Rapid Detection of Streptococci in Cultured Tilapia Fish Using PCR and Chemical Analysis

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ABSTRACT

Streptococcosis causes economic losses due to the high mortality in Nile tilapia (*Oreochromis niloticus*). The present study was carried out to detect the streptococci by PCR and chemical analysis in cultured tilapia fish. A total of 100 cultured tilapia fishes and 20 water samples were collected from 4 different fish farms at different locations in Kafr El-sheikh Governorate for bacteriological and chemical analysis. The results of water quality parameters examination revealed that the mean values of pH, dissolved oxygen, unionized ammonia, and nitrite were 8.2 ± 0.73, 7.44 ± 0.54 parts per million (ppm), 0.05 ± 0.008 ppm, and 0.00 ppm, respectively. All water quality parameters except for ammonia were within the permissible limit. The bacterial isolation results revealed 38 % positive samples for *Streptococcus* species isolated from tilapia fishes and 40 % were positive for *Streptococcus* species isolated from water samples. Of those 38 positive fish samples, 25 (65.78%) were *Enterococcus faecalis,* 15 (39.47%) were *Streptococcus pyogenes*, 6 (15.78%) were *Enterococcus faecium*, 5 (13.15 %) were *Streptococcus agalactiae,* and 3 (7.89 %) were *Streptococcus iniae*. On the other hand, from 8 positive farms water samples, 7 (87.5%) were *Enterococcus faecalis,* 2 (25%) were *Streptococcus pyogenes* , 2 (25%) were *Enterococcus faecium*, 3 (37.5%) were *Streptococcus agalactiae,* and 1 (12.5%) was *Streptococcus iniae* . Using mPCR to identify *Streptococcus* for some fish and water isolates, 6 *Enterococcus faecalis* with 310 base per (bp) were detected while 3 *Streptococcus agalactiae* with 153 bp and 2 *Enterococcus faecium* at 215 bp were detected. However, the mPCR from *Streptococcus* species directly from organs from fish and water samples revealed that 5 *Enterococcus faecalis* were detected at 310 bp while 2 *Streptococcus agalactiae* was identified at 153 bp. The biochemical results indicated that the infected fish with the streptococcal species had reduced total protein, albumin, and globulin in the blood serum while total cholesterol, urea, creatinine levels, and AST, ALT, GPX, CAT, SOD activities significantly increased, compared to non- infected fish.

Keywords: Biochemical parameters, PCR, Streptococci, Tilapia fish, Water examination.

INTRODUCTION

The second most common fish species in tropical and subtropical freshwater aquaculture is *Oreochromis niloticus* (FAO, 2018). Tilapia is a common aquacultured fish and an important seafood source for human consumption. Up to date, little known is about their usual physiology and reaction to disease infections. Subsequently, ceaseless development of tilapia wellbeing evaluation strategies is fundamental. In any event, the hematology methods commonly utilized for demonstrative clinical infections in the veterinary world are still restricted in aquatic animal pharmaceuticals (Chen et al., 2003).

Bacterial infections in cultivated tilapia were considered the foremost critical causes for financial misfortunes. *Vibrio anguillarum, Aeromonas species*, *Flavobacterium columnare, Pseudomonas fluorescens*, *Streptococcus* species, *Edwardsiella tarda,*, and *Enterococcus* species Were found commonly in aquaculture establishment (Plumb, 1997). Various studies help detect fish bacteria, laboratory infection or resistance to disease (Azad et al., 2001; Al-Harbi and Uddin, 2004; Cai, et al, 2004) still few links haematological parameters with experimental bacterial infection. Hematological parameters are important diagnostic tools that indicate the health state of fish (Blaxhall, 1972; Rehulka, 2002; Martins et al., 2004).

