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STAPHYLOCOCCUS AUREUS AND FECAL INDICATORS IN EGYPTIAN COASTAL WATERS OF AQABA GULF, SUEZ GULF AND RED SEA

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ABSTRACT

The occurrence of *Staphylococcus aureus* (*S. aureus*) and conventional fecal indicators bacteria namely total coliform (TC), *E. Coli* (EC) and fecal streptococci (FS) were investigated, in total of 200 coastal water samples, at 40 sites during January, March, May, July and September 2003. Based on national and international bacterial standards, 107 samples (53.5%), 18 samples (9.0%), 39 samples (19.5%) and 43 samples (21.5%) were found to exceed the guide values for *S. aureus,* TC, EC and FS respectively. Assosiation between *S. aureus* counts and levels of recreational water activities was observed. No significant correlation has been found between *S. aureus* counts and the measured hydrographical parameters as well as the other examined fecal indicators. At the same time, TC, EC and FS were significantly correlated with each others. The number of samples that harbour *S. aureus,* exceeded the guide values, without the other fecal indicators were found in 35.3% of the examined samples compared with 14.5% of samples that harbour *S. aureus* with EC and / or *S. aureus* with FS. These results may suggest that monitoring *S.aureus* as supplementary indicator, especially in heavy recreational beaches, is recommended for judging the sanitary quality of coastal waters in these areas.

INTRODUCTION

Egyptian Red Sea, Aqaba and Suez Gulfs coastal waters are highly valued as recreational resources and are widely utilized for a range of activities including bathing, sailing, boating, various forms of surfing and underwater diving. Preservation and restoration of sanitary water quality is of utmost integral to the sustainable use of these coastal waters for these purposes.

For several decades management of such waters has relied upon the use of conventional bacteriological indicators (i.e., total coliforms, *E. coli* and fecal streptococci) for assessment of suitability of water for recreational activities in term of public health risk (Fattal *et al*. 1987; Ferley *et al.* 1989; Cheung *et al.* 1990; Balarajan *et al.* 1991; Corbett *et al.* 1993; Fleisher *et al.* 1996 and 1998; Prieto *et al.* 2001; Wade *et al.* 2003). These organisms are derived from the intestinal tract of warm-blooded animals, and therefore indicate the presence of enteric pathogens. However, illness due to recreational waters are not limited to enteric diseases, but also associated with the upper respiratory tract, eyes, ears and skin. This has been documented in several epidemiological studies (Stevenson 1953; Favero *et al.* 1964; Foster *et al.* 1971; Mujeriego *et al.* 1983,).

All strains of coagulase-positive *staphylococcus aureus* are potential pathogens, causing a wide range of infection. It is responsible for purulent infections, including boils, ear infections, infected cuts and scratches (Evans 1977; Yoshpe-Purer & Golderman 1987). The pathogen is found in

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nasal membranes, hair follicles, skin and perinea of worm-blooded animals (Favero 1985). Their origin in bathing waters is undoubtedly human activity and was found to be shed by bathers under all conditions of swimming from mouth, nose, throat and skin surface and cavities (Charoenca & Kungstkulniti 2001). *Staphylococcus aureus* have been recommended as an index for bather pollution in swimming pools (Favero *et al.* 1964; Robenton & Mood 1966; Tosti & Volterra 1988; Rigas *et al.* 1998), since these bacteria are more chlorine resistant than coliforms, as well as in marine recreational waters (Yoshepe-Purer & Golderman 1988; Cheung *et al.* 1990; Charoenca & Fujioka 1993; Bruni *et al.* 1997; Prieto *et al.* 2001). The 16th edition of standard Methods for the Examination of water and wastewater (American Public Health Association 1989) identifies staphylococci as among the pathogens of human origin in natural bathing beaches.

This work aims to study the hygienic status of Egyptian coastal waters of Aqaba Gulf, Suez Gulf and Red Sea. The possibility of using *S.aureus* as supplementary indicator to the conventional bacterial indicators was another goal.

MATERIALS & METHODS

Study area

Red Sea is a deep semi-enclosed and narrow basin, lies between 12^o-30^o N and 32^o-44^o E and has a length of about 1930 km and an average width of 280 km (Fig.1) (Morcos 1970). It is connected to the Indian Ocean through Bab El-Mandab strait and extends north wards to Sinai Peninsula which divides it into the shallow Suez Gulf (250 km long, average width of 32 km and average depth of 64 m) and deep Aqaba Gulf (150 km long, average width of 16 km and average depth of 650 m). The average depth of the Red Sea is 490 m (Murty & El-Sabh 1984). Forty sampling sites were fixed to represent the recreational areas as well as towns, ports, fishing ports and some protectorate areas. Twelve sites were in the Aqaba Gulf (A), fourteen sites in the Suez Gulf (S) and fourteen sites along the Red Sea proper (R).

