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Prevalence of Streptococcosis-related Mortalities in Farmed Nile Tilapia (*Oreochromis niloticus*) at Different Life Stages.

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IN the current study, clinically diseased cultured *Oreochromis niloticus* at various life stages were collected from different fish farms within the governorates of Qalyubia, Kafr El-Sheikh, Sharqia, and Port Said. The clinical pictures and gross lesions were recorded. Bacterial pathogen isolation and identification were accomplished using both traditional and molecular techniques. For molecular characterization, traditional PCR was employed to confirm the biochemically identified bacteria using the 16S rRNA. The pathogenicity of the isolates was examined, and histopathological findings were recorded for each. At the farm site examination, the infected fish displayed general septicemic signs such as skin hemorrhages and ulcerations, uni- and bilateral exophthalmia, congested internal organs, and significant mortality. The overall prevalence of bacterial infection was (26.2%). *Streptococcus agalactiae* was the most prevalent bacteria recovered from clinically diseased juveniles (15.5%), with the summer season exhibiting the highest incidence. The retrieved bacterial isolates were *Streptococcus agalactiae* (*S. agalactiae*) (50 isolates, 15.5%), *Streptococcus faecalis* (*S. faecalis*) (5 isolates, 1.5%), *Enterococcus faecium* (*E. faecium*) (37 isolates, 11.4%), and *Lactococcus garviae* (*L. garviae*) (55 isolates, 17%) were isolated from infected juveniles in the autumn (55 isolates, 17%) and adults in the summer (20 isolates, 6.2%). According to the results of this investigation, streptococcal infection, specifically *S. agalactiae*, *S. faecalis*, *E. faecium*, and *L. garviae* (strain I and II), could be a significant contributor to tilapia mortality during the summer.

Keywords: Nile tilapia, Streptococcal infection, Mortality, Epidemiology.

Introduction

Aquaculture has evolved into a globally significant economic industry, necessitating ongoing research based on scientific and technological advancements and innovations [1, 2]. Nile Tilapia, Oreochromis niloticus, is the second most farmed freshwater fish worldwide, after carp, and is anticipated to be the most important farmed fish in the twenty-first century [3].

Nile tilapia's widespread appeal partly comes from its efficient nutrient utilization, rapid growth, and high economic return. Since it is resistant to disease and can survive in a variety of water conditions, including those of low quality, it is a desirable fish for intensive farming [4]

Maintaining fish health depends on the interaction between fish, the environment, and pathogens [5]. Subsequently, as the intensity expands, different viral, bacterial, fungal, and parasitic diseases have caused enormous mortalities in Egypt's fish farms over the last decade [6, 7].

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Genus Streptococcus includes several species that can infect many different hosts and result in serious diseases [8].

Fish streptococcosis can be influenced by various factors, including host species, age, immune status, pathogen species/strain, and environmental factors [9]. In addition to the stress, environmental factors such as abrupt changes in water parameters [10, 11]. Ammonia, salinity, temperature, and low dissolved oxygen are significant factors in the pathogenicity of the bacteria [12]. Streptococcus sp., Lactococcus sp., Vagococcus sp., and Enterococcus sp. are all members of diverse bacterial genera linked to the spread of streptococcosis in aquaculture [13, 14]. The most notable clinical signs of the disease are hemorrhagic septicemia, nervous manifestations, abnormal swimming behavior, exophthalmia, or cloudy eyes [15, 16].

Bacterial diseases are traditionally identified using various media types, either general or selective, for a specific bacterium [17, 18]. API 20 Strep test has been widely used for identifying Streptococcus and related species, which provides a relative percent of accuracy in distinguishing bacteria at the species level [19], as well as Polymerase chain reaction (PCR) is a standard method for identifying the Streptococcus species genome [20].

The purpose of this study was to conduct a thorough investigation into the prevalence of streptococcosis in farmed Nile tilapia at different life stages. The bacterial isolates were characterized using conventional bacteriology and molecular identification.

Material and Methods

Fish sampling and transportation

Clinically diseased cultured Nile tilapia (*O. niloticus*) of different life stages were collected from different fish farms located at Qalyubia, Kafr El-Sheikh, Sharqia, and Port Said governorates during the period (2020-2022). The samples were representative of the different life stages; juveniles with average weight (20 ± 5g) and adult/brood stocks with average weight (300 ± 100 g) with a total number of 100 fish/ life stage/season. Clinical signs and behavior changes were recorded at the farm site, and the fish with obvious clinical signs were transferred to the Department of Aquatic Animal Medicine laboratory at the Faculty of Veterinary Medicine, Benha University, Egypt, as quickly as possible for bacteriological and postmortem examination, which were performed following Austin and Austin [21].

Isolation, phenotypic and molecular characterization of the retrieved isolates

Bacteriological samples obtained from the hepatopancreas, kidneys, spleen, brain, intestine, and eyes were inoculated into Brain Heart Infusion broth (BHIB: Hi Media, India) [22]. Gram staining, motility, catalase, and cytochrome oxidase tests were carried out in accordance with Cruickshank et al. [23]. Pure colonies were streaked onto a Streptococcus Selective Agar plate (Oxoid, Denver, USA) and Blood Agar plate supplemented with 5% sheep blood for detection of the type of hemolysis. All cultured plates were incubated at 30 °C for 24-28 h. After recording the culture characters of isolated bacteria, it was stored at -80 °C in BHI broth containing 20% glycerol until further biochemical and molecular studies [24].

Bacterial isolates were phenotypically identified according to Bergey's [25]; Elemar et al. [26]. According to the manufacturer's instructions, the bacterial isolates were identified at the species level using API 20 Strep strips (Bio-merieux L. Etiole, France). This method was preceded by streaking the preserved bacterial isolates over Columbia blood agar (Bio-merieux L. Etiole, France) and incubated at 30 °C for 24 h with anaerobic conditions using AnaeroGen 2.5L (Oxoid Ltd, USA). The API 20 Strep results were analyzed using the analytical profile index recommended by the manufacturer.