Streptococcus species, for the most part, are astute aquaculture pathogens, the pathogenicity of which is influenced by environmental stresses such as low oxygen levels and a high nitrite concentration (Bunch and Bejerano, 1997), water hardness, overfilling with removed scales (Wedmeyer, 1997) and water temperatures over 20 ˚C (Ohnishi and Jo, 1981). All vital organs of the infected fish with streptococci are heavily infected and the mortality is enormous (50-60 %) (Hubbert, 1989)**.**

Streptococcosis is a bacterial disease in fish that causes economic losses in the freshwater and marine fish production, which is economically important in many countries, including the tilapia industry. *Streptococcus* species that cause disease in fish include *S. agalactiae*, *S. dysgalactiae,* and *S. equi*, *S. equisimilis*, *S. faecium*, *S. pyogenes*, *S. zooepidemicus,* and *S.iniae.* Previous case reports showed that *S. agalactiae* and *S. iniae* are the main causative agents of

streptococcosis in Tilapia. Moreover, the World Animal Health Organization (OIE) has declared that *S. iniae* is a zoonosis (Amal, 2011). The acute form of streptococcal infection in *O. niloticus* has resulted in more than 50% mortalities for about 3 days to one week, while the chronic form could last for several weeks, with the low rate daily mortality being approximately one or two percent (Osman et al., 2017).

The aim of the present project was to isolate *streptococcus* species from tilapia fish and water samples by the traditional method by isolation and identification, then by using multiplex PCR for conformation of some *streptococcus* isolates and a rapid method to detect *streptococcus* species directly from organs of fish and water samples. Also, this experiment was conducted to diagnose streptococcal infection using water quality parameters estimation and biochemical parameters, since diagnostic techniques to detect pathogenic bacteria responsible for fish streptococcosis are usually based on the cultured technique, which requires several days to reach a definitive diagnosis leading to increased disease outbreak risk.

MATERIALS AND METHODS

Ethical approval

The experimental design was performed in accordance with the Guidelines for Animal Experimentation of the Ethics Review Committee of the Animal Health Research Institute, Giza, Egypt (Approval No 83429).

Samples collection

A total of 100 Nile tilapia fishes were collected from live or freshly dead fishes with at least one or more clinical signs of eye lesions and opacity, septicemia, skin lesions, detached scales, skin congestion, ulcers, hemorrhage, and congestion of fins. Twenty water samples were collected from four different fish farms at different locations in Kafr El sheikh Governorate and transported to the Animal Health Research Institute Kafr El sheikh branch, Egypt. The fishes were transported in a sterile polythene bag, which was supplied with aerated tap water with chlorine-free water from fish farms and subjected to biochemical and bacteriological examinations.

Water quality sampling and measurement

Water quality at a depth of 1 meter in each farm was measured at five clear sampling points. A similar technique was used to sample the fish. The water quality parameters for pH and dissolved oxygen were measured using a handheld meter (ORION 5 STAR). The concentration of water nitrite and unionized ammonia was measured using powder pillow procedures and using a spectrophotometer to measure the concentration (HACH Company, Loveland, CO, USA). According to (APHA, 1998)

Bacteriological examinations
Bacterial isolation

Tissue samples from the brain, kidney, liver, spleen, and eyes of the fish were homogenized in a sample, which was used for the fish sampling according to Aboyadak et al. (2016) representative. Tissue samples were divided into two parts, one processed directly for multiplex PCR by sending it to the Dokki Laboratory, and the other was primarily cultivated on Tryptic Soy Broth (TSB) from each sample at 37˚C for 48 hours. A loopful from each tryptic soy broth tube was streaked on Edwards medium (modified) with additional 5-7% bovine blood, TSA, and blood agar plates, then the streaked plates were incubated at 37˚C for 48 hours. Pure bacterial isolates were identified according to their cultural, morphological, and biochemical characteristics (Holt et al., 1994).

