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Prevalence and Antibiotic Susceptibility of Bacterial Pathogens Implicating the Mortality of Cultured Nile Tilapia, *Oreochromis niloticus*

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Abstract

Nile-Tilapia (*Oreochromis niloticus*) aquaculture represents one of the most important cultivation species in Egypt. However, Tilapia fish farming is challenged by some problems. Of those, the presence of bacterial pathogens resulting in high fish mortalities and huge economic losses. Thus, the current investigation aimed to isolate, identify, and characterize the pathogenic bacteria from Nile tilapia fish farm in El-Abassa village, Egypt and to investigate their antibiotic susceptibility as a primary step for controlling diseases. 182 bacterial isolates were obtained from one hundred Tilapia fish samples. The microbiological and biochemical analysis of the examined fish indicated the presence of only 5 bacterial genera. Three of them are Gram-negative bacteria (representing 86.26% of total isolates) including *Aeromonas* spp. (46.70 %), *Pseudomonas* spp. (23.08 %), and *Vibrio* spp. (16.48 %). While two genera are Gram-positive bacteria (representing 13.74% of the total isolates) including *Streptococcus* spp. (8.79 %) and *Staphylococcus* spp. (4.95 %). This indicates that Gram-negative bacteria are the main cause of high fish mortalities in the studied area while *Aeromonas hydrophila* exhibited the highest prevalence in infected tilapia. Antibiogram test revealed high levels of resistance expressed by all isolates to ampicillin, amoxicillin, and erythromycin. On the other hand, norfloxacin was effective against all isolated bacteria followed by ciprofloxacin; therefore, norfloxacin should be

recommended as a supplement in fish fed-diets to control the bacterial infection. Establishing effective control methods for pathogenic isolates would greatly enhance fish production.

Keywords: Fish mortality; Nile tilapia; *Oreochromus niloticus*, pathogenic bacteria; sensitivity test; *Aeromonas* spp.

INTRODUCTION

Tilapia aquaculture is one of the most important aquacultures in fish production in Egypt because of its tolerance to poor water quality and can fed on a wide range of natural food organisms (Shaheen *et al*., 2013). Amongst them, Nile-tilapia *(Oreochromis niloticusis)* is of the most important freshwater species for commercial aquaculture because of its high nutritional qualities, fast growth rate and resistance to diseases (Mapenzi and Mmochi 2016). Egypt is the second-largest producer of farmed tilapia next to china (FAO, 2019). Nile tilapia production in Egypt contributes about 65.15% of Egyptian fish production (GAFRD, 2017). However, under intensive farming, tilapia becomes sensitive to several aquatic pathogens such as bacteria, fungi, parasites, viruses and water molds (Plumb and Hanson, 2010).

Bacteria are the main pathogens of cultured warm water fish involved in great losses to the aquaculture industry elsewhere. Many bacteria are considered to be saprophytic that exists in a commensal association with the host or live free in the environment while others are opportunistic. These bacteria can cause diseases when the immunity of fishes gets decreased by the effect of different stressors (Briede, 2010). Some of the 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 hydrophila, Pseudomonas fluorescens* and *Vibrio anguillarum* were the most predominant bacteria that cause infection in fish farms and contributed to the *O. niloticus* seasonal summer mortalities (Abd El-Kader and Balabel, 2017).

Bacterial infections in fish may occur as bacteremia which means that the bacterial organisms are existent in the blood-stream with no clinical signs or occur as septicemia which implies that bacteria and toxins are indeed present in the circulatory system and always cause disease and clinical signs. Hemorrhage, inflammation, and necrosis are clinical signs associated with septicemia (Briede, 2010). *Aeromonas hydrophila*, a widely distributed in aquatic environments, is a causative agent of motile

Aeromonas septicemia (MAS) (Pridgeon *et al*., 2011). The infected fish showed loss of appetite, loss of equilibrium, scales loss, sluggish swimming at the water surface, exophthalmia, skin erosions and ulcer, fin and tail rot, enlarged abdomen with ascites and vent was prolapsed. Gills might be congested or pale and anemic and covered with excessive mucus. The internal organs may friable and showed a generalized hyperemic appearance (Hassan *et al*., 2011).

Therefore, the current investigation aimed to isolation and identification of bacterial pathogens from naturally infected Nile tilapia (*Oreochromis niloticus*) with special reference to the best effective antibiotics for controlling the infection.