Sampling

A total of 200 duplicate water samples were collected five times from forty sites in January, March, May, July, and September during 2003. The code numbers and location names of these sites are shown in Fig.1. Seawater sampling technique was done according to the International Organization for Standardization (IOS) 5667/9 (1992) using 500ml sterile bottles and special sampler, on 50 cm below water surface in a shallow of about 1m deep.

Microbiological analysis

The bacteriological analysis for *Staphylococcus aureus* (*S. aureus*), total coliform (TC), *E. coli* (EC), and fecal streptococci (FS) were performed in situ after sampling in mobile laboratory. The membrane filtration technique using 0.45µm membranes (Gelman) was used. Appropriate volumes of samples (0.1, 1.0, 10 and 100 ml) were filtered and the membranes were placed onto m-Endo-les agar for counting (TC) followed by incubation at 37^{o}C for 24 h. Thermophilic *E. coli* (EC) counts were performed using m-FC medium followed by incubation at 44.5^{o}C (water bath) for 24h. However m-Enterococcus agar medium was used for detection of FS followed by incubation at 37^{o}C for 48 h. Ten random characteristic colonies from each sample / media were sub-cultured and confirmed according to IOS 9308/1 (1990) and IOS 7899/2 (1984). For detection of *S. aureus* the membranes were placed onto nutrient agar and incubated at 30^{o}C for two hours to allow maximum recovery of injured cells, thereafter the membranes were carefully moved and placed onto mannitol salt agar medium followed by incubation at 37^{o}C for 24h. The confirmatory tests including microscopic examination, catalase, aerobic and anaerobic fermentation of mannitol, DN_{ase} and coagulase were done for ten random colonies.

The final counts for all bacteria were calculated as CFU/100 ml. All confirmatory tests were done at the National Institute of Oceanography and Fisheries (NIOF) laboratories in Suez and Hurghada.

Hydrographical analysis

Hydrographical parameters including water temperature (°C), salinity (S‰), dissolved oxygen (DO) mg/l and pH were measured in situ at each sites using CTD (YSI-6000).

Statistical analysis

Correlation matrix between different investigated bacteria and hydrographical parameters was done for testing the relationship between variables. Raw data for bacteria were transformed by adding value of 0.1 to all scores in order to eliminate zero.