Identification of *Streptococcus* species

All purified isolates were identified by studying colony growth characteristics ,morphological analysis (Cruickshank et al, 1975), biochemical analysis as catalase activity test, oxidase test, detection of hemolysis, growth at 6.5% NaCl, growth at $10^{o}C$ and $45^{o}C$, detection of arginine decarboxylase (ADH), hippurate hydrolysis test, bile esculin test, fermentation of sugers (MacFaddin, 2000) and multiplex polymerase chain reaction (m- PCR) for detection of some pathogenic bacteria directly from the samples and from isolates The extraction of DNA from tested samples was done utilizing the QIAamp DNA small kit (Qiagen, Germany, GmbH) with alterations compared to the manufacturer's suggestions. Briefly, 200 µl of the sample suspension tested was incubated for 10 minutes with 20 µl proteinase, potassium and 200 µl of lysis buffer at $56^{o}C$. After incubation 200 µl of 100% ethanol added to the lysate l. The sample was washed and centrifuged according to manufacturer's protocol. The nucleic acid was eluted with 100 µl elution buffer, which was given within the kit. PCR specific primers from Metabion (Germany, table 1), were used in a 50- µl multiplex PCR response containing 25 µl of Emerald Amp Max PCR Master blend (Takara, Japan), 1 µl of each primer of 20 picomols (pmol) concentrations, 11 µl water, ond 8 µl DNA template. The reaction was performed in an applied 2720 thermal cycler biosystem. PCR products were isolated on 1.5% agarose gel (Applichem, Germany, GmbH) in

1xTBE buffer at room temperature using 5 Volts/cm slopes by electrophoresis. 40 µl of the products were loaded into each gel slot for gel analysis. The part sizes were calculated using Gelpilot 100 base per Ladder (Qiagen, Germany, GmbH). The gel documentation system (Alpha Innotech, Biometra) captured the gel and the computer program analyzed the information

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target Agent	Target gene	Primers sequences	Amplified segment (base per)	Primary denaturation	Amplification (35 cycles)			Final extension	References
					Secondary denaturation	Annealing*	Extension		
E. faecalis	16S rRNA	GTT TAT GCC GCA TGG CAT AAG AG	310						Zoletti et al., 2006
		CCG TCA GGG GAC GTT CAG							
S. agalactiae	cfb	TTTCACCAGCTGTATTAGAAGTA	153	94°C 5 minutes	94°C 30 seconds	50°C 40 seconds	72°C 45 seconds	72°C 10 minutes	Ke et al., 2000
		GTTCCCTGAACATTATCTTTGAT							
E. faecium	sodA	GAAAAAACAATAGAAGAATTAT	215						Jackson et al., 2004
		TGCTTTTTTTGAATTCTTCTTTA							

*The annealing temperature of the primers was modified, and validated to 50°C to be suitable for multiplex PCR.

Biochemical examinations

The fish body surfaces were cleaned and dried with absorbent paper. Blood samples obtained from caudal vessels of 2ml/fish from each farm using disposable 3 milliliters syringes, were transferred to serum separation tubes without anticoagulants, such as defined for total serum protein, albumin, globulin, total cholesterol, urea, creatinine levels, and AST, ALT, ALP, GPX, CAT, SOD activities, which were estimated spectrophotometrically (LABOMED Co., Lab. American Inc., USA) according to the manufacturer's instructions.

RESULTS

Table 2. Water quality parameters in examined tilapia fish farms (n = 20) at different locations in Kafr El sheikh Governorate, Egypt

Parameters	Values	Permissible limit*
pH	8.2 ± 0.73	6.5 − 9
Dissolved oxygen (ppm)	7.44 ± 0.54	5.61 - 9.4
Unionized ammonia (ppm)	0.05 ± 0.008	0.00 - 0.02
Nitrite (ppm)	0.00	0.00 - 0.2

Values are means ± standard error. *Permissible limit according to Egyptian law No. 48 (1982).

Clinical and post mortem examinations

The samples of infected fishes revealed the presence of eye lesions (bilateral exophthalmia and opacity), skin lesions and congestion of the internal organs (Figures 1 and 2).