MATERIAL AND METHODS

Naturally infected fish

A hundred of naturally infected tilapia of different body weights and lengths were randomly collected from El-Abassa Fish Farm, Sharkia Governorate. The fish were transferred alive in tanks containing pond water to the microbiological lab in the Fish Diseases Department, Central Lab for aquaculture Research in Abbassa, Sharkia. All collected samples were subjected to clinical, postmortem and bacteriological examinations as described by Austin and Austin, (2012) and Noga, (2010).

Bacterial isolation

Under complete aseptic conditions, the specimens of different tissues and organs (skin ulcer, tail, gills, liver, kidneys, and spleen) from diseased fish were inoculated into Tryptic soy broth (Difco) and incubated at 27ºC for 24 h. Then loopful of broth were streaked onto Tryptic soy agar (TSA) plates and TSA plates with 2% NaCl and incubated at 27±1ºC for 48 h. For purification purposes, pure colonies were further sub-cultured into Tryptic soy agar. Each type of culture colony was picked up and sub-cultured on a selective diagnostic agar media (Thiosulphate citrate bile salt agar (Oxoid) (TCBS), *Aeromonas* base agar media supplemented with ampicillin (5 mg/L) and *Pseudomonas* base agar at 27ºC for 48 h. Then pure colonies were transferred onto TSA slant for further identification that carried out according to Austin and Austin, (2012) and Quinn *et al*., (2002).

Bacterial Identification

The obtained bacterial isolates were subjected to full identification by using colonies characteristics, Gram stain, and biochemical activities as previously described (Austin and Austin, 2012; Quinn *et al*., 2002).

Antibiotic sensitivity test

Sensitivity tests for isolated bacteria to eight types of commercial antibiotic, namely: norfloxacin (NOR), ciprofloxacin (CIP), gentamicin (GN), amoxicillin (AMX), amikacin (AK), rifamycin (RF), erythromycin (E) and ampicillin (AM) were performed on Mueller-Hinton agar (Oxoid) using the disc diffusion method according to the National Committee of Clinical Laboratory Standards (NCCLS) (2003). The diameters of the inhibition zone appearing in the agar plate were measured and interpreted as susceptible (S), intermediate (I) or resistant (R).

RESULTS

Examination of naturally diseased fish

Clinical picture

The clinical picture of the collected naturally infected Nile tilapia (*Oreochromis niloticusis*) fish showed anorexia, body depigmentation, frayed fins and tail, corneal opacity, exophthalmia, body ulceration, detachment of scales, hemorrhages all over the fish body especially at fins and tails. In some cases, the infected fish showed erythema around the mouth and swelling of the abdomen (Figure 1).

Postmortem picture

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

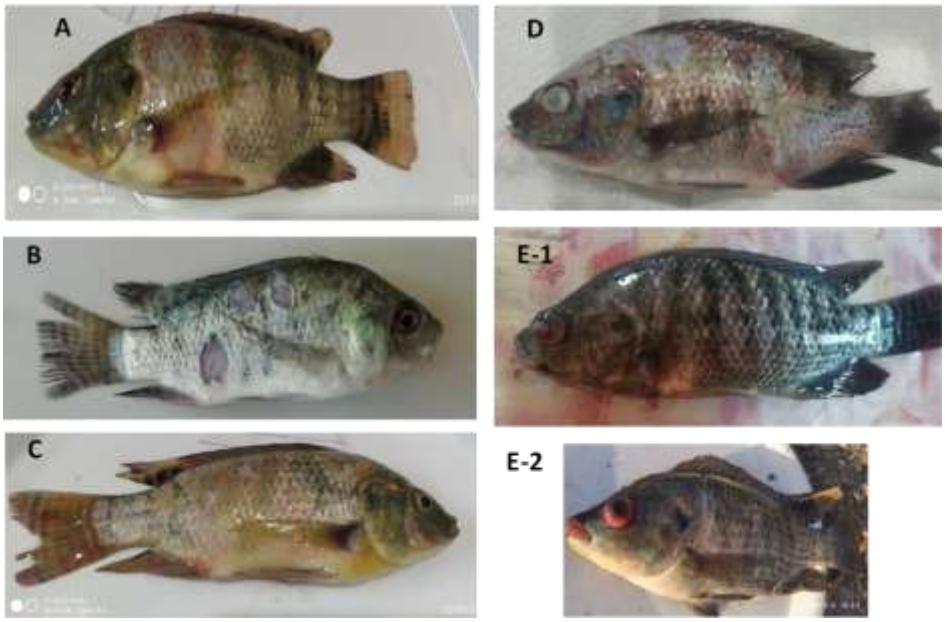
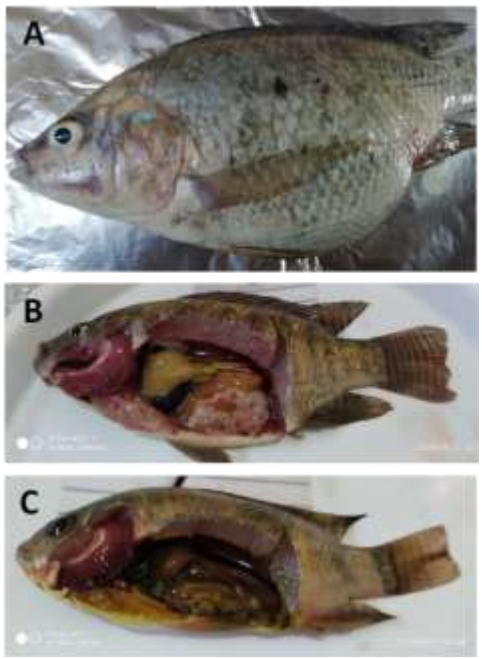