Since all retrieved isolates showed consistent phenotypic characteristics and biochemical profiles, representative isolates were randomly selected for molecular characterization and sequencing. Bacterial isolates were grown overnight in 5 ml of BHI broth at 28 °C for 24 h. The harvested bacterial pellets were used for DNA extraction using the Qiagen DNeasy DNA extraction technique adopted in the company handbook. The extract was stored at −20 °C till use. Two universal 16S rRNA bacterial primers, F 5-AGAGTTTGATCMTGGCTCAG-3 and R5 TACGGYTACCTTGTTACACTT-3, were used according to Lagacé et al. [27]. PCR reactions were performed following Panigrahy et al. [28] using a thermal cycler (Eppendorf, Hamburg, Germany). The final volume of 25 µl was prepared by adding 12 µl Hot Star Taq DNA polymerase master mix (QIAGEN) with 20 ng of DNA and 0.1-0.3 µl of each primer. The PCR conditions were the

following: 95 °C for 15 minutes, 30 cycles at 95 °C for 45 seconds, 55 °C for 45 seconds, 72 °C for one minute, and final elongation at 72 °C for seven minutes. Twelve µl. of PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide in a 1 M Tris-Acetate-EDTA buffer and visualized by U.V. transillumination.

The purified PCR product was sequenced in the forward direction using an automated DNA sequencer (Applied Biosystems 3130, USA) with the aid of a ready-to-use Bigdye Terminator V3.1 cycle sequencing kit (Cat. No. 4336817 from Perkin-Elmer/Applied Biosystems, Foster City, California) following the manual instructions. To determine sequence identity to GenBank accessions, a BLAST® analysis (Basic Local Alignment Search Tool) was first carried out according to Altschul et al. [29]. The phylogenetic tree was constructed using MEGA X and contained partial 16S rRNA gene sequences from the nearest type strains [30]. Sequences having a 99% similarity were deemed relevant for bacterial identification.

The overall and seasonal prevalence of each retrieved bacterial isolate were recorded.

Antimicrobial susceptibility testing

The antibiotic susceptibility of the retrieved isolates was determined using disc diffusion method according to Romalde et al. [31]. Briefly, 5 ml of BHI broth was inoculated with one loop of culture. The inoculum concentration was ~10^8 CFU/ml (0.5 McFarland). The suspension was uniformly spread on Muller Hinton agar (HiMedia, India) supplemented with 5% defibrinated sheep blood plates. Discs of Eight commercial antimicrobial agents (Oxoid) were used, such as aquaflor (30 µg); Novobiocin (30 µg); Amikacin (30 µg); Doxycycline (30 µg); Norfloxacillin (10 µg); Oxytetracycline (30 µg); Ciprofloxacin (25 µg); Gentamycin (10 µg). The plates were incubated at 28 °C 24 h. The interpretations of the zones of inhibition were estimated and classified as susceptible, intermediate, or resistant based on the interpretive criteria described by CLSI [32].

The pathogenicity of the isolated bacteria was determined using the mortality rate of the infected fish, as described by Kozińska et al. [33]. And it was rated into nonpathogenic bacteria (up to four fish with slight disease signs + no mortality), weak pathogenic (more than four fish showing clinical signs + no mortality), pathogenic (mortality of 5-10 fish and more than six fish with disease signs), and high pathogenic (all fish mortality and most of fish show signs).

The preserved identified and verified bacterial isolates; *Lactococuss graviae* (*L. graviae* strain I and II), *Streptococcus agalactiae* (*S. agalactiae*), *Streptococcus faecalis* (*S. faecalis*), and *Enterococcus faecium* (*E. faecium*) were revived in Tryptic Soya Broth (TSB: Merck, Germany) at 30 ± 1ºC for 24 h followed by streaking on TSA and incubated at 30 ± 1ºC for 24 h. One colony from each isolate was aseptically picked, transferred to 10 ml of TSB separately, and incubated at 30 ± 1°C for 24 hr. Each bacterial species was suspended in PBS, and their count was adjusted to (1.5, 3, 6, and 9 × 10^8) CFU/ml following Abu-Elala et al. [34]. The experiments were conducted using apparently healthy Nile tilapia (25 ± 2g), and a sample of fish was examined for parasitic, bacterial, and fungal infection. Fish were experimentally injected intraperitoneally (I.P.) with 0.2 ml of (1.5, 3, 6, and 9 × 10^8) CFU/ml) which is equivalent to four doses (D); 0.3x10^7 cells/fish (D1), 0.6×10^7 cells/fish (D2), 1.2×10^7 cells/fish (D3), and 1.8×10^7 cells/fish (D4) alongside the control group was injected I.P. with saline only (D5) for each bacterial isolate. Five separate experiments were performed for each infective dose (5 fish/ isolate/ in duplicate). Fish were maintained in 50 L aquaria by adjusting the water temperature at 30 ± 2 °C and monitored daily for signs and mortalities for ten days post-infection. Bacterial swabs were taken from the kidneys and brains of moribund and/or freshly dead fish for re-isolation of the challenge organisms.

Histopathology of experimentally infected fish

Brain, eye, spleen, kidney, and liver samples were collected from experimentally infected and control fish groups and fixed in 10% neutral-buffered formalin for preparation of paraffin-embedded sections for histopathological examination [35]. In each cohort, the histopathological lesions were recorded

Results

Clinical findings of clinically examined fish

All clinically examined diseased fish displayed red batches and hemorrhages on all body surfaces and eyes; clinical signs also included detached scales and deep ulcers on the skin; unilateral exophthalmia; corneal opacity; skin discoloration and skeletal deformity. The developed lesions

included congestion and enlargement of the kidney, liver, and spleen, distended gall bladder, and congested brain. The intestine was empty, inflamed, and packed with bloody exudate (Plate 1).

Bacteriological and molecular identification

The study's main goal was to concentrate on the prevalence of *Streptococcus* spp., and specific media were used to selectively grow Streptococcus spp., Enterococcus, and Lactococcus species. On TSA media, the suspected Streptococcus species colonies were small, convex, and creamy white. Gram staining revealed gram-positive cocci, which were non-motile. While on Streptococcus selective agar, pinpoint creamy white convex colonies appeared. The growing colonies on the Blood agar were appeared as small, convex creamy white with non-β-hemolytic character.

