

# Monitored Variations in Microbial Load of Aquacultured Tilapia Fish in Kafr El-Sheikh and El-Faiyum Governorates, Egypt

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## Abstract

The main aim of the Egyptian government is not only to increase tilapia fish production, but also to improve the quality of the fish produced. Contaminated water causes damage to farmed fish and is also considered an important factor affecting the fish quality. To avoid losses in fish farms, it is critical to obtain information on the microbial load. This research aimed to study the microbial load in four fish farms in the governorates of Kafr El-Sheikh and El-Faiyum (two farms in each governorate), in Egypt. Water and fish samples were collected at various locations within each farm pond for two seasons (autumn and spring) to assess the microbiological characteristics. Total coliform count of aquaculture water samples was the highest mean count of  $3.93 \times 10^3 \pm 1.81 \times 10^3$  CFU/ml in farm (3) of El-Faiyum. However, *Clostridium* counts were highest in farm (2) of Kafr El-Sheikh at  $1.85 \times 10^2 \pm 1.76 \times 10^2$  in autumn of 2021. Total coliform count ( $1.196 \times 10^3 \pm 8.38 \times 10^2$ ) was the highest in farm (1) of Kafr El-Sheikh in spring 2022. Also, *Salmonella* spp. was only detected in farm (2) of Kafr El-Sheikh in autumn 2021, but in spring 2022 it was positive in farm (3) of El-Faiyum. Nevertheless, *Listeria* spp. was not detected in the aquaculture water samples examined. In addition, *Salmonella* spp. was detected in farm (1) of Kafr El-Sheikh and in farms (3 and 4) of El-Faiyum in spring 2022. It was concluded that the presence of pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* and *Clostridium* in fish samples in some farms in Kafr El-Sheikh and El-Faiyum may contribute to foodborne illness. So that, the microbiological criteria are recommended to be applied as guidelines to develop additional control programs in fish farms. This can help in the pollution control of the agricultural drainage water that is used in aquaculture; consequently, prevent the growth and toxin production by pathogens

## KEYWORDS

Tilapia, *Salmonella*, *Listeria*, Fish quality, Fish farms, Kafr El-Sheikh, El-Faiyum, Egypt.

## INTRODUCTION

Fish is a vital source of human nutrition because of its protein nature, high content of unsaturated fatty acids, and low carbohydrate content (Hassanen *et al.*, 2018). Fish and fish products are an important source of readily digestible and delicious animal protein with high biological value. They comprise many essential amino acids, vitamins, polyunsaturated fatty acids, omega-3, essential minerals, and micronutrients (Nagy *et al.*, 2018). The primary objective of the Egyptian government is to increase the production of fish and improve the quality of the fish produced. One of the most important factors affecting fish quality is water quality (El-Nemaki *et al.*, 2008). The transformation of farmland into fish farms has several adverse effects on the environment (Khairy *et al.*, 2020). The aquaculture industry is a rapidly growing area in Egypt; however, this industry is impeded by many challenges such as poor water quality and associated bacterial infections (El-Gohary *et al.*, 2020). Tilapia fish is a white meat fish with an ever more significant Egyptian market. The main issues in the aquatic environment are wastewater pollution in Egypt and other countries. Tilapia is considered suitable for Egyptian cul-

tivation because its relatively fast growing and its flesh is firmly textured and finely appetizing fish for consumers. Tilapia is the most famous and popular fish in Egypt, the Middle East, and in countries with warm weather conditions. Fish production had to be increased in Egypt to meet the rising demand of the population (Belal *et al.*, 2012). Fish has been classified into the food category most frequently affected by foodborne outbreaks. *Salmonella* sp. and *Listeria monocytogenes* are associated with major food-borne illnesses and deaths each year. Pollution can cause the presence of improper water resources with organic matter and harmful pathogenic bacterial species of *Coliforms*, *Staphylococcus*, *Salmonella*, *Shigella*, and *Clostridium* which have human health hazards (Mahmoud *et al.*, 2016) (Osman *et al.*, 2017). *Salmonella* is considered a major food-borne illness that poses a serious and unacceptable public health threat in developed and developing countries. *Listeria monocytogenes* is also the source of a serious human illness called listeriosis. Serious clinical outcomes were often caused by listeriosis. (Hassanen *et al.*, 2018). Bacteria, fungi, and parasites are implicated due to economic losses and high mortality on fish farms. Apart from mortality, pathogens have a negative impact on feed conversion rates and