Figure 1. Bilateral exophthalmia and skin lesion in tilapia fish

Figure 2. Congestion of the internal organs in tilapia fish

Table 3. Percentage of positive *Streptococcus* species isolated from tilapia fish (n =100) and water samples (n = 20) was done at the Animal Health Institute - Kafer El sheikh Regional Laboratory, in Egypt and in summer 2018.

Samples types	Samples number	Positive samples number	Percentage (%)*
Tilapia fish samples	100	38	38
Water samples	20	8	40

*Percentage (%) calculated according to total number of samples.

Table 4. Identification of *Streptococcus* species isolated from positive tilapia fish samples was done at the Animal Health Institute - Kafer El sheikh Regional Laboratory, in Egypt and in summer 2018.

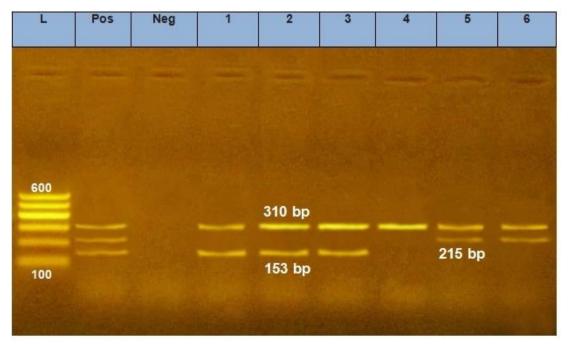| Identified organism	Positive samples (n = 38)	Percentage (%)*
Enterococcus faecalis	25	65.78
Streptococcus pyogenes	15	39.47
Enterococcus faecium	6	15.78
Streptococcus agalactiae	5	13.15
Streptococcus iniae	3	7.89

*Percentage (%) calculated according to the number of positive fish samples (38).

Table 5. Identification of *Streptococcus* species isolated from positive farms water samples from four different farms in Kafer El sheikh Governorate, in Egypt and in summer 2018

Identified organism	Positive samples (n = 38)	Percentage (%)*
Enterococcus faecalis	7	87.5
Streptococcus agalactiae	3	37.5
Streptococcus pyogenes	2	25
Enterococcus faecium	2	25
Streptococcus iniae	1	12.5

*Percentage (%) calculated according to the number of positive water samples (8).

Detection of some *Streptococcus* species by using multiplex Polymerase Chain Reaction

Figure 3. Agarose gel electrophoresis of multiplex PCR amplification products of *Streptococcus* isolated from tilapia fish samples and water samples. Lane L: 100 base per (bp) ladder as a molecular size DNA marker. Lane Pos: Control positive *streptococcus* species genes. Lane Neg: Control negative. Lanes1, 2, and 3: Positive for *Enterococcus faecalis* at 310 bp, and *Streptococcus agalactiae* at 153 bp. Lane 4: Positive for *Enterococcus faecalis* at 310 bp. Lanes 5 and 6: Positive for *Enterococcus faecium* at 215 bp.
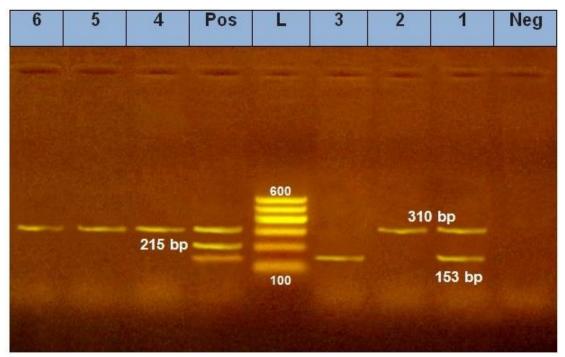
Figure 4. Agarose gel electrophoresis of multiplex PCR of *Streptococcus* species directly from organs of tilapia fish and water samples. Lane L: 100bp ladder as a molecular size DNA marker. Lane Pos: Control positive *Streptococcus* species genes. Lane Neg: Control negative. Lane1: Positive for *Enterococcus faecalis* at 310 bp and *Streptococcus agalactiae* at 153 bp. Lanes 2, 4, 5 and 6: Positive for *Enterococcus faecalis* at 310 bp. Lane 3: Positive for *Streptococcus agalactiae* at 153 bp.