Based on phenotypic and biochemical characterizations using API 20 Strep, the isolated bacteria identified as *S. agalactiae*, *S. faecalis*, *E. faecium,* and *L. garviae* strain I and II (Table 1) that were confirmed by PCR using 16S rRNA with 1485 bp. After identifying the strains for 16S rRNA, sequencing was applied, and the obtained sequences for three pathogenic strains were deposited in GenBank under accession no. OQ 187769, OQ 186904, and OQ 186907 for *S. agalactiae, L. garviae II,* and *E. faecium,* respectively.

The phylogenetic tree constructed from the 16S rRNA sequences of three pathogenic bacterial isolates and reference strains from the same species provided strong support for our identification (Fig. 1).

Prevalence and identification of isolated Enterococcus, Lactococcus, and Streptococcus spp.

The total prevalence of bacterial infection was (26.2%) among the examined *O. niloticus*. *S. agalactiae* was the most isolated bacteria from clinically diseased juveniles (50 isolates, 15.5%), as shown in Table (2); no isolates were recorded from adult clinical cases across the four seasons. *S. faecalis* was isolated from only juveniles during the summer (5 isolates, 1.5%). While *E. faecium* (37 isolates, 11.4%) was the most common infectious agent in adults, it was only found in the summer. *L. garviae* was isolated from infected juveniles in the autumn (55 isolates, 17%) and from adults in the summer (20 isolates, 6.2%). *L. lactis* was also isolated from the majority of cases, but it did not show any pathogenicity in the infectivity tests (Table 2).

Infectivity trials of isolated bacteria

Four infective doses were I.P. injected in order to find out the infective concentration and in turn, the pathogenicity of the retrieved bacterial isolates associated with the clinical cases. The highest

Plate 1. The clinical signs and PM lesions of streptococcus spp. infected Nile tilapia A) dead fish with septicemic signs, bad water quality (black arrow), B) hemorrhage and red batches all over the body (red circle), prolapse to the intestine (black arrow head), C) unilateral exophthalmia, D) corneal opacity (black arrow), enlarged hepatopancreas (thick black arrow), congested kidney (zigzag arrow), and the intestine was empty, inflamed and packed with bloody exudate (black arrow head), E) congested and inflamed brain

TABLE 1. Gram staining, cell morphology, motility, catalase, cytochrome oxidase tests and API 20 strep strips of the retrieved bacterial strains

Spp	*Streptococcus fecalis*	*Streptococcus agalactiae*	*Enterococcus faecium*	*Lactococcus garviae* **I, II**	*Lactococcus lactis*
Gram stain	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**
Cell morphology	Cocci	Cocci	Cocci	Cocci	Cocci
Motility test	-	-	-	-	-
Catalase	-	-	-	-	-
Cytochrome oxidase	-	-	-	-	-
API Reactions					
VP ((Voges Proskauer)	+	+	+	+	+
HIP (hydrolysis (HIPpuric acid)	-	-	-	-	-
ESC (ß-glucosidase hydrolysis (ESCulin)	+	+	+	+	+
PYRA (PYRrolidonyl Arylamidase)	+	+	V	+	+
αGAL (α-GALactosidase)	-	-	-	-	-
ßGUR (ß-GlUcuRonidase)	-	-	-	-	-
ßGAL (ß-GALactosidase)	+	-	+	-	+
PAL (ALkaline Phosphatase)	+	-	-	-	+
LAP(Leucine AminoPeptidase)	+	+	+	-	+
ADH Arginine DiHydrolase	V	+	+	-	-
RIB acidification (RIBose)	+	+	+	+	+
ARA acidification (ARAbinose)	+	+	+	-	+
MAN acidification (MANnitol)	+	+	+	+	+
SOR acidification (SORbitol)	+	V	-	+	+
LAC acidification (LACtose)	+	+	+	-	+
TRE acidification (TREhalose)	+	+	+	+	+
INU acidification (INUlin)	+	V	-	+	+
RAF (acidification (RAFfinose)	+	V	-	-	+
AMD acidification (AmiDon)	+	+	+	-	V
GLYG (acidification (glycogen)	-	-	-	-	+
B-HEM (Beta hemolysis)	-	-	-	-	-

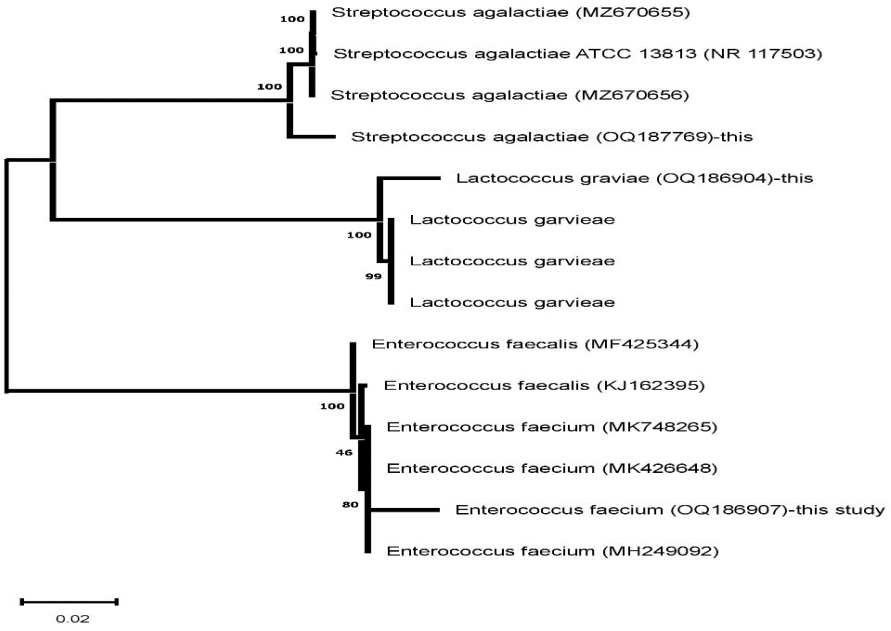

Fig. 1. Phylogenetic tree constructed in MEGA X software based on the nucleotide sequences alignment of 16srRNA gene (1485 bp) from Streptococcus, Lactococcus and Enterococcus strains in this study in relation to other related strains published in the GenBank. Tree was constructed by the neighbour-joining trees using the distance-based method with bootstrap for 1000 replicates.