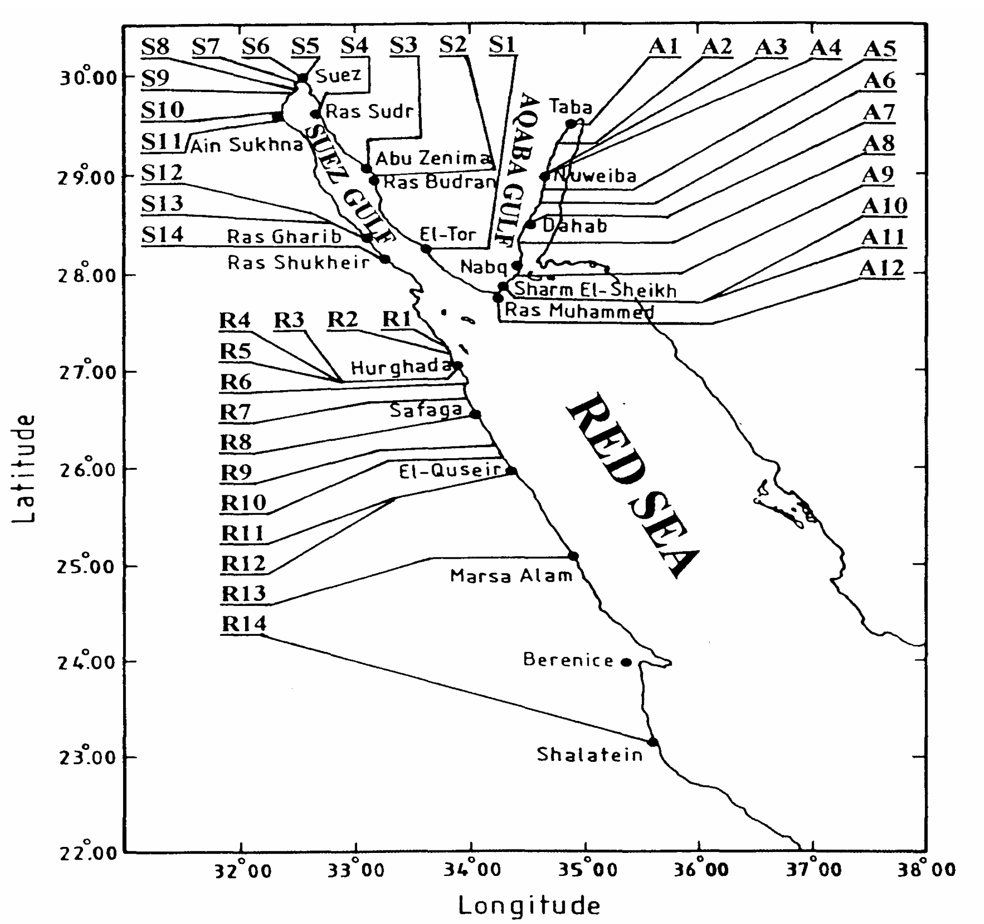

Fig. (1) Map showing the names and sites code of 40 sampling locations for Aqapa Gulf (A), Suez Gulf (S) and Red Sea (R).

RESULTS AND DISCUSSION

The spatial and temporal variation of the obtained counts of *S. aureus*, TC, EC and FS are graphically illustrated in Fig. 2. The annual means of the hydrographical parameters as well as bacterial counts are graphically presented in Figures 3 and 4. The recommended maximum numbers of *S. aureus* for recreational water (100 cfu/100ml) used by Favero *et al.* (1964), Charoenca & Fujioka (1993, 1995) and Charoenca & Kungskulniti (2001) was used for judging the obtained counts of *S. aureus*. However the maximum allowed counts for conventional indications, adopted by Egyptian Ministry of Health (1996) as well as European Commission (EC 1998), used for judging the quality of investigated water samples were 500, 100 and 100 cfu/100ml for (TC), (EC) and (FS) respectively.

According to the above-mentioned guide values, 107 samples (53.5 %) of the 200 total examined samples were found to harbour *S. aureus* exceeding the aforementioned guide standards. The most highly polluted sites were recorded at Aqaba Gulf (58.3 %) while a percentage of 50.5 % and 52.8 % were reported at Suez Gulf and Red Sea respectively (Table 1). This is probably due to the crowded users of these beaches located in A1 (Taba), A7 (Dahab) and A9 and 10 (Sharm El-Sheikh) (Fig.1). These percentages were less than those recovered, in some crowded beaches, in coastal recreational waters in Thiland (Charoenca & Kungskulniti 2001) where 78% of the examined samples harbored numbers of the pathogen exceeded the guide counts.

The numbers of *S. aureus* that exceeded recommended guidelines were detected in sites A1, A7, A9, A10, S6, S11, Re1, R5, R7 and R10. These high counts of S.aureus always associated with high numbers of beach users and thus high recreational activities such as swimming, sailing, snorkeling, diving and various forms of marine sports were running at these sites. According to the visual observation of the author, site A1 (Taba) was the most crowded site all over the others. This may explain why the highest annual mean (2260 cfu/100ml) was recorded at this site (Fig. 4). A strong correlation between staphylococci levels and bathers density has been documented by many authors (Ortiz 1977; Fattal 1986; Calderon *et al.* 1991; Charoenca & Fujioka 1993). These authors pointed out that the main source of staphylococci in recreational waters coming from the bathers; whose have the bacteria in their nasal membranes, skin and other parts of the body that coming in contact with the water during swimming.

The highest count of *S. aureus* arrived to 5000 cfu/100ml in May 2003 at Taba (site A1) (Table 1 and Fig. 2). These same numbers (up to 4500 cfu/100 ml) of S. *aureus* were recorded in crowded recreational beaches in Spain (Prieto *et al.* 2001), while less numbers (1800 cfu/100ml) and (1950 cfu/100ml) were reported in UK and Thailand (Fewtrell *et al.* 1994 and Charoenca & Kungskulniti 2001) respectively.