Table 6. Variation of some blood serum parameters of Nile tilapia on exposure to *Streptococcus* infection in Kafer El sheikh Governorate, Egypt

Parameters	Non infected	Infected
Total protein (g/dl)	6.01 ± 0.08	5.81 ± 0.05
Albumin (g/dl)	5.44 ± 0.03	5.21 ± 0.09
Globulin (g/dl)	0.76 ± 0.11^a	0.40 ± 0.08^b
Urea (mg/dl)	4.99 ± 0.05^b	5.12 ± 0.02^a
Creatinine (mg/dl)	1.89 ± 0.08	1.99 ± 0.03
AST(u/ml)	31.3 ± 0.46^b	47.3 ± 0.38^a
ALT (u/ml)	54.3 ± 0.27^b	82.3 ± 1.2^a
ALP (u/ml)	76.3 ± 0.27	74.8 ± 0.61
Glucose (mg/dl)	159.4 ± 0.54^b	235.7 ± 0.27^a
Cholesterol (mg/dl)	176.75 ± 1.67^b	192.02 ± 1.23^a
Cortisol (ng/ml)	2.9 ± 0.11^b	3.36 ± 0.12^a

Values are means ± standard error. Means inside the same row of diverse litters are essentially distinctive at $p \leq 0.05$.

Table 7. Variation of some blood serum antioxidant enzymes of Nile tilapia on exposure to *Streptococcus* infection in Kafer El sheikh Governorate, Egypt

Items	Non infected	Infected
Glutathione peroxidase (GPX) (u/ml)	16.6 ± 1.21^b	21.6 ± 1.11^a
Catalase(CAT) (u/ml)	$12 \pm 0.^{06b}$	19 ± 0.03^a
Superoxide dismutase (SOD) (u/ml)	200.62 ± 0.22^b	282.58 ± 0.11^a

Values are means ± standard error. Means within the same row with different litters are significantly different at $p \leq 0.05$.

DISCUSSION

Streptococcal infections have been frequently reported in the freshwater and saltwater fish that have been cultivated in several regions of the world, particularly tilapia species, and several *Streptococcus* species were involved (Chang and Plumb, 1996). The most effective *Streptococcus* species in fish are *S. iniae, S. difficile, S. agalactiae, S. parauberis, S. dysgalactiae and S. Shiloi* (Eldar et al., 1995; Mata et al., 2004; Netto et al., 2011). *S. agalactiae* belongs to group B streptococci, which may be either haemolytic (Evans et al., 2002) or non-haemolytic (Finch and Martin, 1984) .All eight strains of *S. agalactiae* isolated from tilapias are non-haemolytic. *S. agalactiae* is really the only streptococcal species which belongs to the Lancefield serotyping sero-group B (Devriese et al., 1991).

Water quality parameters

Natural conditions encompassing the cultural areas could influence the water quality and introduce stress to the cultivated fish. This inevitably diminishes the immune status, and activates bacterial disease which leads to infection (Amal et al., 2015).

The results of the analysis of water quality parameters shown in table 2 revealed that the mean value of pH, dissolved oxygen, unionized ammonia and nitrite were 8.2 ± 0.73, 7.44 ± 0.54, 0.05 ± 0.008 parts per million (ppm) and 0.00 ppm, respectively. Our results indicated that some parameters of water quality, like water pH, dissolved oxygen, and nitrite, were within the recommended range for tilapia cultivation, but ammonia was higher than the recommended range for tilapia culture. These results were similar to those studied by Zamri-Saad et al. (2014) and lower than those reported by Ali et al. (2008).