conc. (9×10^8 CFU/ml) of *S. agalactiae* and *L. graviae* II resulted in 100% mortality of all injected fish, while *E. faecium* and *L. graviae* I, the mortality rates reached 20 % at the end of 10 days' post-infection (Fig. 2.). The most common clinical picture of the examined fish was eye opacity, and darkness. *S. faecalis* infected fish didn't show any signs, and no mortalities were recorded. Nearly the same pattern was observed in the fish injected with 6×10^8 CFU/ml, with reduced mortality in *S. agalctiae* and *L. graviae* II to 80% **(Fig. 3).** The other two concentrations (3 and 1.5×10^8 CFU/ml) exhibited the lowest cumulative mortality in *S. agalctiae* and *L. graviae* II infected fish, which reached 40% and 20% for both concentrations, respectively, followed by *E. faecium* with 20% cumulative mortality in 3×10^8 CFU/ml and 0 % in 1.5×10^8 CFU/ml infected fish (Fig. 4, 5). The clinical picture of infected fish ranged from eroded fins and darkness of the skin, while in the low conc. The fish exhibited slight hemorrhages, and its general health was good as it accepted feed, with the presence of food in the intestine with a normal PM picture except for an enlarged gall bladder.

Pathological alterations of infectivity trial

Pathological changes associated with S. agalactiae infection

Hepatopancreas of fish infected with S *agalactiae* highest conc. 9×10^8 CFU/ml showed congested hepatic vessels, and degenerative and necrotic changes of several hepatocytes and pancreatic epithelium. While the Iris showed congested blood vessels and the spleen showed activation of white pulp Fig. (6).

Pathological changes associated with E. faecium infection

The pathological alterations of *E. faecium* infected fish present in Fig (7A) showed the hepatopancreas with necrotic areas of hepatocytes, which were replaced by hemorrhage and inflammatory exudate. In addition, degenerative changes present in pancreatic acini with peripancreatic inflammatory cells infiltrates. The spleen appeared with multifocal melanomacrophages centers surrounded by mildly depleted white pulps (Fig, 7B). While the kidney revealed necrotic renal tubular epithelium with pyknotic nuclei (Fig, 7C). Cerebellum exhibited vaculations in the outer layer, necrotic neurons in the middle Purkinje cell layer, and inner granular layer (Fig. 7 D).

TABLE 2. Seasonal prevalence of streptococcus spp. isolates in different life stages of Oreochromis niloticus.

Life stages	Juveniles										Adult/broodstock							
Bacterial isolates	*Streptococcus Fecalis*		*S. agalactiae*		*E. faecium*		*L. lactis*		*L. garviae*		*S. agalactiae*		*E. faecium*		*L. lactis*		*L. garviae*	
Season	No. of isolates	%	No. of isolate	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
Winter	0	0	0	0	0	0	40	12.4	0	0	0	0	0	0	45	13.9	0	0
Spring	0	0	0	0	0	0	40	12.4	0	0	0	0	0	0	20	6.2	0	0
Summer	5	1.5	50	15.5	0	0	30	9.3	0	0	0	0	37	11.4	25	8	20	6.2
Autumn	0	0	0	0	0	0	0	0	55	17	0	0	0	0	5	1.5	0	0
Total	5	1.5	50	15.5	0	0	110	34.1	55	17	0	0	37	11.4	95	39.6	20	6.2

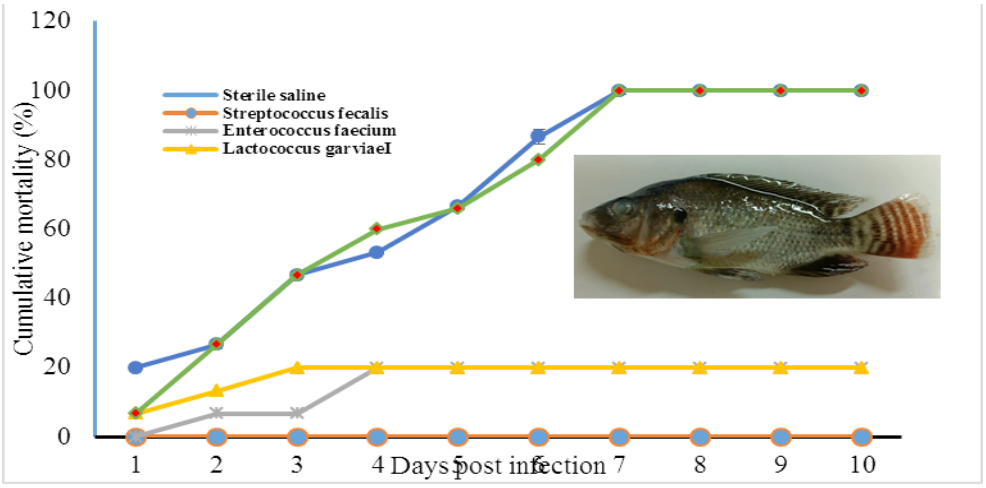

Fig. 2. 10 days' cumulative mortality % and clinical picture of the Nile tilapia IP infected with *S. agalactiae, S. fecalis, Lactococcus graviaeI, II,* and *E. fecaium* at conc. 9×10^8 CFU/ml

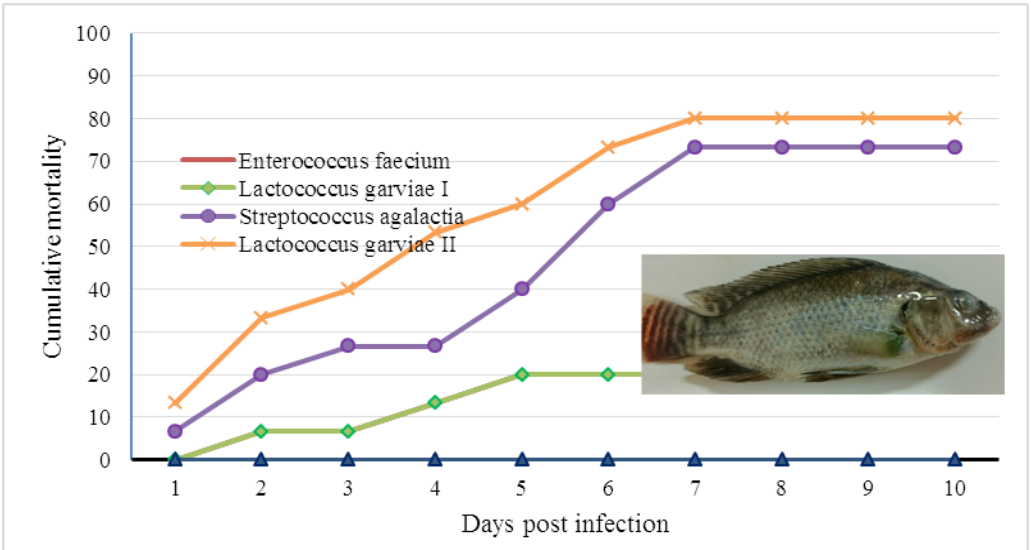

Fig. 3. 10 days cumulative mortality % and clinical picture of the Nile tilapia IP infected with *S. agalactiae, S. fecalis, Lactococcus graviaeI,II,* and *E. fecaium* at conc. 6×10^8 CFU/ml