Figure (1): Naturally infected fish showing hemorrhage, scales loss and fin and tail rot (A), skin ulceration (B); Frayed tail and scales loss (C); Corneal opacity and Scales detachment (D) and Exophthalmia (E-1&2).

Figure (2): A, naturally infected Tilapia showing abdominal swelling, B, Postmortem examination showing enlargement of gall bladder, yellowish liver, empty intestine and congestion in kidney, and C, Congested gills and yellowish ascetic fluid.



Isolation and identification of pathogenic bacteria

One hundred and eighty-two bacterial isolates were isolated from the infected fish. These isolates were phenotypically identified following the standard protocol described bacterial isolation and identification. Out of these isolates, 157 isolates (86.26%) were Gram-negative bacteria. Those are identified as *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp. Ther other 25 isolates at rate of (13.74 %) of total isolated strains were Gram-positive bacteria that identified as *Streptococcus* spp. and *Staphylococcus* spp.

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

Pseudomonas spp. were identified biochemically to *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 was shown in Table (1).

As shown in Table (2), *Streptococcus* spp. were cocci, non-motile, oxidase negative, O/F fermentative, catalase negative, can grow in media with 3.5 and 6% NaCl but not grow in media with 8% NaCl, hydrolyzed starch, negative for methyl red and Vogaus Proskauer. They cannot utilize citrate as a sole carbon source and produce acid from sucrose, glucose, maltose and fructose but not from inositol, and variable for arabinose, salicin, mannitol and lactose. While *Staphylococcus* spp. as showed in Table (2), were cocci, non-motile, oxidase negative, oxidase negative, O/F oxidative, catalase positive, can grow in media with 3.5, 6 and 8% NaCl, cannot hydrolyze starch, positive for methyl red and Vogaus Proskauer. They can utilize citrate as a sole carbon source, positive for gelatin liquefaction and produce acid from sucrose, glucose, maltose, mannitol, lactose and fructose but not from arabinose, salicin, and inositol.

Prevalence of bacterial infections among the examined Nile tilapia fish

From the result in Figure (3), the prevalence of isolated bacteria was as the following. The highest prevalence rate of the isolated bacteria from naturally infected Nile tilapia fish was *Aeromonadaceae* (46.70 %) as *A. hydrophila* with prevalence rate (16.48%), *A. veronei* (14.84%), *A. sobria* (6.59%), *A. jandae* (6.04%) and *A. cavae* with prevalence rate (2.75%). followed by the *Pseudomonadaceae* (23.08%) as *P. fluorescen* with prevalence rate 23.08%, *P. Aeroginosa* (10.44%), and *P. anguilliseptica* (8.79%). While *Vibrionacea* has prevalence rate 16.48 % as *V. harveyi* at (7.14%), *V. vulnificus* at (5.49%). and *V. alginolyticus* at (3.85%). On the other hand, the prevalence of *Streptococcus* was (8.79) % and the *Staphylococcus* have lowest prevalence rate at (4.95 %).