According to the aforementioned guide values of fecal indicators, 18 samples (9.0 %), 39 samples (19.5 %) and 43 samples (21.5 %) of the total 200 examined samples were found to exceed the guide standards for TC, EC and FS respectively (Table 1) .This include sites A1, A2, A4, A7, A10, A11, S1, S4, S5, S6, S9, S11, R1, R3, R4, R5, R8, R10, and RI2. The detected counts in these sites overlapped, sometimes, the acceptable levels of one or more fecal indicator depended on recreational activities. In contrast, sites of Suez-Kabanoon (S7), Ras Garib-City (S12) and Bir Shalatin (R15) recorded dramatic increase in numbers of fecal indicators arrived to 10^6 cfu/100ml all over the year. This dramatic increase in numbers explained by receiving obvious domestic untreated sewage (EIMP 2002).

Statistically, the correlation matrix analysis (Table 2) showed that no correlation was found between the hydrographical parameters (Fig.2) and all studied bacteria. Also, no correlation between the presence of *S. aureus* and all other conventional indicators bacteria including (TC), (EC), and (FS), was observed. This was clear in Table 3 where a percentage of 35.5% (75 samples), of 200 examined samples, harbored *S. aureus* counts exceeded the guide values without any increase of the other fecal pollution indicators. At the same time the percentage of samples that harbour *S. aureus* plus any of the conventional indicators bacteria, exceeded the guide numbers, were ranged from 3.5% to maximum of 14.5% of the total examined samples. Thus the traditional fecal indicators may not be adequate for detection of hygienic status of the Red Sea recreational waters. In the same time, there was a strong correlation between the presence of the three fecal conventional indicators bacteria including (TC), (EC) and (FS). Similar findings were obtained by Calderon *et al.* (1991) and Charoenca & Kungskulniti (2001). These obtained correlations may be explained as the conventional indicators are enteric pathogens and derived from the intestinal tract of warm blooded animals and thus indicate mainly the possibility of fecal contamination (Berg & Metcalf 1978; WHO 1994), while staphylococci is normally found in human hair follicles, skin and respiratory tract and usually shed from bathers during contact with waters (Yoshpe-Purer & Golderman 1987) in heavily recreational beaches.

The obtained numbers, of the examined bacteria, showed no clear seasonal variation throughout the year (Fig. 2) in spite of increasing water temperature in summer season (reaching 29.5 ^{o}C in summer while in winter was 19.5 ^{o}C) which normally increases the bacterial numbers. This may be due to the associated increasing summertime daylight and solar radiation at this subtropical area, which may increase the death rate of the microorganisms in summer season (Fattal *et al.*1983).

Staphylococci spp were used as a supplementary indicator for evaluating marine water quality in Spain (Alonso *et al.* 1989), in Israel (Yoshpe-Purer & Golderman 1987), and in Thailand (Charoenca & Kungskulniti 2001). Favero (1985) reported that the water in swimming pools as well as natural waters can pass the colifrom standards but still have high counts of *Staphylococci*. The obtained results (Table 3) strongly support this statement.

CONCLUSION

The present results concluded that addition of *S. aureus* as supplementary indicator to the conventional fecal indications may be useful for judging the marine water quality in Red Sea region. Also, the study highlight the fact that using some standard for all countries may not be valid, as the different degrees and sources of water pollution and the different susceptibility of the people of each country as well as different climates. Further studies should be done to identify the most appropriate indicator system for Red Sea climate and environment as well as to determine a relationship between recreational water quality assessed by various bacterial indicators and health risks associated with recreational activities.

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Table 1: Occurrence of organisms in studied coastal water regions during 2003

Regions	Indicator organisms	No. of examinded samples (N)	Counts (CFU/100ml)		No. (%) samples exceed standard	
			range	mean		
Aqaba Gulf	*S. aureus*	60	00-5000	16.2-2260.0	35	58.3%
	TC	60	00-900	00-295.0	3	5.0%
	EC	60	00-400	00-202.0	14	23.3%
	FS	60	00-300	00-138.0	10	16.6%
Suez Gulf	*S. aureus*	70	00-3500	14.4-1320.0	35	50.0%
	TC	70	00-3920000	0.84-1460000.0	11	15.7%
	EC	70	0.2340000	00-775200.0	14	20.0%
	FS	70	00-460000	00-196600.0	15	21.4%
Red Sea	*S. aureus*	70	00-1900	52.04-860.0	37	52.8%
	TC	70	00-8500	00-2184.0	4	5.7%
	EC	70	00-6000	00-1418.0	15	21.4%
	FS	70	00-1600	00-670.0	19	27.1%
All study area	*S. aureus*	200	00-5000	14.04-2260.0	107	53.5%
	TC	200	00-3920000	00-1480000.0	18	9.0%
	EC	200	00-2340000	00-775200.0	39	19.5%
	FS	200	00-460000	00-196600.0	43	21.5%