the total body weight of fish recovered after infection. Bacterial infections in fish are also found with a higher mortality incidence than parasitic infestations. (Shaalan *et al.*, 2018). The aim of this study was to detect the safety of fish samples and water from some farmed fish in Egypt. This study examined the microbiological quality of tilapia fish and the water supply of Egyptian aquaculture. Fish and water samples were collected from various governorates and private breeding fish (Kafr El-Sheikh and El-Fayum) in Egypt.

## MATERIALS AND METHODS

### Site description of the studied aquacultures

Four aquaculture farms in Kafr El-Sheikh and El-Faiyum governorates (2 farms from each governorate), in Egypt were selected for the present study. The selected sites were known for their high potential pollution due to the agricultural drainage as shown in Figure 1.

### Sampling seasons

Samples were collected in the first season of sampling during the autumn season (September 2021), while the second season of sampling was during the spring season of the following year (April 2022).

### Samples collection and handling

Water samples were collected in sterilized Falcon tubes, labeled, and charged in a refrigerated car to the research laboratory, and then stored at 4°C until the time of analysis (Figure 2). Tilapia fish samples were collected from the farms and kept in polystyrene boxes full of ice and then charged to the research laboratories in a refrigerated car. Once receive the fish samples, they were kept in polyethylene bags, categorized into groups with replicates, coded, and then stored in the refrigerator for quick bacteriological examination. Then the fish meat was sub-

jected to the analysis.

### Microbiological determinations

All samples were transferred to the laboratory under aseptic conditions. The external surface of the water falcon was sterilized using cotton cut to 70% ethanol. Additionally, the external surface of the fish plastic bag was sterilized with 70% ethanol. In this study, all samples of water and fresh fish samples were subjected to the following microbiological examination: Total plate count (TPC), Total Coliform count, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., Yeast and mold, and *Clostridium perfringens*. 25 grams were taken from each fish sample to homogenize in 225 ml of 0.1 % Buffered peptone water. The dilutions of fish samples were prepared from the samples up to 10<sup>4</sup>. Then bacterial counts were cultured in nutrient agar and selective media (Mannitol Salt Agar, Violet Red Bile (VRB) media, *Salmonella-Shigella* (SS) agar, Robertson's cooked meat (RCM), and Potato Dextrose Agar (PDA) for the detection of pathogenic microorganisms (*Staphylococcus*, *Coliforms*, *Salmonella*, *Shigella*, *Clostridium perfringens*, and yeast and mold). A sterile loop has been inserted in dead fish organs. Then inoculated in various solid selective media (*Salmonella Shigella* agar, Brain heart infusion, and Mannitol Salt Agar) described by Mahmoud *et al.*, (2016) and Eltholth *et al.* (2018). Bacteriological analysis of water and fish samples was performed according to the procedures described by (International Organization for Standardization (ISO, 2017) and Eltholth *et al.* (2018).

### Total bacterial count

Nutritional agar and pouring plate technique have been used to detect total bacterial counts after incubating at 37°C (24 hours) (Sanjee and Karim, 2016).

### Total Coliforms

According to ISO Method No. 4832:2005(E) (International Organization for Standardization (ISO) 2005), the dilutions of each