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 (15.3 %), gills (10.4 %), tails (7.14 %), and skin ulcer (3.84 %).

Sensitivity of the pathogenic isolates to antibiogram

The sensitivity of the isolated bacterial species, obtained from naturally infected fishes in this study, to different antibiotics was evaluated as indicated in Table (4). As showed, high levels of resistance were expressed by all isolates to ampicillin, amoxicillin, and erythromycin. On the other hand, norfloxacin was drug of choice against all isolated bacteria followed by ciprofloxacin that is effective against most bacterial isolates under study.

DISCUSSION

Nile tilapia is the main cultured fish species in Egypt. Tilapia fish are susceptible to several bacterial diseases under stressed conditions (Dong *et al*., 2017; Eissa *et al*., 2015). The present study was carried out to isolate and identify the causative agent of fish diseases and mortalities outbreak in tilapia fish in farming culture in Egypt.

Naturally infected Nile tilapia fish showing hemorrhagic skin, body depigmentation, frayed fins and tail, corneal opacity, exophthalmia, body ulceration, detachment of scales, hemorrhages over the fish body especially

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	***Aeromonas* spp.**					***Pseudomonas* spp.**			***Vibrio* spp.**		
	A. hydrophila	***A. sobria***	***A. veronii***	***A. jandaei***	***A. cviae***	***P. fluorescens***	***P. aeroginosa***	***P. anguilliseptica***	***V.harveyi***	***V.vulnificus***	***V.alginolyticus***
Gram-stain	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Shape	Short rod	Short rod	Short rod	Short rod	Short rod	Long curved rods	Long curved rods	Long curved rods	curved rods	curved rods	curved rods
Motility	+	+	+	+	+	+	+	+	+	+	+
Cytochrom oxidase	+	+	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	O	O	-	F	F	F
Growth on TCBS									Yellow colonies	Green colonies	Yellow colonies
Growth at 5ºC	+	+	-	-	+	+	-	+			
Growth on 0% NaCl	+	+	+	+	+	+	+	+	+	-	-
3.5 % NaCl	+	-	+	+	+	+	+	+	+	+	+
6% NaCl	-	-	-	-	-	+	+	-	+	+	+
8% NaCl									-	+	+
10% NaCl									-	-	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
H_2S (TSI)	+	-	-	-	-	-	-	-	-	-	+

Table (1): Continued.

Identification Test	***Aeromonas* spp.**					***Pseudomonas* spp.**			***Vibrio* spp.**		
	A. hydrophila	***A. sobria***	***A. veronii***	***A. jandaei***	***A. cviae***	***P. fluorescens***	***P. aeroginosa***	***P. anguilliseptica***	***V. harveyi***	***V. vulnificus***	***V. alginolyticus***
Indol	+	+	+	+	+	-	-	-	+	-	+
Urease	-	-	-	-	-	+	+	-			
Starch hydrolysis	-	+	v	-	+	-	-	-			
Methyl red	+	v	v	+	-	v	-	+	+	+	+
Vogaus proskauer	+	-	+	+	-	-	-	-	-	-	+
Citrate	+	+	+	+	+	+	+	+	+	+	-
Gelatin liquefaction	+	v	+	-	+	+	+	+	-	+	v
Ornithen decarboxylase	-	-	+	+	-	-			-	+	+
Lysin decarboxylase	+	+	+	+	-	-	-	-		-	+
Arginie dehydrogenase	+	-	v	-	+	+	+	+	-	-	+
Acid production from											
Arabinose	+	-	-	-	+	+	-	-	-	-	-
Salicin	v	-	+	-	+	-		-	-	+	-
Sucrose	+	+	+	-	+	+	-	-	+	-	+
Inositol	-	-	-	-	-	-	-	-	+	-	-
Glucose	+	+	+	+	-	+	-	-	+	+	+
Maltose	+	+	+	+	+	-	+	+			
Mannitol	+	+	+	+	+	+	-	-	+	-	+
glycerol	+	+	+	+	v						
lactose									-	v	-

Table (2): The morphological and biochemical characters of isolated Gram-positive bacteria from the examined Nile tilapia fish.