S. aureus: Staphylococcus aureus **TC: Total coliforms** **EC: *E. coli*** **FS: Fecal streptococci**

Table 2. Correlation matrix between different investigated coastal water parameters in Aqaba Gulf, Suez Gulf, and Red Sea during 2003

	S. aureus	TC	EC	FS	T	S	DO	PH
S. aureus	1							
TC	-0.007	1					**Aqaba Gulf**	
EC	0.357	**0.636**	1				**N=60**	
FS	0.347	**0.682**	**0.954**	1				
T	-0.052	0.349	**0.570**	0.440	1			
S	-0.035	-0.270	-0.446	-0.253	**-0.745**	1		
DO	-0.053	-0.389	-0.319	-0.324	-0.481	0.093	1	
PH	-0.033	-0.292	**-0.523**	-0.300	**-0.745**	0.702	0.352	1

	S. aureus	TC	EC	FS	T	S	DO	PH
S. aureus	1							
TC	-0.252	1					**Suez Gulf**	
EC	-0.252	**0.990**	1				**N=70**	
FS	-0.252	**0.990**	**0.990**	1				
T	0.390	-0.095	-0.095	-0.094	1			
S	0.142	-0.271	-0.271	-0.270	-0.033	1		
DO	**-0.525**	0.431	0.431	0.432	-0.332	0.272	1	
PH	-0.280	**0.537**	**0.537**	**0.538**	-0.256	0.006	0.261	1

	S. aureus	TC	EC	FS	T	S	DO	PH
S. aureus	1							
TC	0.006	1					**Red Sea**	
EC	-0.003	**0.999**	1				**N=70**	
FS	0.117	**0.972**	**0.973**	1				
T	-0.310	0.466	0.467	0.372	1			
S	0.376	-0.107	-0.110	-0.009	**-0.906**	1		
DO	-0.162	-0.074	-0.061	0.000	-0.337	0.267	1	
PH	-0.182	0.134	0.131	0.173	0.138	-0.076	0.131	1

S.aureus: Staphylococcus aureus. **TC:Ttotal coliforms. EC: *E. coli* . FS: Fecal streptococci**

T: Temperature (^{o}C) S: Salinity (S%$_{o}$) DO: Dissolved oxygen (mg/l)

N: Number of analysed samples.

Table 3: Numbers and percentage of samples that harbour bacteria exceeded the recommended current standards in studied areas during 2003

Indicator organisms	No. and percentage of examinded samples (N=200)	
	No.	%
S. aureus without other indicators	71	35.5
S. aureus + TC	7	3.5
S. aureus + EC	29	14.5
S. aureus + FS	28	14.0
S. aurous + EC + FS	20	10