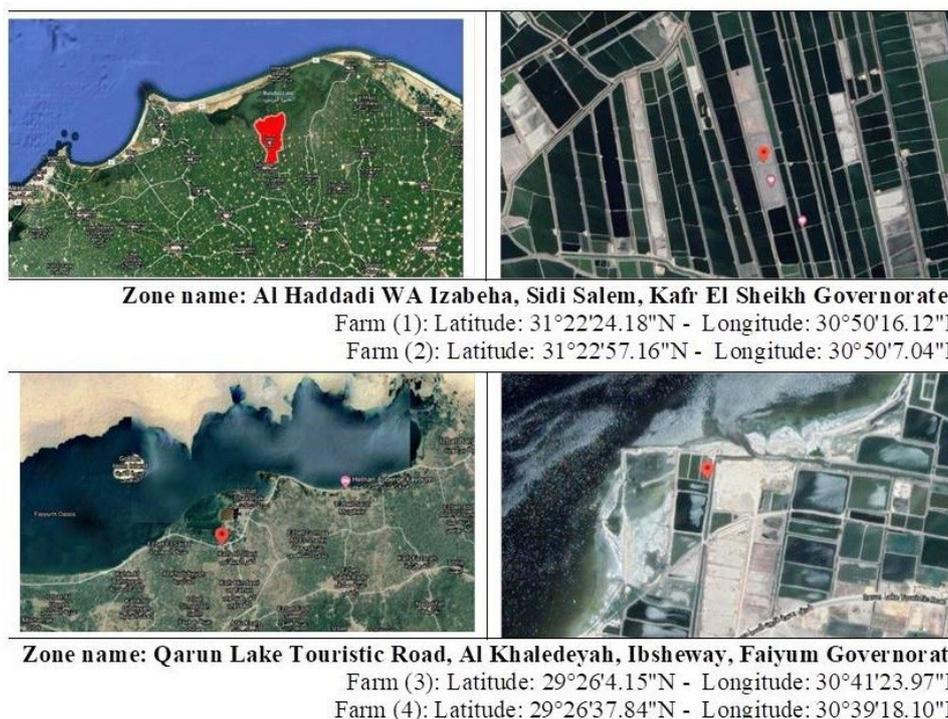


Fig. 1. Aquaculture sites in Kafr El-Sheikh and El-Faiyum governorates.

sample were inoculated in duplicate sterile petri dishes and then poured Violet red bile agar (VRBG) agar. The Inverted dishes were then incubated at 37°C for 24 h. The colonies were purplish red with a diameter of 0.5 mm and sometimes surrounded by a red-dish zone of precipitated bile.

#### Coagulase-positive *Staphylococcus aureus*

Each sample dilution was inoculated in a sterile dish then poured with Mannitol salt agar (MSA) and incubated at 37°C (24 h). The colonies were yellow colonies with a yellow halo. Followed by a Coagulase test to differentiate between *Staphylococcus aureus* and other *Staphylococcus* species.

#### *Salmonella* spp.

Isolation of *Salmonella* spp. by using ISO Method No. 6579.1:2017(E) protocol (International Organization for Standardization (ISO) 2017), the homogenate was prepared by incubation in Buffered peptone water (BPW) at 37°C for 18 h) for non-selective pre-enrichment. Then, 0.1 ml of pre-enriched culture was transferred in a tube of 10 ml of Rappaport-Vassiliadis medium (RVS) broth (41.5 °C for 24 h for Selective enrichment). After enrichment, a loopful of each enriched sample was streaked onto S.S. agar For Plating out on selective solid media and incubated at 37°C for 24 h. Then, confirmation of the presence of *Salmonella* spp. by biochemical testing of *Salmonella* spp. From streak TSI agar slant surface and stab the butt, then incubate for 24 h (37°C).

#### *Shigella* spp.

The dilution sample was inoculated in a sterile dish then poured with *Salmonella Shigella* Agar (SSA) then incubated for 24h (37°C) (Sichewo et al., 2013).

#### Yeast and mould

Each sample dilution was inoculated in a sterile dish then poured with Potato Dextrose Agar (PDA) and incubated at 28°C for three days (Haroon et al., 2014).

#### *Listeria monocytogenes*

*Listeria* spp. was tested according to ISO Method No. ISO 11290-1:2017(E) by homogenizing 10 g of each fish sample in 90 ml of half Fraser broth to incubate at 30°C for 24 h. Then 0.1 ml of half Fraser broth was transferred to 10 ml of Fraser broth to incubate for 48 h (30°C). Then a loopful was streaked onto PALCAM and the colonies were examined after 24 h and 48 h at 30°C. Biochemical tests occurred to confirm the presence of *L. monocytogenes*.

#### *Clostridium perfringens*

Aseptically inoculated samples in Robertson's cooked meat (RCM) and incubated at 37°C for 48 hours. Followed by taking the inoculum to seed onto 10% sheep blood agar and incubated at 37°C (24 h) anaerobically. Then confirmation by biochemical tests such as fermentation of glucose and lactose (Das and Jain, 2012).