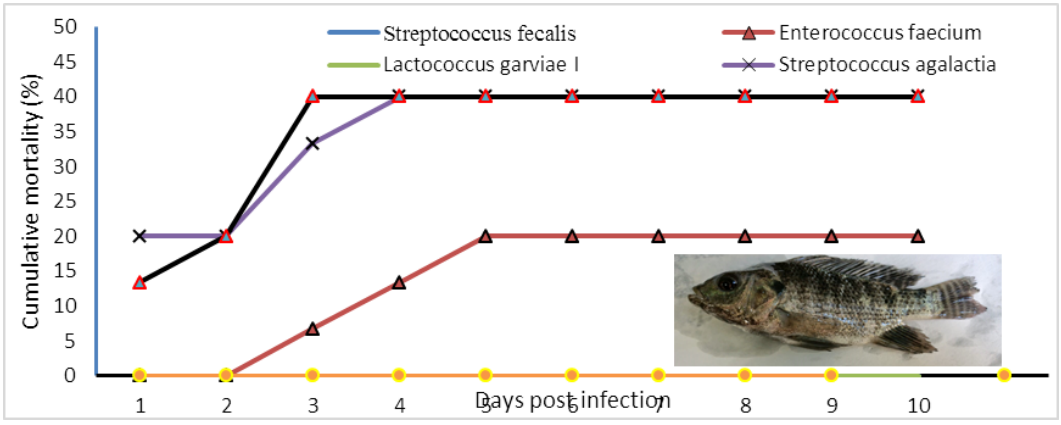

Fig. 4. 10 days cumulative mortality % and clinical picture of the Nile tilapia IP infected with *S. agalactiae, S. fecalis, Lactococcus graviaeI,II,* and *E. fecaium* at conc. 3 × 10^8 CFU/ml

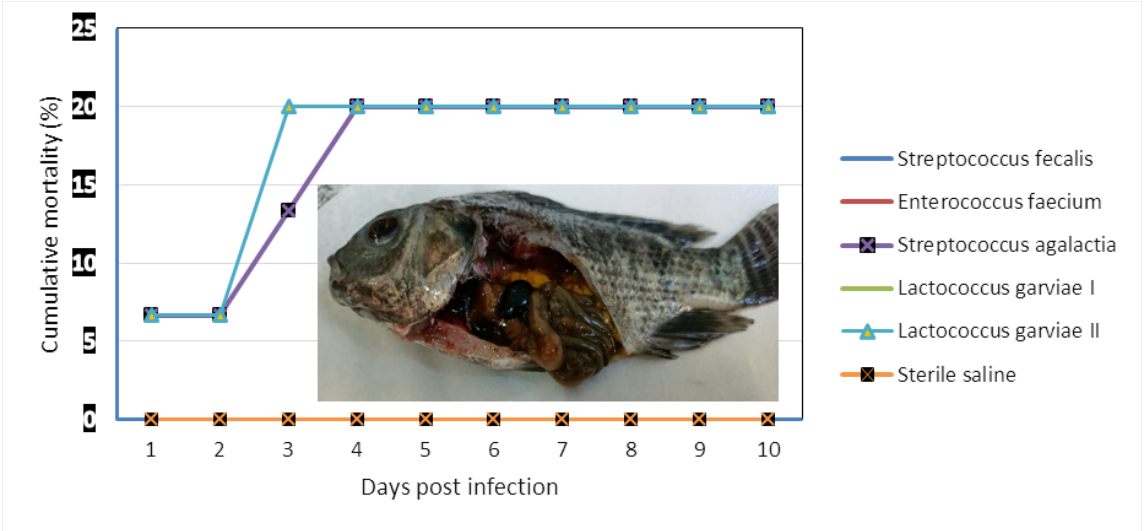

Fig. 5. 10 days cumulative mortality % and clinical picture of the Nile tilapia IP infected with *S. agalactiae, S. fecalis, Lactococcus graviaeI,II,* and at conc. 1.5 × 10^8 CFU/ml

Pathological changes of L. graviae

Histopathological section from the hepatopancreas of *L. graviae* infected fish showed congested hepatic blood vessels, and peripancreatic edema with round inflammatory cells infiltration**.** Moreover, focal areas of hepatic parenchyma showed apoptosis with shrunk cells and pyknotic nuclei (Fig. 8A, B, C&D). The kidney of the infected showed an atrophic or shrunk moderate number of glomeruli, round cell infiltrates between degenerated and necrotic renal tubular epithelium (Fig. 8E&F). Spleen showed activation of the red pulp with dilation of the splenic blood vessels beside prominent melanomacrophage cells (Fig. 8G). And, as shown in Fig (8H), there is edema within the cerebellum**,** with degeneration in some neurons in the Purkinje cell layer.

Antibacterial sensitivity test for isolated bacteria

It was recorded that all isolates were sensitive to Ciprofloxacin, Gentamycin, Norfloxacillin, Aquaflor, and Novobiocin. All isolated bacterial strains were resistant to doxycycline. *Lactococcus garviae* strain I and *Streptococcus agalactiae* were resistant to novobiocin where *L. garviae* strain1 and *E. faecium* were sensitive. Additionally, *L. garviae* strain I and *L. garviae* strain II were resistant to oxytetracycline (Table 3).