The ammonia in water samples is assumed to originate from the feces of fish and excessive feed provided to the fish. Tilapias can live in pH extending between 5 and 10, but grow best at pH levels between 6 and 9 (Popma and Masser, 1999). But on the other hand, a low pH of the water contributed to behavioral changes, damage to the gill epithelial cells, and a reduced nitrogen excretion efficiency, and causes high mortality (Sammut, 2001).

Non-ionized ammonia is the most poisonous fish parameter in the aquaculture and contaminated water (Zhao et al., 1997; Harris et al., 1998; El-Shafai et al., 2004). In fresh water, the toxic levels for free ammonia in short-term exposure were usually between 0.6 and 2 mg/l, while others consider 0.1 mg/l as the highest tolerable concentration (Pillay, 1992).

The present study also identified the significant parameters of water quality that affected the presence of the bacteria in cultivated fish. Although each sampling site has a different parameter of water quality related to the presence of bacteria, ammonia and nitrite are two of the major important contaminants that threaten the health of aquatic organisms, particularly in freshwater, and have since been described as the most important parameters, that there are strong associations with the presence of bacteria at all sampling.

Popma and Masser (1999) recorded that metabolism, growth, and resistance disease were impaired if Dissolved Oxygen declines over an extended period and tilapias predisposed to streptococcosis. In addition (Swann, 1992; El-Sayed, 2006) studied that, the concentration of dissolved oxygen higher than 5 ppm is necessary for good growth of tilapia. Nitrite is extremely toxic to tilapia as it disrupts the fish's physiological function and results in growth retardation, as reported by El Sayed, (2006). Nitrite could inactively enter the circulatory system as a nitrous acid and diffused unconditionally through the layers of fish gills. Upon entering the circulation system, nitrite oxidizes the iron within the hemoglobin particle from ferrous state (Fe2+) to ferric state (Fe3+) and the resulting product is called methemoglobin. Because methemoglobin is unable to reversibly combine with oxygen, exposure to nitrite can lead to significant respiratory trouble since even the blood's oxygen-carrying capacity is difficult (Boyd and Tucker, 1998).

Isolation and identification of *Streptococcus* species

In table 3, data revealed that *Streptococcus* species were isolated from 100 diseased tilapia fish, since 38 samples showed an incidence of 38%, while 20 water samples showed 8 positive *Streptococcus* isolation with an incidence of 40%. These findings were lower than those mentioned by Asencios et al. (2016) and El-refaee (2005), who isolated *Streptococcus* species with the percentage of 100 % and 39.8 %, respectively from tilapia fish. The recorded results were higher by El-Rouhy (2002) in Sharkia and Ismailia (21.8%), Zeid (2004) in El-Mansoura city (18%), and Huang et al. (1991) in marine water fish (17.3%). Also, the recorded results were higher than reported by Badran and Eissa (1991), who mentioned that incidence of *Streptococcus* was 1.7% in tilapia. The incidence of *Streptococcus* was reported by El-Bouhy and Megaheed (1994) 9.2% and 10% in cultivated *Oreochromis niloticus* by Ebtsam (2002). The different percentage of streptococcal infection could be attributed to different fish species and different environmental factors as well as the use of manure and waste water beside chicken and duck houses over fish ponds.

The tables 4 and 5 of the present study showed the identification of *Streptococcus* species isolated from positive tilapia fish samples and water. The proportions of fish and water samples were 65.78% and 87.5% for *Enterococcus faecalis*, 39.47% and 25% for *Streptococcus pyogenes*, 15.78% and 25% for *Enterococcus faecium*, 13.15% and 37.5 % for *Streptococcus agalactiae*, and finally 7.89 % and 12.5% for *Streptococcus iniae* respectively. The percentage of isolation of *Enterococcus faecalis* and *Streptococcus pyogenes* from fish was found to be higher than those reported by Abou El-gheit (2005), Amal (2009), Khafagy et al. (2009) and Osman et al. (2017). The *Enterococcus faecalis* isolated from tilapia fish with the percentages of 7.5%, 45%, 5%, and 23.76% and Abou El-gheit (2005) isolated *Streptococcus E. pyogenes* with a percentage of 16%, while the percentage of isolation of *Enterococcus faecium ,Streptococcus agalactiae* and *Streptococcus iniae* from fish were lower than the percentage which reported by Asencios et al. (2016), who isolated *Streptococcus agalactiae* from fish at 56.3%, but Amal (2009) isolated *Streptococcus iniae* with 32.4%, while the results of the present study was similar to Delphino et al. (2019), who isolated *Streptococcus agalactiae* from fish with 16% and Hernández et al. (2009) with 17.5% from fish. Although Saleh et al. (2019) isolated *Streptococcus iniae* with 13.3% from fish.

