

# Detection of *Staphylococcus aureus* from fish and water samples collected from Lake Qarun

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**Abstract**—*Staphylococcus aureus* is considered to be one of the most frequently prevailing food-borne pathogen worldwide. The number of outbreaks and number of cases of staphylococcal gastroenteritis is much higher than several other microbial food borne diseases outbreaks. *Staphylococcus* spp. is one of the zoonotic bacteria which could be found on fish and also could reach the aquatic environment as it was isolated from different water types (fresh or brackish water) in many countries. The present study focused on detection of total staphylococci and *S.aureus* in Lake Qarun,, Egypt. *Staphylococci* isolates were isolated from Lake Qarun, water and Nile Tilapia (*Oreochromis niloticus*) fish within the period of 2010 to 2011. The determination of the four hundred typical colonies of total staphylococci and one hundred and eighty of typical colonies of *S.aureus* were carried out using surface plate technique. Moreover, molecular and biochemical confirmation of the *S.aureus* isolates were carried out by PCR analysis and biochemical reactions. Three hundred and twenty six isolates were total staphylococci +ve when tested by PCR. Also, one hundred and eighty isolates were *S.aureus* isolates +ve when confirmed by biochemical reactions.

**Index Terms**— Lake Qarun,, Nile Tilapia, PCR, *Staphylococcus aureus*.

## 1 INTRODUCTION

Natural lakes and reservoir surface water are a major source of freshwater for agricultural, industrial and domestic purposes worldwide. Environmental pollution, especially concerned with water sources has become of public interest. For both developed countries that have been affected and developing countries suffer from impact of pollution [1, 2, 3]. Water bodies are continually used as receptacles for untreated wastewater or poorly treated effluents increased

from industrial activities. This may provide water bodies unsuitable for both primary and/or secondary usage [4, 5].

Lake Qarun, one of the largest lakes in Egypt, is a residue of a bigger one, "Lake Moreis ", which was natively a fresh water lake. But for now, it is considered to be a closed basin for different types of wastewater drainage of EL-Fayoum governorate. Most of the discharge is carried out to the lake through two drains (EL- Batts and EL-Wadi Drain). Lake Qarun is an important source of fishing in Fayoum Province. However, Lake Qarun, has been subjected to high level of pollution by agricultural drainage water and raw domestic sewage. Water sources contamination by wastewater is a severe health risk problem due to the abundance of pathogenic microbial agents in recreation water.

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Total staphylococci or specific *Staphylococcus aureus* enumeration is a useful tool for water quality evaluation in different aquatic environments [6, 7].

*Staphylococcus aureus* determination considered as a new parameter for a long time for seawater quality evaluation. *S. aureus* presence in marine environments has been related to the number of bathers and may cause diseases in skin, eye or ear [8]. Also, *S. aureus* enterotoxins are another serious problem which causes

Water and Tilapia fish samples were collected and immediately transferred within 2-4 hours to the laboratory in NRC. Where decimal dilution method was used for detection and enumeration of staphylococci group in water samples while 3 fish samples were externally decontaminated through washing by distilled water several times then by (70%) ethanol. Fishes were dissected and 3 organs were examined for the presence of staphylococci group: liver, gills and muscle. Where 1gm of each homogenized fish organ sample was added to 9 ml of 9% saline solution then used in the experiments.

Table. 1. Lake Qarun, sampling sites description

Site	Site description
1Q	El-Batts drain.
2Q	Lake Qarun, after the mixed point with El-Batts drain by 3 km.
3Q	Lake Qarun, after the mixed point with El-Batts drain by 10 km.
4Q	Lake Qarun, after the mixed point with El-Batts drain by 30 km.

## 2.2 Staphylococci group determination by surface plate technique

gastroenteritis after fish and related products consumption. Thus, this study has been conducted to detect *S. aureus* in water and fish samples at Lake Qarun, at different pollution sites.

## 2 MATERIALS AND METHODS

### 2.1 Samples collection

Water sample were collected monthly (from April 2010 to March 2011) from four different sites as shown in Table 1, while Tilapia fish samples were collected from 2 different sites only (1Q and 3Q).

Detection and enumeration of staphylococci group from water and Tilapia fish samples were carried out by surface plate count technique using Baird Parker agar according to APHA 2005 [9].the plates were incubated for 1-2 days at 37°C afterward five typical colonies from cultured plates were isolated and streaked on tryptic soya agar (TSA) slants and stored at 4°C.