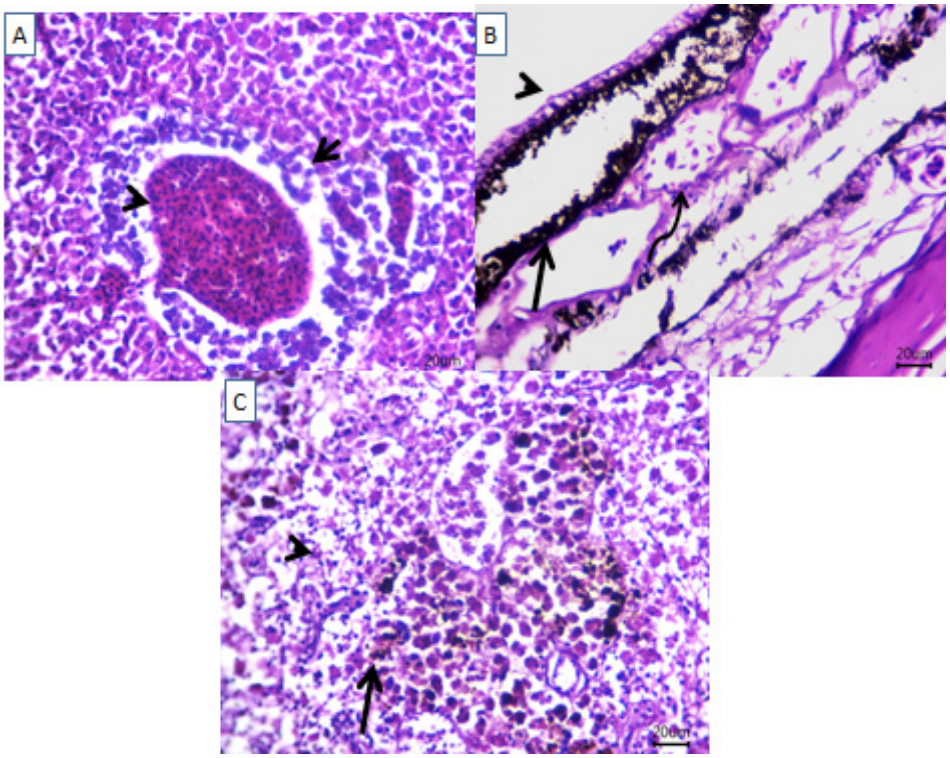

Fig. 6. Histopathological examination of Streptococcus agalactiae

A: dilated hepatic blood vessels (arrowheads), degenerative and necrotic changes of pancreatic epithelium (arrow). (H&E stain).B: Iris showing congested blood vessels (arrow) (H&E stain).C: spleen showing activation of white pulp (arrow). (H&E stain).

TABLE 3. In vitro antimicrobial sensitivity test for the pathogenic isolated bacteria to different chemotherapeutics.

Antibiotic	*Lactococcus garviae* strain I	*Enterococcus faecium*	*Lactococcus garviae* strain II	*Streptococcus agalactiae*
Aquaflor (50% flourfenicol)	S	S	S	S
Novobiocin	S	S	R	R
Amikacin	S	S	S	S
Doxycycline	R	R	R	R
Norfloxacillin	S	S	S	S
Oxytetracycline	R	S	R	S
Ciprofloxacin	S	S	S	S
Gentamycin	S	S	S	S
% of R	20	10	30	20
% of S	80	90	70	80

R: Resistant S: sensitive

Pathological changes of L. graviae

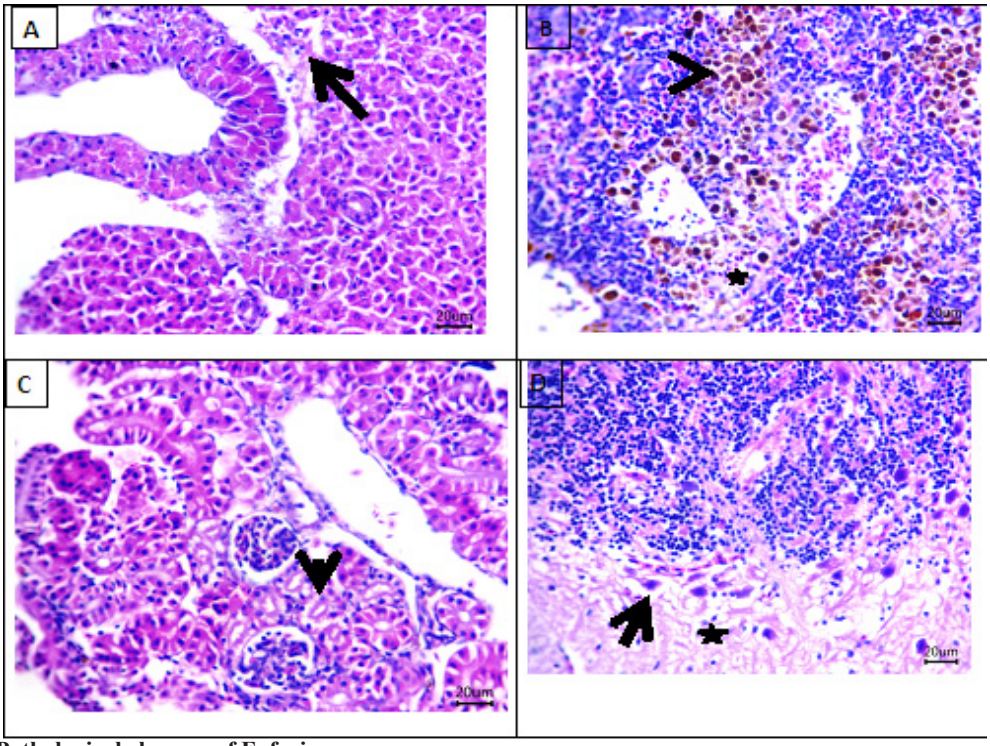

Fig. 7. Pathological changes of E. facium

A: hepatopancreas with necrotic areas of hepatocytes replaced by hemorrhage and inflammatory exudate (arrow) .in addition, degenerative changes of pancreatic acini with peripancreatic inflammatory cells infiltrates (arrow). (H&E stain)

B: Spleen with multifocal melanomacropahges centers (arrowhead) surrounded by mildly depleted white pulps (star).

C: Kidney with necrotic renal tubular epithelium with pyknotic nuclei (arrowhead). (H&E stain).