Diagnostic techniques to detect pathogenic bacteria responsible for the streptococcosis of fish are usually based on the cultured technique, which takes many days to reach a final diagnosis leading to an increased disease outbreak potential. PCR can target unique genetic sequences of microorganisms and has been previously developed to detect pathogenic fish bacteria using a primer specific that is specific for a gene segment of a particular bacterium (Mata et al., 2004).

In the present study, the multiplex PCR test for the detection of streptococcosis caused by *E. faecalis, S. agalactiae,* and *E. faecium* was proposed, which could cause great losses and could be a threat to the fish farmers. All primers were used to detect these pathogenic bacteria isolated from infected fish cultured (Ke et al., 2000; Jackson et al., 2004). The m-PCR was accurate and specific for the detection of representative pure isolates of *E. faecalis, S. agalactiae, and E. faecium* with the detection limits.

Molecular identification

In the present study, PCR was used to identify the isolates using species-specific primers. The majority of the isolates from the previously mentioned specimens were *E. faecalis* (6 of 6) with the percentage of 100%, followed by *S. agalactiae* (3 of 6) with the percentage of 50%, and *E. faecium* (2 of 6) with the percentage of 33.3%. However, Ouissal et al. (2015) detected *E. faecium* from European sea bream as the most frequently isolated species of *Enterococcus* with the percentage of 24.32%, followed by 18.91% to *E. faecalis,* while Qasem et al. (2008) found that most of the isolates from fishes and wastewater samples (9 out of 17) with the percentage of 52.94 % were identified as *S. agalactiae*.

By detecting warm-water streptococcosis, the sensitivity threshold for the identification of *S. iniae, S. difficilis, S. parauberis,* and *L. garvieae* was between 2.5×10^3 and 1.2×10^4 cells/g tissue (Mata et al., 2004). Although the sensitivity of m-PCR to the detection of specific bacteria from fish tissue was not fulfilled, the m-PCR analysis in this study allowed the detection of *S. agalactiae* (5 of 6) with the percentage of 83.3% and *E. faecalis* (2 of 6) with the percentage of 33.33%, directly from the infected tilapia organs (Itsaro et al., 2012).

Biochemical parameters associated with *streptococci* infection

The present experiment was conducted to diagnose of *Streptococcus* infection using the estimation of water quality parameters and biochemical parameters, since diagnostic techniques for the detection of pathogenic bacteria responsible for fish streptococcosis are usually based on the cultured technique, which requires many days to reach a proper diagnosis leading to the increased potential for a disease outbreak. In tilapia fish, biochemical changes were studied to identify the variable parameters for the determination of healthy and infected fishes.