Identification Test	***Staphylococcus sp.***	***Streptococcus sp.***
Gram-stain	+ve	+ve
Shape	Cocci	Cocci
Motility	-	-
Cytochrom oxidase	-	-
O/F	O	F
Growth at 5ºC	+	-
Growth on 0.0% NaCl	+	+
3.5% NaCl	+	+
6% NaCl	+	+
8% NaCl	+	-
Catalase	+	-
H_2S (TSI)	-	-
indole	-	-
Urease	+	-
Starch hydrolysis	-	+
Methyl red	+	-
Vogaus proskauer	+	-
Citrate	+	-
Gelatin liquefaction	+	-
Heamolysis	β	β or γ
Alkaline phosphotase	+	+
Ornithen decarboxylase	-	+
Lysin decarboxylase	-	-
Arginie dehydrogenase	+	+
Acid production from:		
Arabinose	-	v
Salicin	-	v
Sucrose	+	+
Inositol	-	-
Glucose	+	+
Maltose	+	+
Mannitol	+	v
Lactose	+	v
Fructose	+	+

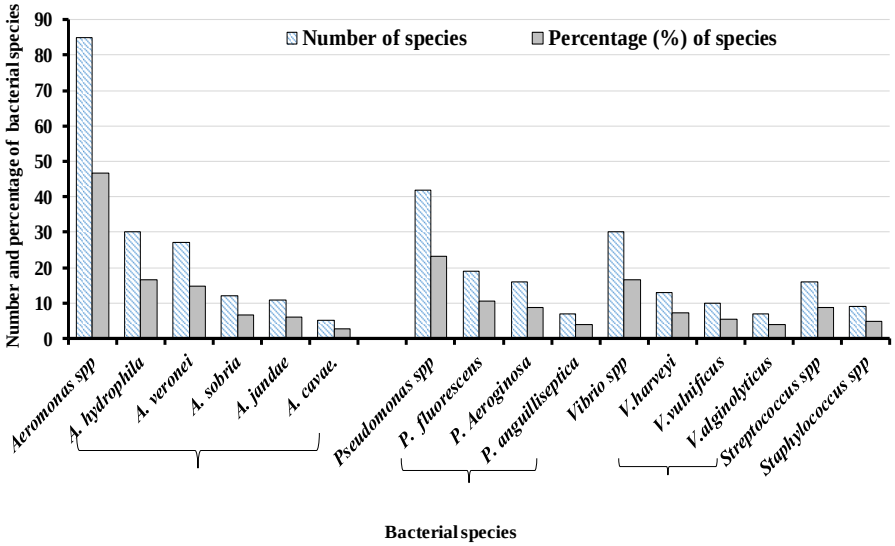


Figure (3): Prevalence of isolated bacteria in the examined fish

at fins and tails, erythema around the mouth and swelling of the abdomen. Postmortem examination showing congection of the internal organs and presence of ascetic fluid. These abnormalities are similar to that were reported by Pretto-Giordano *et al*., (2010) and El-Son, (2016) who observed signs such as anorexia, exophthalmia, skin alterations, an extension of the visceral cavity, corneal opacity, 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, musculature, heart, and brain.

In this study, the bacteriological examination of infected fishes showed the isolation of three Gram-negative bacteria at 86.26% of the total isolated strains with the predominance of *Aeromonas* spp. at 46.70 %. Besides, two Gram-positive bacteria at 13.74% were isolated with the dominance of *Streptococcus* spp. at 8.79 %. These results are following some researches who reported that *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp., *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp. and *Enterobacteriaceae* were responsible for the fish fatal outbreak (Daskalov, 2006: Najiah *et al* 2012). El-Gamal *et al*., (2018) also reported that the bacteria isolated from naturally infected *O. niloticus* from different fish farms at Kafr El-Sheikh Governorate, Egypt, were *Aeromonas* spp. by 26%,

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

	Aeromonas sp.		***Pseudomonas sp.***		***Vibrio sp.***		***Streptococcus sp.***		***Staphylococcus sp.***		**Total**	
Organ	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Skin ulcer	3	3.53	1	2.38	2	6.67	1	6.25	0	0.00	7	3.84
Tail	4	4.70	3	7.14	3	10.0	2	12.5	1	11.1	13	7.14
Liver	35	41.1	21	50.0	7	23.3	5	31.2	0	0.00	68	37.3
Kidney	23	27.0	11	26.1	6	20.0	2	12.5	5	55.5	47	25.8
Spleen	11	12.9	3	7.14	8	26.6	4	25.0	2	22.2	28	15.3
Gills	9	10.5	3	7.14	4	13.3	2	12.5	1	11.1	19	10.4
Total	85	46.7	42	23.08	30	16.4	16	8.79	9	4.95	182	100

Table (4): Antibiogram sensitivity test.