### 2.3 Confirmation of total staphylococci and *S. aureus* by PCR:

Extractions of DNA from all samples were done as described by Kapperud et al. 1993 and Waage, A.S. et al.1999 [10, 11]. PCR assays targeting the (tuf) gene sequence has been developed for staphylococci genus as described by Martineau, F.et al.1998 [12]. The two primers used in PCR were: TStag422 (5'-GG CC GT GT TG AA CG TG GT CA AA TC A-3') and TStag765 (5'-TI AC CA TT TC AG TA CC TT CT GG TA A-3'). and another pair of *S. aureus*-specific primers: Saa-442F (5'-GT CG GT AC AC GA TA TT CT TC AC G-3'), Saa442-R (5'-CT CT CG TA TG AC CA GC TT CG GT AC-3') were used to amplify 108bp which target the Sa442 gene [12].

PCR procedures were done using applied biosystems Gene Amp PCR system 9700.The PCR products were separated on 2% Agarose gel, stained with (0.5µg/ml) ethidium bromide where a 1 kb DNA Ladder (Promega) was used as a molecular weight standard on gel as described by

Sambrook, J. et al.1989 [13]. Then gel was photographed by gel documentation system (Biometra bioanalysis).

## 2.4 Identification of staphylococcus aureus by biochemical reactions

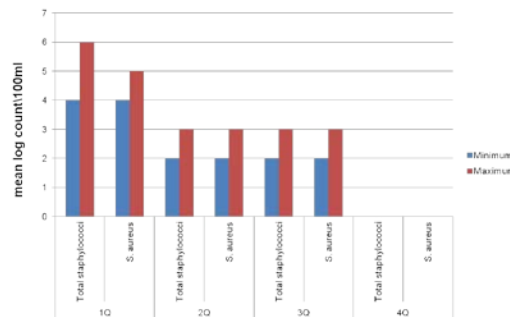
Isolates were microscopically examined after Gram staining to ensure purity. Catalase, Coagulase, Sugar fermentation [14] as well as DNase production [15] tests were also performed.

## 3 RESULTS AND DUSCUSSION

The mixing of Qarun lake water with different types of wastewater drains including agricultural and municipal origin are heavily contaminated with various types of pathogenic microorganisms cause a severe effect on public health. A large number of epidemics due to the presence of several types of pathogens (Staphylococcus and Aeromonas bacteria) in the environment as reported by Moe, C.L.et al.2002 [16].

### 3.1 Density of total staphylococci and S. aureus

Results of this study were represented in Figures (1, 2 and 3) which show the density of total staphylococci and *S.aureus* in water and Tilapia fish organ samples collected from El-Batts drain and Lake Qarun, at different pollution sites using surface plate count technique were recorded during one year (April 2010 – March 2011).



Figure(1). log counts of total staphylococci and *S. aureus* in water samples collected from Qarun lake during one year

Figure (1) demonstrate the gradual increase in total staphylococci and *Staphylococcus aureus* density occurred after El-Batts drain is mixed with Qarun lake in sites 2Q and 3Q, with a maximum level shown in site 3Q. However, site 4Q showed no total staphylococci or *staphylococcus aureus* density which may be attributed to lake self purification. The above results are contradicted with study of Mansour, A.A.et al.2003 [17], who investigated some pathogenic bacteria in Lake Qarun, as there results showed that both salmonella and staphylococci bacteria were not detected in all water samples examined in Lake Qarun, at different sites. This finding may reflect the deterioration of Lake Qarun, microbial quality from 2003 till 2011.

Figure (2) and (3). Shows the density of total staphylococci and *S.aureus* collected from Tilapia fish organ samples. The results revealed that density of total staphylococci and *Stapylococcus aureus* in different fish organs are much higher in fish captured from El-Batts drain than in fish obtained from Lake Qarun,. This finding may be due to the salinity of water or because Lake Qarun, receives many effluents of drains contain industrial wastewater.