## RESULTS AND DISCUSSION

### Microbiological examination of aquaculture water samples

#### Season 1

Bacteriological quality is important for public health because it is directly linked to fish degradation and can cause food poisoning. As a result, it is important to monitor the quality of aquaculture water. Furthermore, bacteriological examination of the water source of fish farms is very important to detect the type of bacteria which may be transferred into fish. It can provide guid-



Fig. 2. Aquaculture water samples and Tilapia fish samples.

ance for monitoring and protecting the health of the environments of fish. Water samples collected from Kafer El-sheikh farms and El-Fayoum farms were contaminated with all the realization micro-organisms. Microbial examination of water on selective media revealed the presence of bacterial strains that are pathogenic to human health because of wastewater pollution. Microbiological results for water were summarized in Table 1. Water samples from Kafer El-Sheikh and El-Fayoum farms revealed the presence of different pathogenic bacteria as *Coliforms* in all samples (100%) as shown in Tables 1. Water samples from El-Fayoum farms showed coliform (cfu/ml) ( $1.33 \times 10^4 \pm 1.54 \times 10^4$ ) and from Kafer El-Sheikh farms ( $2.01 \times 10^3 \pm 1.45 \times 10^3$ ). In this concern, coliform isolation indicated a high number of environmental bacteria and/or wastewater pollution. It can also include biological indicators of faecal contamination at these sites in Egypt (El Faiyum, Kafer El-Sheikh). Coliform detection serves as an overall indicator of the state of health and their presence indicates an appreciably higher risk of pathogens. *Salmonella* sp. was isolated in 16.66% of water samples from Kafer El-Sheikh farms, while 73.33% of water samples from El-Fayoum tested positive for *Salmonella* spp. In Egypt, Enterobacteriaceae may have been contaminated in a variety of locations, as some researchers have shown (Karthiga, et al., 2016; Mahmoud, et al., 2016; Osman, et al., 2017). The overall picture of the present study showed that the mean counts of water samples from Kafer El-Sheikh were  $3.65 \times 10^2 \pm 7.25 \times 10^2$ ,  $3.65 \times 10^2 \pm 7.25 \times 10^2$ ,  $2.80 \times 10^2 \pm 2.17 \times 10^2$  and  $1.30 \times 10^2 \pm 1.28 \times 10^2$  cfu/ml for *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp. and *Clostridium* spp., respectively. On the other hand, the mean counts of water samples collected from El Faiyum were  $2.00 \times 10^2 \pm 1.43 \times 10^2$ ,  $6.18 \times 10^2 \pm 9.3 \times 10^2$ ,  $2.87 \times 10^2 \pm 1.88 \times 10^2$  and  $2.50 \times 10^2 \pm 3.50 \times 10^2$  cfu/ml for *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp. and *Clostridium* spp., respectively.

Season 2

Obtained data in Table 2 showed that water samples from Kafer El-Sheikh showed different pathogenic bacteria as Coliform (cfu/ml) ( $7.13 \times 10^2 \pm 1.00 \times 10^2$ ,  $1.196 \times 10^3 \pm 8.38 \times 10^2$  &  $6.16 \times 10^2 \pm 0.57 \times 10^2$ ) collected from different sites in farm (1) as inlet, pond and outlet, respectively but the water samples from farm (1) was free from *Salmonella* spp. In this concern, Coliform isolation indicated a number of environmental wastewater pollution in this farm. The study showed that the mean counts of water samples from pond site in farm (1) from Kafer El-Sheikh were  $1.33 \times 10^3 \pm 0.57 \times 10^3$ ,  $6.15 \times 10^2 \pm 0.57$  and  $6.65 \times 10^2 \pm 0.57$  cfu/ml for *Staphylococcus aureus*, *Shigella* spp. and *Clostridium* spp., respectively. On the other hand, the mean counts of water samples collected from pond in farm (2) were  $7.33 \times 10^2 \pm 0.57 \times 10^2$ ,  $2.33 \times 10^3 \pm 0.57 \times 10^3$  &  $1.66 \pm 0.57 \times 10^2$  cfu/ml for *Staphylococcus aureus*, *2.33 \times 10^3 \pm 0.57 \times 10^3 and  $1.66 \pm 0.57 \times 10^2$  for Coliform, *Staphylococcus aureus* and *Clostridium* spp., respectively. The results of water samples in pond from farm (3) in El Faiyum showed that the mean as  $3.04 \times 10^2 \pm 1.60 \times 10^2$ ,  $3.93 \times 10^2 \pm 0.57 \times 10^2$ ,  $1.0 \times 10^2 \pm 2.00 \times 10^2$  and  $8.3 \pm 0.57 \times 10^2$  cfu/ml for Coliform, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp. and *Clostridium* spp., respectively However, the farm pond (4) was not contaminated with *Salmonella* spp. and *Shigella* spp. but had Coliform and *Staphylococcus aureus* as  $3.48 \times 10^2 \pm 1.15 \times 10^2$  and  $3.16 \times 10^2 \pm 0.57 \times 10^2$ , respectively. Other research reported that water results show the highest values of total bacterial count and *Coliforms* as  $63.17$  &  $27.17 \times 10^5$  CFU/ml and  $10.77$  &  $5.35 \times 10^4$  CFU/ml, at two stations 5 and 3, respectively (Farouk, 2018). Aboseif et al., (2022) found the mean aerobic heterotrophic bacteria in water were ranged from  $7.5 \pm 9.2 \times 10^4$  to  $60 \pm 11.3 \times 10^4$  CFU ml<sup>-1</sup> (Aboseif et al., 2022).*