Antibiotic tested		**NOR**	**CIP**	**GN**	**Amx**	**Ak**	**RF**	**E**	**AM**
Concentration		**10 mg**	**5 mg**	**10 µg**	**25 µg**	**30mg**	**30 µg**	**15 µg**	**10 µg**
Zone diameter interpretation Standards (mm)	**R**	**≥ 12**	**≥ 15**	**12**	**≥11**	**≥ 14**	**≥ 16**	**≥13**	**≥13**
	I	**13-16**	**16-20**	**13-14**	**12-13**	**15-16**	**17-19**	**14-22**	**14-16**
	S	**≤ 17**	**≤ 21**	**15**	**≤ 14**	**≤ 17**	**≤ 20**	**≤ 23**	**≤ 17**
A. hydrophila		18(S)	25 (S)	17 (S)	10(R)	19(S)	13(R)	0(R)	0(R)
A. veronei		24(S)	27(S)	19(S)	0(R)	13(R)	17(I)	0(R)	0(R)
A. sobria		32(S)	29(S)	14(I)	6(R)	18(S)	16(R)	11(R)	0(R)
A. jandae		24(S)	22(S)	18(S)	0(R)	26(S)	18(I)	13(R)	0(R)
A. caviae		27(S)	28(S)	15(S)	10(R)	21(S)	14(R)	0(R)	0(R)
P. fluorescens		18(S)	22(S)	13(I)	7(R)	23(S)	9(R)	7(R)	0(R)
P. Aeroginosa		20(S)	15(R)	16(S)	8(R)	21(S)	17(I)	23(S)	0(R)
P. anguilliseptica		19(S)	21(S)	19(S)	0(R)	0(R)	15(R)	19(I)	0(R)
V. harveyi		24(S)	28(S)	16(S)	0(R)	21(S)	20(S)	10(R)	7(R)
V. vulnificus		17(S)	26(S)	20(S)	9(R)	19(S)	16(R)	9(R)	0(R)
V. alginolyticus		20(S)	22(S)	17(S)	0(R)	17(S)	23(S)	0(R)	0(R)
***Streptococcus* spp.**		21(S)	13(R)	6(R)	14(S)	16(I)	18 (I)	9(R)	0(R)
***Staphylococcus* spp.**		18(S)	16(I)	17(S)	12(I)	18(S)	21(S)	7(R)	7(R)

NOR= Norfloxacin, CIP = Ciprofloxacin, GN= Gentamycin, AMX= Amoxicillin, AK= Amikacin, RF= Rifamycin, E= Erythromycin , AM= Ampicillin, R= Resistant, S = Sensitive, I = Intermediate

Pseudomonas spp. by 23.3%, *Staphylococcus aureus* by 7.3% and mixed infections were 36.6% and they 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 and Austin, (2012) reported that *Aeromonas* spp. infect fishes and were 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*) were the causative agents of *Pseudomonas* septicemia in various species of fish (Eissa *et al.,* 2010; EL-Nagar, 2010)

In this study, *Aeromonas hydrophila* had the highest prevalence rate of isolated bacteria. This is agreed with Hassan *et al*., (2017), who reported that the *Aeromonas hydrophila* was the most predominant *Aeromonas* spp. found in tilapia aquaculture. Van Hai, (2015) also reported that *Aeromonas hydrophila* was the bacterial pathogen in aquaculture that results in huge economic losses and causing a disease known as hemorrhagic septicemia or motile septicemia.

The present study indicated 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. Ezzat et al., (2018) have reported that the kidney and the liver were the most predominant organs for isolation of bacteria at a rate (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 from kidneys 25.3%, liver 23.0%, spleen 19.8% and intestines 15.0% and the lowest prevalence was recorded from gills and skin lesions at rates of 10.3% and 6.35% respectively. Eissa *et al*., (2010) also reported 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 *et al*., (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*. The occurrence of *Staphylococcus sp.* in this study was mainly from kidney and spleen, but not isolated from liver and skin ulcer. While Osman *et al*., (2017) reported 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 was different from result 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 in the current study showed that all or 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 erythromycin indicating that these antibiotics are inappropriate 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*.