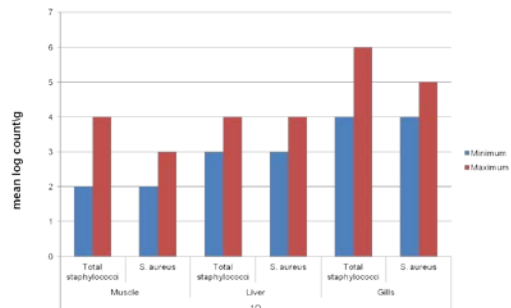
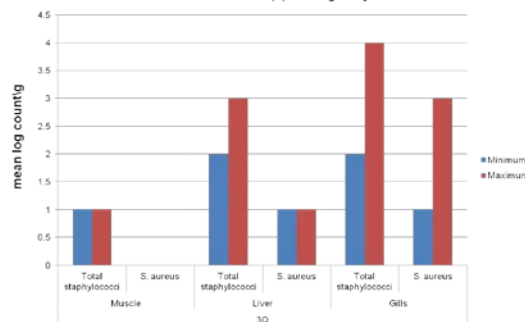


Figure (2). Log counts of total staphylococci and S. aureus in tilapia organ samples collected from site (1)Q during one year



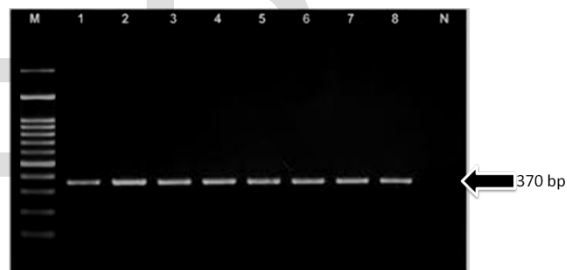
Figure(3). Log counts of total staphylococci and S. aureus in tilapia organ samples collected from site (3)Q during one year

### 3.2 Confirmation of total staphylococci group by PCR technique

Table (2) and Figure (4) demonstrate the results of total staphylococci in water and fish samples from El-Batts drain and Lake Qarun,. Determination of total staphylococci was accomplished by surface plate technique then confirmed by PCR. PCR confirmation results showed that only 326 isolate was +ve staphylococci group with a percentage of 81.5%. It is important to mention that PCR failed to identify all S.aureus isolates and this may be attributed to the primer or the condition used in this test.

Samples	Area description	No. of isolates tested	No. of +ve total staphylococci		
			No	%	
Water	Site 1Q	60	48	80	
	Site 2Q	45	41	91.1	
	Site 3Q	45	40	88.8	
	Site 4Q	0	0	0	
Fish	Site 1Q	Muscle	30	21	70
		Liver	35	25	71.4
		Gills	60	45	75
	Site 3Q	Muscle	30	24	80
		Liver	50	42	84
		Gills	45	40	88.8
Total isolates		400	326	81.5	

Table (2). The positive determination percentages of total staphylococci in water and fish organ samples.



Fig( 4). Agarose gel showing results of PCR with positive control (lane 1), water (lane 2-4), fish (Lanes 5-7) isolates of Staphylococci spp and negative control (lane 8).

Marker:  
100,200,300,400,500,600,700,800,900,1000,1500,3000 bp.

### 3.3 Confirmation of S. aureus by biochemical reactions:

Results in Table (3) showed the presence of S.aureus in water and Tilapia fish organ samples collected from different sites in Lake Qarun,. Also Table (4) represents the results of S.aureus isolates

identification by different biochemical tests. Where all *S. aureus* isolates were found to be gram positive bacteria and were able to ferment mannitol, glucose, trehalose sugar and positive to coagulase, catalase and DNase tests.

Table (3). The positive determination percentages of *S.aureus* in water and different fish organ samples.

Samples	Area description	No. of isolates tested	No. of +ve <i>S.aureus</i>		
			No	%	
Water	1Q	20	20	100	
	2Q	20	20	100	
	3Q	20	20	100	
	4Q	20	20	100	
Fish	1Q	Muscle	10	20	100
		Liver	20	20	100
		Gills	20	20	100
	3Q	Muscle	10	20	100
		Liver	20	20	100
		Gills	20	20	100
Total isolates		180	180	100	

Table (4). Identification of *S.aureus* isolated from water and fish samples by biochemical tests.

Biochemical tests		<i>S.aureus</i>	Others
Biochemical reaction	Gram stain	+	-
	Catalase	+	-
	Coagulase	+	-
Sugar fermentation & gas production	Mannitol	+	-
	Glucose	+	-
	Trehalose	+	-
DNase		+	-

## 4 Conclusion

From pervious results, it can be concluded that:

- Fish muscle contain lower or even no bacterial counts than liver and gills, while gills

contained the maximum bacterial counts especially those collected from polluted regions.

- Lake Qarun, water quality must be improved as soon as possible through prior treatment for industrial wastewater, domestic wastewater must be treated before discharge and managing agriculture wastes to reduce lake pollution.

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