D: Cerebellum with vacuolations in outer molecular layer (stars), necrotic neurons in middle purkinje cell layer (arrow) and inner granular layer (double headed arrow) (H&E stain).

Discussion

Considering the severe economic losses that might result from an outbreak of a bacterial disease, preventing such an event is of the utmost importance in aquaculture. Diseases in fish can be successfully managed, avoided, and treated if only accurately diagnosed [15].

The isolation and identification revealed that the isolated bacteria were *L. garviae, E. faecium* and *S. agalactiae*. These observations are in the same respect with those reported by many researchers [36 , 16-18].

PCR confirmed that the isolates belonged to *streptococcus* species. Sequencing and A BLAST analysis confirmed the identified strains as; *L. garviae, E. faecium* and *S. agalactiae*.

The prevalence of naturally infected *O. niloticus* with bacterial infections is (26.2%) and the most prevalent bacteria were *S. agalactiae*. These findings were relatively related to those of Hamouda et al. [18], who recorded a total prevalence reached 25% in the examined fish, but our findings were higher than those of Wamala et al. [38], who reported a total prevalence of streptococcosis was only 6.3%. The results of prevalence may differ completely or partially between the published studies and these differences could be due to abiotic and biotic conditions of the environments where the studies performed. This variation can be ascribed to the site of sample collection, the number of investigated fish, fish size, and environmental circumstances. In terms of the seasonal prevalence of infected fish species,

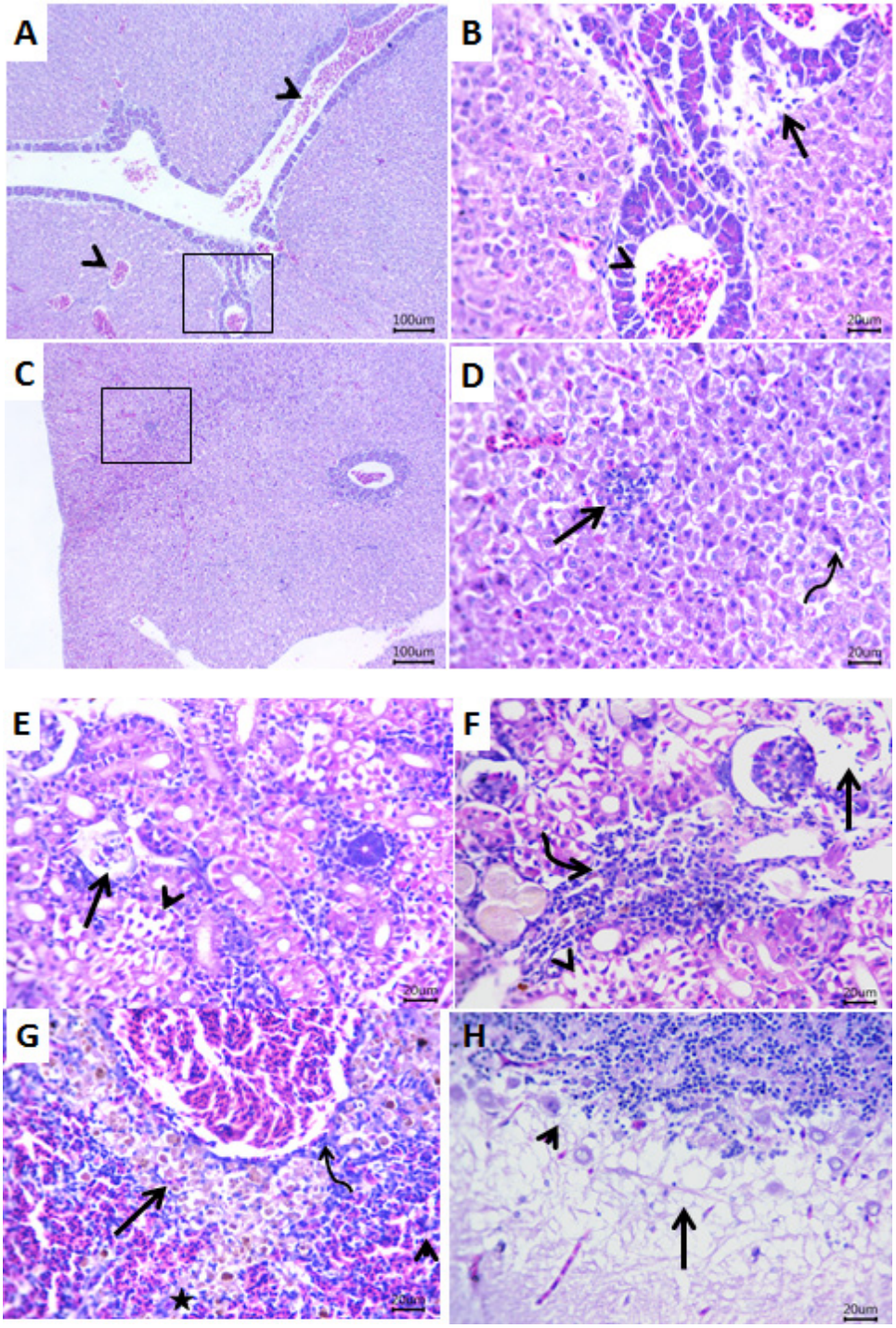

Fig. 8. Pathological changes of Lactococcus graviae A, B: hepatopancreas showed congested hepatic blood vessels, peripancreatic edema with round inflammatory cells infiltration

C, D: focal areas of hepatic parenchyma showed apoptosis with shrinked cells and pyknotic nuclei. (H&E stain). E, F: Kidney showed atrophic or shrinked moderate number of glomeruli, round cell infiltrates between degenerated and necrotic renal tubular epithelium. G: Spleen showed activation of the red pulb with dilation of the splenic blood vessels beside prominent melanomacropahge cells. (H&E stain)

H: Cerebellum showed edema within molecular area, degenerated some neurons in purkinje cell layer and normal middle granular layer.

the summer season exhibited the highest incidence among the examined fish. This could be related to the fact that in the summer, high temperatures, low dissolved oxygen, and other changes in water parameters that generate stressors on fish weaken the immune response, leading the fish more susceptible to bacterial infection [39].