Efforts are needed to control the disease from occurring rather than treating the disease which is most of the time risky and expensive. The wide and frequent application of antibiotics in aquaculture not only results in antibiotics resistance in aquatic bacteria but also residual antibiotics in the environmental and aquatic products (Lalumera *et al*., 2004). Such residual antibiotics may cause allergic reactions, toxicity, and alteration of normal microflora of consumers and results in antibiotics resistance in bacterial pathogens (Cabello, 2006). Therefore, searching for a safe alternative to the use of antibiotics is of great importance (Kesarcodi-Watson *et al*., 2008). Several medicinal plants have attracted great attention as a safe alternative to synthetic chemotherapeutics (Mehrim and Salem, 2013). They are advantageous as natural substances that have no effect on the health of fish, humans, and environment, of low cost, and would be minimizing the side effects compared to synthetic chemotherapeutics (Gabor *et al*., 2010). Thus, further studies based on the enhancement haemato-biochemical and immune parameters of Nile tilapia against challenge with pathogenic bacteria are under investigation.

CONCLUSION

The present study concluded that the Gram-negative bacteria were the main cause of Nile tilapia diseases and mortality in farmed fish. *A. hydrophila* exhibited the highest prevalence in naturally infected Nile tilapia fish. The bacterial infection occurs mainly in the liver and kidney of infected fish. Norfloxacin was drug of choice to treat fish infected with bacterial pathogens. Further investigations on the safe effective supplements, alternatives to antibiotics, for improving growth performance and immunity against bacterial pathogens should be recommended.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

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انتشار ومدى قابلية المضادات الحيوية للبكتيريا الممرضة والمتسببة في نفوق اسماك البلطي النيلي المستزرعة

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الملخص العربى

تهدف هذه الدراسة الي عزل وتوصيف البكتريا الممرضة المسببة للنفوق في سمك البلطي النيلي، تم تجميع عدد ١٠٠ عينه من سمكة البلطي النيلي تحمل علامات مرضية تم فحصها اكلينيكيا وتشريحيا وتم عمل الفحوصات البكتيرية وكذلك تم عمل اختبار الحساسية للمضادات الحيوية للبكتيريا المعزولة وقد تم التوصل في هذه الدراسة الي النتائج التالية. تم عزل ١٨٢ عزلة بكتيرية من البلطي النيلي من الكبد، الكلي، الطحال، الخياشيم والتقرحات الجلدية. تم تصنيف أنواع البكتريا المعزولة الي ٨٥ عزله ايروموناس، ٤٢ عزلة من السيدوموناس، ٣٠ عزلة من الفيبريو، ١٦ عزلة من ستربتوكوكس و٩ عزلة من ستافيلوكوكس. وتم تصنيف العزلات من سلالة الإيروموناس كالآتي: ٣٠ عزلة إيروموناس هيدروفيلا،٢٧ إيروموناس فيرونى، ١٢ عزلة إيروموناس سوبريا، ١١ عزلة إيروموناس جاندي و٥ عزلة إيروموناس كافى:وتم تصنيف العزلات من سلالة السيدوموناس كلآتى: ١٩ عزلة من السيدوموناس فلوريسينس , ١٦ عزلة من السيدوموناس ايرويجنوزا و٧ عزلة من السيدوموناس انجويليسبتيكا وتصنيف العزلات من سلالة الفيبريو كالآتي: ١٣ عزلة فيبريوهارفي ، ١٠ عزلة فيبريو فولنيفكس و٧ عزلة فيبريو الجينوليتكس. وجد ان أكثر معد انتشار للعزلات البكتيرية كان في الكبد بنسبه ٣٧,٣٦٣٪. بعد عمل اختبار الحساسية للمضادات الحيويه وجد ان العزلات حساسة للنورفلوكساسين، السيبروفلوكساسين، الجنتاميسين والأميكاسين بينما أظهرت العزلات مقاومة لريفاميسين، الاريثرومايسين، اموكسيسيلن وامبسيلين.