Table 1. Microbiological examination of aquaculture water samples (Autumn 2021) in season 1.

Sites	TPC	Coliform	<i>S. aureus</i>	<i>Salmonella</i>	<i>Shigella</i>	Fungi	<i>Clostridium</i>
kafr El-Sheikh Farms							
F1	$7.00 \times 10^3 \pm 3.60 \times 10^3$	$3.23 \times 10^3 \pm 1.79 \times 10^3$	$1.50 \times 10^2 \pm 7.0$	-ve	20	$9.0 \times 10^2 \pm 9.84 \times 10$	$30.0 \pm 1.41 \times 10$
F2	$4.00 \times 10^4 \pm 4.35 \times 10^4$	$2.10 \times 10^3 \pm 1.05 \times 10^3$	-ve	$1.0 \times 10$	$6.0 \times 10$	$4.20 \times 10^2 \pm 3.98 \times 10^2$	$1.85 \times 10^2 \pm 1.76 \times 10^2$
El-Faiyum Farms							
F3	$1.80 \times 10^4 \pm 10^3$	$3.93 \times 10^3 \pm 1.81 \times 10^3$	$1.30 \times 10^2 \pm 1.27 \times 10^2$	-ve	$2.0 \times 10 \pm 1.41 \times 10$	$1.40 \times 10 \pm 5.29$	$6.0 \times 10 \pm 7.07 \times 10$
F4	$2.07 \times 10^4 \pm 1.93 \times 10^4$	$2.30 \times 10^3 \pm 1.08 \times 10^3$	$2.55 \times 10^2 \pm 2.19 \times 10^2$	-ve	$2.0 \times 10 \pm 1.41 \times 10$	$2.03 \times 10^2 \pm 2.08 \times 10^2$	-ve

Table 2. Microbiological examination of aquaculture water samples (Spring 2022) in season 2.

Sites	TPC	Coliform	<i>S. aureus</i>	<i>Salmonella</i>	<i>Shigella</i>	Fungi	<i>Clostridium</i>
kafr El-Sheikh Farms							
F1	$9.66 \times 10^2 \pm 2.08 \times 10^3$	$1.196 \times 10^3 \pm 8.38 \times 10^2$	$1.33 \times 10^2 \pm 0.57 \times 10^2$	-ve	$6.15 \times 10^2 \pm 0.57$	$6.16 \times 10^2 \pm 1.15 \times 10^2$	$6.65 \pm 0.57 \times 10^2$
F2	$9.80 \times 10^4 \pm 1.52 \times 10^4$	$7.33 \times 10^2 \pm 0.57 \times 10^2$	$2.33 \times 10^2 \pm 0.57 \times 10^2$	-ve	-ve	$4.66 \times 10^2 \pm 1.00 \times 10^2$	$1.66 \pm 0.57 \times 10^2$
El-Faiyum Farms							
F3	$2.70 \times 10^5 \pm 0.30 \times 10^5$	$3.04 \times 10^2 \pm 1.60 \times 10^2$	$3.93 \times 10^2 \pm 0.57 \times 10^2$	$1.0 \times 10^2 \pm 2.00 \times 10^2$	$1.66 \times 10^2 \pm 0.57 \times 10^2$	$1.10 \times 10^2 \pm 2.00 \times 10^2$	$8.3 \pm 0.57 \times 10^2$
F4	$2.03 \times 10^5 \pm 0.20 \times 10^5$	$3.48 \times 10^2 \pm 1.15 \times 10^2$	$3.16 \times 10^2 \pm 0.57 \times 10^2$	-ve	-ve	$5.33 \times 10^2 \pm 1.52 \times 10^2$	-ve