In table 6 the results of the present study revealed that there were insignificantly decreased in total protein and albumin but there was significant decreased in globulin, also there were significant increase in total cholesterol, urea, creatinine levels, and AST, ALT, GPX, CAT, SOD activities compared to non-infected fish. These results were similar to recorded by Adel and Shalaby (2004) and Yu et al. (2010), who revealed that decrease of serum total protein could be due to increased protein breakdown as a stimulator of corticosteroid hormones, which enhances proteins breakdown to provide amino acids and gluconeogenesis to provide glucose to cope with the increase in energy demands to balance stressful condition. Elevated Creatinine and urea levels may also be associated with reduced kidney function (Zotti et al., 2008) and as an indication of gill and kidney dysfunction (Adham et al., 2002; Yang and Chen, 2003). The increase in ALT and AST levels was similar to studies by Chen et al., (2004), Abuseliana et al., (2010), Bin and Xiao-jin, (2010) and Khalil et al., (2011). The increased level of these parameters due to infection were suggested to be due to the severe damage of viscera organs such as liver and kidney. There were no changes in alkaline phosphatase enzyme between infected and uninfected fishes, these differed from those reported by chen et al. (2011), who studied that alkaline phosphatase was significantly increased 12 hours post-infection with *streptococcus iniae*. Elevated cholesterol and glucose levels could be associated with the stress response and metabolism. These results were consistent with previous findings from Alsaid et al. (2014) and a similar increase in blood glucose levels had been detailed by Evans et al. (2006) taken after exposure of (*O. niloticus*) Nile tilapia to unionized ammonia. These elevated glucose levels may be due to the decrease in insulin levels, since insulin has a greater influence on protogenic and lipogenic pathways (El- Naggar et al., 1998). Also, the results of the present experiment revealed that there was a significant increase in cortisol levels in an infected group than in the uninfected group. These results were similar to those of Qiang et al., (2016), which increased the level of ammonia serve as acute stress and secrete a high amount of cortisol to facilitate the synthesis of glucose and degradation of fat.

In table 7, the results of the present research showed a significant increase in GPX, CAT, and SOD activities compared to non-infected fish. These results were consistent with those examined by Harikrishnan et al. (2012). In addition, malondialdehyde and SOD are example of biomarker for oxidative stress besides catalase (CAT) enzymes, Glutathione Peroxidase (GPx) and Glutathione Reductase (GR). When fish are under stress conditions, the level of Reactive Oxygen Species (ROS) also increases. Defense mechanisms to fight the ROS overload were found in many mammalian species and fish. Thus, fishes are mainly being used as bio indicators for environmental changes (Beutler,

1984). Catalase is an essential antioxidant defense component, protects fish from oxidative stress by converting the hydrogen peroxide into oxygen and water (Atli and Canli, 2007). SODs are enzymes which then catalyze the dismutation of superoxide (O^{2-}) radicals into either conventional atomic oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is a by-product of oxygen metabolism and causes many types of cell damage if not regulated (Garry, 2011; Tohru and Masuko, 2011).

CONCLUSION

Tilapia has become a perfect host for *Streptococcus* infections. *Streptococcus* (*S. agalactiae* and *S. iniae*) are exceptionally pathogenic since they can infect many spices of fish around the world. Streptococcosis has been found to cause millions of economic losses in aquaculture worldwide. Tilapia farmers should be advised and trained on the proper management of tilapia fish to avoid the spread and outbreak of disease. Furthermore, water quality parameters play a vital role in tilapia farming. In particular, an ideal parameter for water quality should be preserved to avoid "stress'' in fish, which could lead to infection events. The diagnosis of diseased and carrier fish could be achieved using fast and accurate molecular techniques and measurement of fish biochemical parameters. Also, the present study demonstrated that the m-PCR assay was a sensitive and specific diagnostic tool for simultaneous detection of fish streptococcosis caused by *E. faecalis, S. agalactiae,* and *E. faecium* from fish and water isolates, however only a limited range of isolates could be detected directly from multiplex PCR from fish tissues and water. Although chemotherapy was not really suggested, proper management, and immunization could be aspects of the system to prevent and control streptococcosis.

DECLARATION

Competing interests
The authors declare that they have no competing interests.

Author's contribution
Hala AM Abd El-Hady and Mayada AM Abou Zeid found a research idea, planned the study design, performed data, the bacteriological and molecular examinations, and drafted the manuscript. Gehan IE Ali shared the research's idea, shared designed work, and shared a collection of blood samples during the experiment and helped the manuscript preparation. All authors have read and approved the final manuscript.

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