	+	-	-	-	+	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-			
Urease	-	-	-	-	-	+	+	-			
Arginie dehydrogenase	+	V	-	+	-	+	+	+	+	-	-
Lysin decarboxylase	+	+	+	-	+	-	-	-	+		-
Ornithen decarboxylase Acid production	-	+	-	-	+		-		+	-	+
from											
Arabinose	+	-	-	+	-	-	+	-	-		-
Sucrose	+	+	+	+	-	-	+	-	+	+	-
Salicin	V	+	-	+	-		-	-	-		+
Maltose Mantinol	+	+	+	-	+	+	-	+	+	+	-

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Isolate	No. of isolates	%
<u>Aeromonas spp</u>	170	52.80
A. sobria	46	18.48
A. hydrophila	40	14.84
A. veronei	37	6.59
A. jandae	29	8.14
A. cavae	18	4.75
<u>Pseudomonas spp</u>	50	27.9
P. Aeroginosa	23	14.43
P. fluorescens	18	8.75
P. anguilliseptica	9	4.72
<u>Vibrio spp</u>	32	19.3
V. harveyi	15	9.33
V. vulnificus	10	6.49
V.alginolyticus	7	3.48
Total	252	100

Table (2): The Prevalence of isolated bacteria from naturally infected Nile Tilapia, *Oreochromis niloticus*.

Incidence of isolated bacteria in the organs and tissues

As shown in Table (3), the most occurrence of the isolated bacteria was from the liver (37.3%) followed by the kidney (25.8%), spleen (15.1%), gills (10.3%), tails (7.38%), and skin ulcer (4.12%).

Table (3): Incidence of isolated bacterial from the examined organs of Nile tilapia fish

	Aeron	nonas sp.	Pseudomonas sp.		Vibrio sp.		Total		
Organ	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	
Tail	9	3.73	6	7.14	4	10.0	19	7.38	
Skin ulcer	4	4.90	3	3.48	1	6.67	8	4.12	
Kidney	48	29.0	13	26.9	7	20.0	68	25.8	
Liver	73	48.1	12	50.0	9	23.3	94	37.3	
Spleen	20	12.9	13	9.16	5	26.6	38	15.1	
Gills	16	10.7	3	7.14	6	13.3	25	10.3	
Total	170	46.7	50	31.9	32	21.4	252	100	

Antibiotic sensitivity test

The isolated bacterial species which obtained from naturally infected, *O. niloticus*. were tested for susceptibility to antibiotics as indicated in Table 4. High levels of resistance were expressed by all isolates to Ampicillin, Amoxicillin, and Cefixime. On the other hand, Norfloxacin was the drug of choice against all isolated bacteria followed by Ciprofloxacin that was effective against most bacterial isolates under study.

Antibiotic tested	NOR	CIP	AX	Ak	F	CFM	TE	AM	NV	AMX
Concentration	10 mg	5 mg	25 µg	30 mg	300µg	15µg	30 µg	10 µg	30 mg	25 µg
Zone diameter (R)	≥ 12	≥ 15	≥11	≥ 14	≥14	≥15	≥11	≥13	≥13	≥11
Interpretation (I)	13-16	16-20	12-13	15-16	15-16	16-18	12-14	14-16	14-18	12-13
Standards (mm) S	≤ 17	≤ 21	≤14	≤ 17	≤ 17	≤19	≤15	≤ 17	≤19	≤ 14
A. hydrophila	19(S)	24 (S)	18(S)	18(S)	22(S)	23(S)	28(S)	0(R)	12(R)	10(R)
A. veronei	24(S)	29(S)	14(S)	13(R)	19(S)	19(S)	25(S)	0(R)	9(R)	0(R)
A. sobria	31(S)	31(S)	15(S)	17(S)	20(S)	21(S)	19(S)	0(R)	0(R)	7(R)
A. caviae	29(S)	28(S)	17(S)	21(S)	18(S)	19(S)	18(S)	0(R)	19(S)	9(R)
A. jandae	24(S)	22(S)	16(S)	26(S)	17(S)	24(S)	16(S)	0(R)	22(S)	0(R)
P. Aeroginosa	21(S)	15(R)	13(R)	21(S)	13(R)	20(S)	11(R)	0(R)	11(R)	8(R)
P. fluorescens	18(S)	24(S)	17(S)	24(S)	20(S)	13(R)	9(R)	0(R)	0(R)	6(R)
P. anguilliseptica	19(S)	21(S)	22(S)	0(R)	12(R)	15(R)	0(R)	0(R)	7(R)	0(R)
V. vulnificus	18(S)	26(S)	20(S)	20(S)	13(R)	21(S)	17(S)	0(R)	0(R)	10(R)
V. harveyi	24(S)	29(S)	15(S)	21(S)	18(S)	14(R)	8(R)	9(R)	11(R)	0(R)
V. alginolyticus	20(S)	21(S)	14(S)	17(S)	21(S)	11(R)	11(R)	0(R)	10(R)	0(R)