Cfu: colony forming units; -ve: negative.

Table 3. Microbiological examination (CFU/ml) of Tilapia fish samples (autumn 2021) in season 1.

Sites	TPC	Coliform	<i>S. aureus</i>	<i>Salmonella</i>	<i>Shigella</i>	Fungi	<i>Clostridium</i>
kafr El-Sheikh Farms							
F1	$5.00 \times 10^3 \pm 6.06 \times 10^3$	$6.33 \times 10^2 \pm 3.51 \times 10^2$	$4.33 \times 10^2 \pm 5.77 \times 10^2$	-ve	$1.00 \times 10^2 \pm 0.00$	$3.66 \times 10^2 \pm 1.15 \times 10^2$	-ve
F2	$2.90 \times 10^4 \pm 4.00 \times 10^3$	$3.33 \times 10^2 \pm 1.52 \times 10^2$	$1.46 \times 10^3 \pm 4.16 \times 10^2$	$0.33 \times 10^2 \pm 0.57 \times 10^2$	$2.33 \times 10^2 \pm 0.57 \times 10^2$	$4.33 \times 10^2 \pm 2.57 \times 10^2$	$1.00 \times 10^2 \pm 1.00 \times 10^2$
El-Faiyum Farms							
F3	$2.80 \times 10^3 \pm 1.90 \times 10^3$	$4.00 \times 10^2 \pm 1.00 \times 10^2$	$4.33 \times 10^2 \pm 2.08 \times 10^2$	-ve	$1.00 \times 10^2 \pm 1.00 \times 10^2$	$4.66 \times 10^2 \pm 1.15 \times 10^2$	-ve
F4	$4.90 \times 10^3 \pm 3.60 \times 10^2$	$3.66 \times 10^2 \pm 5.77 \times 10^2$	$4.66 \times 10^2 \pm 0.57 \times 10^2$	-ve	$1.00 \times 10^2 \pm 1.00 \times 10^2$	$4.66 \times 10^2 \pm 0.57 \times 10^2$	-ve

Values are expressed as (Mean ± Std). cfu: colony forming units; -Ve: negative.

Table 4. Microbiological examination (CFU/ml) of Tilapia fish samples (spring 2022) in season 2.

Sites	TPC	Coliform	<i>S. aureus</i>	<i>Salmonella</i>	<i>Shigella</i>	Fungi	<i>Clostridium</i>
kafr El-Sheikh Farms							
F1	1.86×10 <sup>5</sup> ±1.15×10 <sup>4</sup>	2.66×10±0.57×10	5.49×10±2.08×10	5.0±1.00×10	8.33±0.57×10	5.33±1.00×10	-ve
F2	2.64×10 <sup>5</sup> ±2.08×10 <sup>4</sup>	1.5×10±1.00×10	4.33×10±1.52×10	-ve	-ve	5.33×10±0.57×10	-ve
El-Faiyum Farms							
F3	1.02×10 <sup>5</sup> ±1.52×10 <sup>4</sup>	2.33×10±1.15	1.99×10 <sup>2</sup> ±0.57×10	0.66×10±0.57×10	1.66×10±1.00×10	4.99×10±0.57×10	1.00×10±0.00
F4	3.53×10 <sup>5</sup> ±1.52×10 <sup>4</sup>	2.33×10±0.57×10	4.33×10±2.30×10	1.00×10±0.00	0.66×10±0.57×10	2.64×10 <sup>2</sup> ±0.57×10	1.00×10±0.00

Values are expressed as (Mean ± Std), cfu: colony forming units; -Ve: negative.