Wamala et al. [38]; Hamouda et al. [18] recorded that the most prevalent bacterial isolates was *S. agalactiae* which are similar to our results. These findings indicated that *S. agalactiae* was found to be the most commonly isolated species infecting *O. niloticus*. This finding was supported with experimental infections of *O. niloticus* with *S. agalactiae* which revealed that it is highly pathogenic to this fish species with 100% mortalities was recorded with the highest injected dose, followed by *L. garviae* strain II with a low percentage of isolation. On the other hand, with a high dose of injection, E. faecium and *L. garviae* strain I recorded 20% mortality. These results agreed with that recorded by Abu-Elala et al. [19], who documented high mortalities, up to 70% in case of *S. agalactiae* and *L. garvieae* infections and 30% in case of *E. faecalis* infection when Nile tilapia was injected I.P. with 0.2 ml of 6×10^8 CFU/ml. In another experiment, Sudpraseart et al. [40] recorded 80% mortality in Nile tilapia experimentally infected with *S. agalactiae* (1.76×10^6 CFU per fish). The difference in the reported mortalities could be attributed to variations in the pathophysiology and virulence of each bacterial strain, as well as the severity of the toxins [41].

Studies of the of bacterial pathogens sensitivity to antibiotics in fish are of major time-wise and important for the development of new chemotherapeutic agents to combat bacterial infections in certain cultured fish population [18]. Our results are nearly similar to those reported by Abu-Elala et al. [19] who recorded that *Streptococcus agalactiae* were resistant to Aquaflor, Ciprofloxacin, and Gentamycin antibiotics. Also, Legario et al. [42] mentioned that S. agalactiae was sensitive to oxytetracycline, Aquaflor, and enrofloxacin. Moreover, Li et al. [43] recorded *S. agalactiae* was sensitive to tetracycline, Doxycycline, Aquaflor, Ciprofloxacin, and rifamycin.

The histological changes due to bacterial infection become distinct only if clinical conditions are prolonged. Pathological alterations on different organs varied according to the isolated spp. The main common pathological alteration revealed meningitis, and infiltration of lymphocytes and macrophages in internal Organs. Moreover, hemorrhage, septicemia, and inflammatory exudate are present in the liver and spleen, and the haemolysis of red blood cells may be due to the migration of bacteria to the blood vessels. Our results were closely similar to that obtained with many authors [44,45, 19] who reported nearly similar histopathological pictures in the splenic and hepatopancreas tissue of Nile tilapia infected with *E. faecium* and *L. graviae*. While according to the findings of Alsaid et al. [46], the hepatopancreas and spleen of red hybrid tilapia infected with *S. agalactiae* displayed significant congestion, necrotic foci, and a rise in melanomacrophage cells, which is comparable to our findings. Moreover, similar histopathological findings of Nile tilapia infected with *S. agalactiae* were observed by Chen et al. [47]. Other researchers recorded an increased number of melanomacropages in fish spleens during streptococcosis [48,49].

Conclusion

This investigation evaluated the current status of streptococcosis in various life stages of Nile tilapia. *S. agalactiae L. garviae* and*, E. faecium* are the most significant microbial agents affecting *O. niloticus* in Egypt. *S. agalactiae* was the most prevalent isolate from clinically diseased juveniles causing septicemia and mortalities that lead to serious economic losses. The development of *S. agalactiae* multi antibiotic resistance among clinical isolates of is a potential risk for the fishery farms as well as, underlines the necessity of a constant monitoring of their resistance range. The study also demonstrates that phenotypic and molecular identification of streptococcal isolates is useful for identifying the causes, which in turn facilitates disease control programs and further vaccine development against streptococcosis in farmed tilapia.

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Conflict of interest

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Authors contribution

All authors contributed equally in Writing, review and editing of this research. All

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Ethical approval

This research was conducted according to the guidelines of the Committee of Animals Welfare and Research Ethics of Benha University, Faculty of Veterinary Medicine (BUFVTM: 19-10-22), Egypt

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دراسة عن وبائية الامراض المرتبطة بالميكروب السبحى في البلطي النيلي المستزرع (Oreochromis niloticus) والمسئولة عن نفوقها في مراحل النمو المختلفة

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في الدراسة الحالية تم تجميع البلطى النيلى المستزرع والمصاب إكلينيكيا في مراحل النموالمختلفة من مواقع مختلفة في محافظات القليوبية وكفر الشيخ والشرقية وبورسعيد. تم تسجيل الاعراض الخارجية والداخلية. تم عزل وتحديد مسببات الأمراض البكتيرية باستخدام الطرق التقليدية وصبغة الجرام وايضا باستخدام API20strep. ومن خلال الفحص الظاهرى وجد ان معظم الأسماك المصابة بأعراض تسمم الدم العامة مثل نزيف الجلد والتقرحات ، جحوظ العين ، احتقان الأعضاء الداخلية ، ونفوق كبيرة وقد اوضحت الدراسة بان Streptococcus agalactiae هي البكتيريا الأكثر فى نسب العزل من الاسماك المصابة بنسبة (١٥,٥٪)، مع أعلى نسبة انتشار في فصل الصيف. وبناءً على التوصيف المورفولوجي والكيميائي الحيوي باستخدام API 20 Strep ، كانت العزلات البكتيرية هي Streptococcus agalactiaeعزلة (50) بنسبة ١٥,٥٪) ، *Streptococcus faecalis*(٥) عزلات بنسبة ١,٥٪) ، Enterococcus faecium37عزلة ، ١١,٤٪)، و *Lactococcus garviae* ٥٠عزلة ، ١٥,٥٪) تم عزل ٥٥ عزلة بنسبة ١٧٪) من المصابة في الخريف (٥٥ عزلة ، ١٧٪) والبالغات في الصيف (٢٠ عزلة ، ٦,٢٪). تم استخدام PCR التقليدي للتوصيف الجزيئي لتأكيد البكتيريا المحددة كيميائيًا باستخدام16sRNA. كشفت هذه الدراسة أن *S. agalactiae* و *S. faecalis* و *E. faecium* و *L. garviae* (السلالة الأولى والثانية) قد تكون مرتبطة بالعدوى بالميكروب السبحى في البلطي مع S. agalactiae

الكلمات المفتاحية: البلطي النيلي ، الإصابة بالميكروب السبحى، النفوق ، علم الأوبئة.