Table (4): Antibiotic sensitivity test

DISCUSSION

Knowledge about prevalent etiological agents and treatment options is necessary for proper management and control of diseases in aquaculture. The current study presented an isolation and identification of different bacteria infecting *O. niloticus*. Moreover, antibiograms of pathogenic bacteria were used for cultured tilapia in Egypt. However, Nile tilapia is considered an important economically cultured teleost fish (FAO, 2016). Recently, the use of phytogenic products in practical diets for fish has become a very topical concept in aquaculture (Gatlin III *et al.*,2007). Besides the use of immunostimulants in fish diets is important in aquaculture and it should be looked at as immunity and growth promotor. Fish is susceptible to several bacterial diseases especially under stress conditions (Eissa *et al.*, 2015;Dong *et al.*, 2017).

In the present study, there are various genera and species among the identified bacteria. Some of these bacteria are common pathogens that lead to high mortalities in fish elsewhere. Naturally, infected *O. niloticus* shows body depigmentation, hemorrhagic skin, corneal opacity, detachment of scales, body ulceration ,frayed fins and tail,

exophthalmia, hemorrhages over the fish body, especially at tails and fins, erythema around the mouth and swelling of the abdomen. Postmortem examination showed congestion of the internal organs and presence of ascetic fluid. These abnormalities are similar to the results that obtained by **Pretto-Giordano** *et al.* (2010) and Hassan *et al.* (2020) who observed signs such as anorexia, exophthalmia, skin alterations, corneal opacity, an extension of the visceral cavity, bleeding, and abdominal inflammation, splenomegaly and hepatomegaly. Soto (2009) reported that the bacteria are the main cause of splenomegaly, epithelial hyperplasia in gills and necrosis in internal organs mainly in kidney, liver, spleen, brain and heart.

The bacteriological examination of infected fish showed the isolation of Gramnegative bacteria with the predominance of *Aeromonas* spp. at 52.80 %. These results are similar to some studies who reported that *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp were responsible for the fatal outbreak of fish (**Daskalov**, **2006**; **Najiah** *et al.*, **2012**). These results are also in accordance with those reported by **El-Gamal** *et al.* (**2018**) who revealed that the isolated bacteria, from naturally infected Nile tilapia from different fish farms at Kafr El-Sheikh Governorate in Egypt, were *Aeromonas* spp. by 26%, *Pseudomonas* spp. by 23.3%, and mixed infections were 36.6% and are responsible for ulcerative syndrome. This indicated that the outbreak is mainly attributed to the same genera as previously. Therefore, controlling these strains is pending necessary to prevent further loss of fish culture. A previous study in Indonesia by Hardi *et al.* (**2018**) reported that the Gram-negative bacteria are the most predominant bacteria found in cultured tilapia. They have isolated seven bacterial genera from tilapia and catfish (*Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Aeromonas* sp., *Neisseria* sp. and *Listeria* sp.).

In the present study, *Aeromonas* spp. were the predominant pathogenic bacteria followed by genus *Pseudomonas* spp. **Austin & Austin(2012)** reported that *Aeromonas* spp. infect fishes and are responsible for the Motile *Aeromonas* Septicemia (MAS) disease in fish or epizootic ulcerative syndrome (EUS). Also, some researchers reported that *Pseudomonas* spp. (*P. fluorescens, P. aeruginosa, P. putida, and P. angulliseptica*) are the causative agents of *Pseudomonas* septicemia in various species of fish (**Eissa** et al. 2010; El-Nagar 2010).

Aeromonas sobria, and *Aeromonas hydrophila*, which were isolated from several cases involved highly virulent strains (**Nielsen** *et al.***2001; Hemstreet 2010**). *Aeromonas* spp. were the most common bacteria isolated in this study with about 14.68% of the fish farms infected with *A. hydrophila* and 15.38% with *A. sobria*. Indeed, similar findings have been reported from the same study area (**Joh** *et al.***2013; Walakira** *et al.***2014**). Other researchers have also reported similar findings (Newaj-Fyzul *et al.*, 2008; Ribeiro *et al.***2010**) and this could be attributed to the ubiquitous nature of *Aeromonas* spp. in the

aquatic environment, similarity of production systems and fish species involved in the different studies.