### Bacteriological hazards in Tilapia fish

#### Season 1

The results of fish samples collected from farms (1) and (2) of kafer El-Sheikh were free from *Salmonella* spp. and did not contain *Clostridium* spp. in farm (1). However, the farm (3) showed that the mean of *Salmonella* spp. at El Faiyum were  $0.33 \times 10 \pm 0.57 \times 10$ . On the other hand, farms (3) and (4) in El Faiyum did not show *Clostridium* spp. in Table 3. Also, The results of fish samples from the farm (3) in El Faiyum showed that the mean values were  $2.80 \times 10^3 \pm 1.90 \times 10^3$ ,  $4.00 \times 10^2 \pm 1.00 \times 10^2$ ,  $4.33 \times 10 \pm 2.08 \times 10$ ,  $1.00 \times 10 \pm 1.00 \times 10$  and  $4.66 \times 10 \pm 1.15 \times 10$  cfu/ml for TPC, Coliform, *Staphylococcus aureus*, *Shigella* spp., and fungi, respectively. However, the farm pond (4) had TPC, Coliform and *Staphylococcus aureus* as  $4.90 \times 10^3 \pm 3.60 \times 10^2$ ,  $3.66 \times 10^2 \pm 5.77 \times 10$  and  $4.66 \times 10^2 \pm 0.57 \times 10$ , respectively. The streptococcal disease is not limited to geographic boundaries or host range, resulting in a global outbreak on aquaculture farms. Streptococci are an emergent pathogen of Nile tilapia aquaculture in Egypt (Osman et al., 2017). In the present study, the dead samples revealed streptococcus species isolated from the hemorrhagic kidney. *Streptococcus iniae* causes heavy mortalities on aquaculture farms worldwide. A previous study detected fourteen isolates of *Streptococcus iniae* from cultured Nile tilapia (*O. niloticus*) at Kafr El-Shiekh Governorate (Younes, et al., 2019). Aboseif et al. (2022) showed the mean aerobic heterotrophic bacteria in fish ranged from  $41 \pm 1.4 \times 10^5$  to  $257 \pm 15.6 \times 10^7$  CFUg<sup>-1</sup> (Aboseif et al., 2022).

#### Season 2

Fish samples collected from farms (2) of kafer El-Sheikh were free from *Salmonella* spp., *Shigella* spp. In addition, the farm (1) did not contain *Clostridium* spp. However, the farm (1) showed that the mean values of *Salmonella* spp. and *Shigella* spp. were  $5.00 \pm 1.00 \times 10$  and  $8.33 \pm 0.57 \times 10$ , respectively in Table 4. On the other hand, the fish samples taken at the farm (3) at El Faiyum were presented  $1.99 \times 10^2 \pm 0.57 \times 10$ ,  $0.66 \times 10 \pm 0.57 \times 10$  and  $1.00 \times 10 \pm 0.00$  for *Staphylococcus aureus*, *Salmonella* spp. and *Clostridium* spp., respectively. Furthermore, the farm (4) demonstrated the presence of different pathogenic bacteria in fish samples such as *Coliforms*, *Staphylococci* and *Salmonella* in Table 4. A previous study found *Aspergillus flavus* and *Aspergillus niger*, which were the most common fungi isolated from Nile tilapia fish farms at Kafr El Sheikh (Abd El Tawab et al., 2020). Another study showed that *Salmonella* sp. has been undetected at Khor Al Ramla fish samples also, reported that pathogenic bacteria were detected in greater numbers in fish collected from Khor Abu Simbel than from Khor Al Raml (Ali, 2022).

## CONCLUSION

The risk of pathogenic bacteria can be eliminated by applying proper management to farmed fish, such as water supply and control of wastewater pollution. In addition, control the risk of bacterial illness in farmed fish, e.g. *Salmonella* spp. to prevent the threat of health problems on farms and pathological tissue changes, as well as the loss of fish in farms. At the same time,

consumers are advised not to eat fish that is not adequately cooked. To maximize the total production of fish farms and ensure the sustainability of the Egyptian aquaculture industry with regular surveillance and detection of fish free of disease, antibiotic residues, and toxic contaminants should be established.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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