The current study showed that the incidence of isolated bacteria from examined organs of fish was higher in the liver followed by kidney and spleen. Mahmoud et al. (2016) also reported that bacterial isolates distribution in different organs and tissues of the examined fishes were presented mainly in the liver, kidney, and spleen. Elsayed et al. (2018) have reported that the kidney and the liver were the most predominant organs for isolation of bacteria at a rate of 37.4% and 36.3%, respectively, followed by spleen (15.9%) and gills (9.85%). Eissa et al. (2016) reported that Aeromonas sobria isolates were isolated with the high prevalence rate from kidneys (25.3%), liver (23.0%), spleen (19.8%), and intestines (15.0%), while the lowest prevalence was recorded from gills and skin lesions at rates of 10.3% and 6.35%, respectively. Eissa et al. (2010) also recorded that the prevalence of *Pseudomonas* sp. in organs of Nile tilapia was mainly from the liver (35%) and kidney (30%) followed by spleen (21.2%) and gills (13.7%). El-Sayed (2019) also reported that the prevalence of V. alginolyticus was high in liver at rate 38.5% followed by kidney (29.2%), spleen (23%) and heart (9.2%) from naturally infected O. niloticus. In this study, its prevelance was mainly from kidney and spleen, but was not isolated from liver and skin ulcer. While Osman et al., (2017) found that Streptococcus spp. occurred mainly in 8/80 (10%) in liver samples followed by 4/80 (5%) in spleen samples, 3/80 (3.8%) in kidney samples and 1/80 (1.3%) in brain and ascitic fluid samples in Nile tilapia. This result is different from that recorded by Ali (2014) who reported that Staphylococcus spp. was most commonly detected in skin (35.5%, 36.8%), livers (25.8%, 25%), intestines (21%, 17.60%), muscles (17.7%, 20.6%) of Cyprinus carpio and Silurus glanis, respectively.

The results of sensitivity tests revealed that the most tested strains were sensitive to Norfloxacin or Ciprofloxacin. Therefore, these antibiotics can be used for the treatment of infected fishes against these bacteria. On the other hand, high levels of resistance were expressed by all isolates to Ampicillin, Amoxicillin, and Cefixime indicating that these antibiotics are improper to treat fishes infected with pathogenic bacteria. Similarly, **El-Barbary and Hal (2016)** reported that Ciprofloxacin, Norfloxacin, and Gentamycin could be used to treat fish infected with *A. hydrophila*, *P. fluorescens*, *P. putida*, and *Klebsiella oxytoca*.

CONCLUSION

From obtained results, we concluded that the Gram-negative bacteria were the main cause of diseases and mortality in Nile tiapia. *A.sobria* exhibited the highest prevalence in naturally infected Nile tilapia. The bacterial infection occurs mainly in the liver and kidney of infected fish. Norfloxacin was the drug of choice to treat fish infected with bacterial pathogens. Further investigations on the safe effective supplements, alternatives

to antibiotics, for stimulating non-specific immune response and antioxidant activity against bacterial pathogens should be recommended.

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الملخص العربى

عزل وتصنيف بكتريا .Aeromonas spp., Pseudomonas spp و .Vibrio spp من البلطي النيلي ومعرفة مدى قابليتها للمضادات الحيوية في المزراع السمكية

هالة فؤاد ايوب 1 ، ايمان يوسف التهامي 2 ، هديات محمد سلامة 2 ، سارة سليمان محمد 2

أقسم صحه الاسماك ورعايتها - المعمل المركزي لبحوث الثروة السمكية 2قسم النبات و الميكروبيولوجي، كلية العلوم ، جامعة الزقازيق

هدف هذة الدراسة هو عزل وتصنيف Aeromonas spp., Pseudomonas spp., Vibrio spp. من اسماك البلطي النيلي، تم تجميع عدد 200 عينة من سمكة البلطى النيلي التي بالفعل تحمل اعراض مرضية وتم فحصها كلينيكا وتشريحيا وتم عمل الفحوصات البكتيرية وكذلك التاكد بجهاز الفيتك وتم عمل اختبار الحساسية للمضادات الحيوية للبكتريا كخطوة اولية في السيطرة علي المرض ونتج عن ذلك عدد 252 عزلة بكتيرية من الكبد، الكلي، الطحال ، الخياشيم والتقرحات الجلدية. تم تصنيف انواع البكتريا المعزولة الي . Aeromonas spp. بنسب الطحال ، الخياشيم والتقرحات الجلدية. تم تصنيف انواع البكتريا المعزولة الي . ورودي اليم انظهر ان الليروموناس سوبريا تمثل اعلي نسبة (27.9%) و. Vibrio spp. بنسبة (19.3%) . بينما اظهر ان الإيروموناس سوبريا تمثل اعلي نسبة انتشار في البلطى المصاب تم عمل اختبار الحساسية للمضادات الحيوية والتي اظهرت مستويات عالية من المقاومة عبرت عنها جميع العز لا للأمبيسيلين والأموكسيسيلين والإريثروميسين. بينما كان النور فلوكساسين فعالا ضد جميع البكتريا المعزولة تليها سيبر وفلوكساسين