S. aureus: Staphylococcus aureus **TC: Total coliforms EC: *E. coli* FS: Fecal streptococci**

N: Number of analysed samples

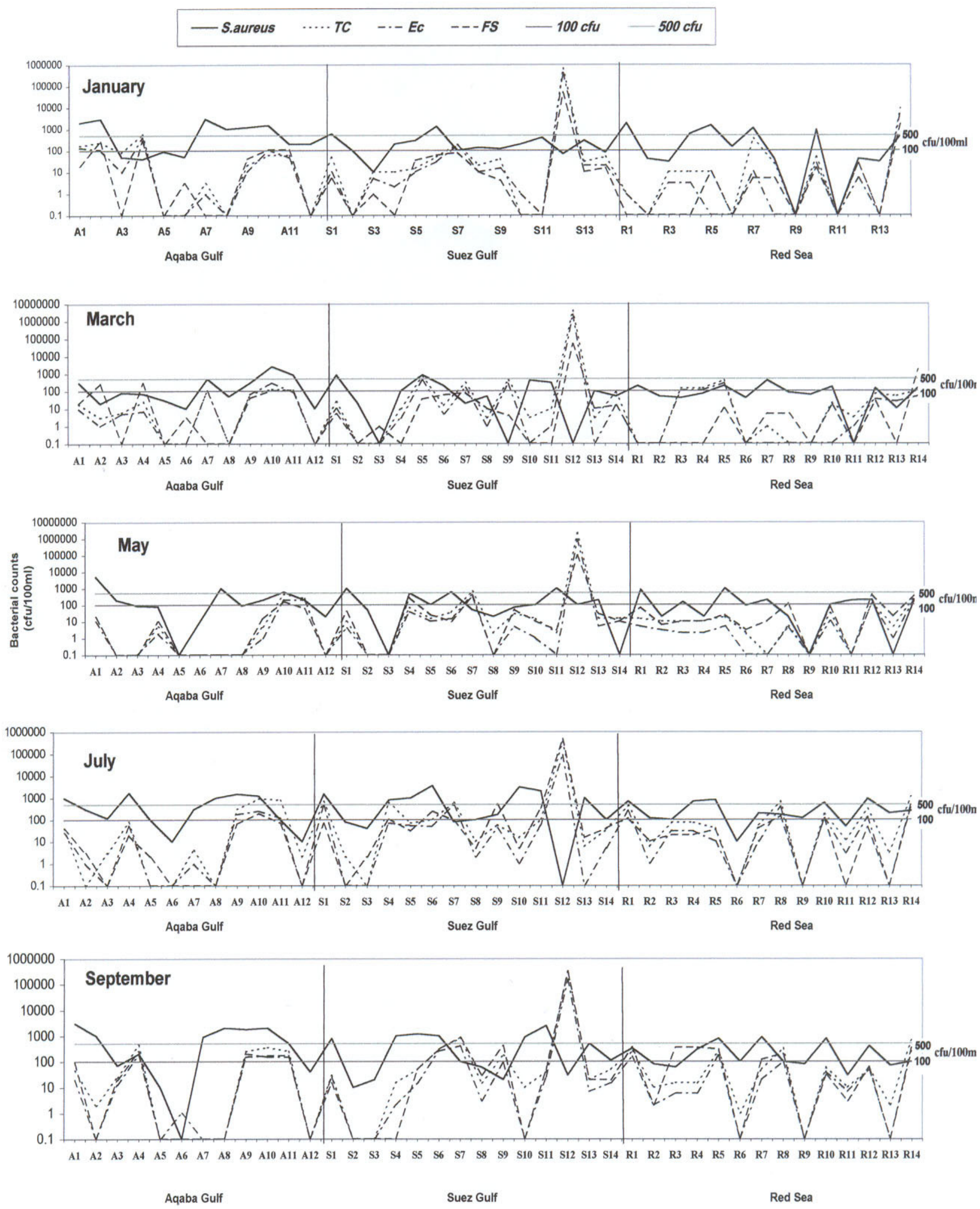

Fig.2. Bacterial counts (cfu/100ml) recorded in surface coastal waters of Aqaba Gulf (A), Suez Gulf (S) and Red Sea (R) during 2003.

S.aureus: ***Staphylococcus aureas*** **bacteria** **TC: total coliforms** **EC:** ***E. coli*** **bacteria** **FS: fecal streptoccoci bacteria**

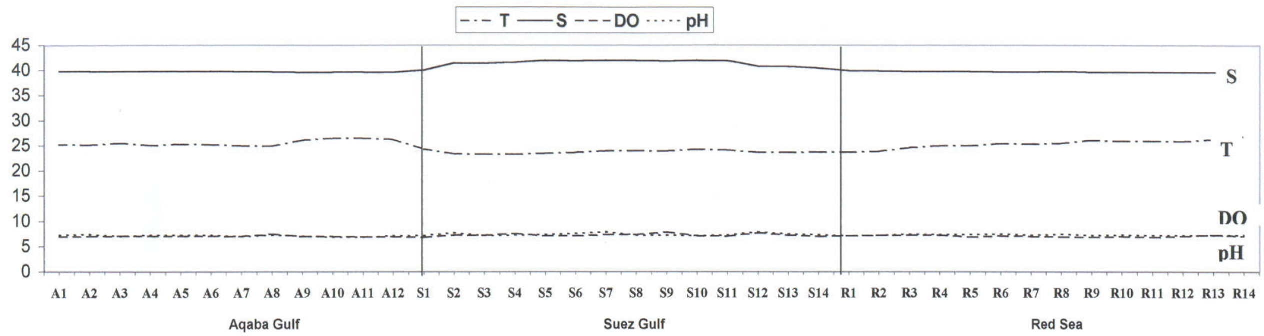

Fig.3. Annual mean values of T (temperature°C), S (salinity ‰), DO (dissolved oxygen mg/l) and pH recorded in surface coastal waters of Aqaba Gulf (A), Suez Gulf (S) and Red sea (R) during 2003.

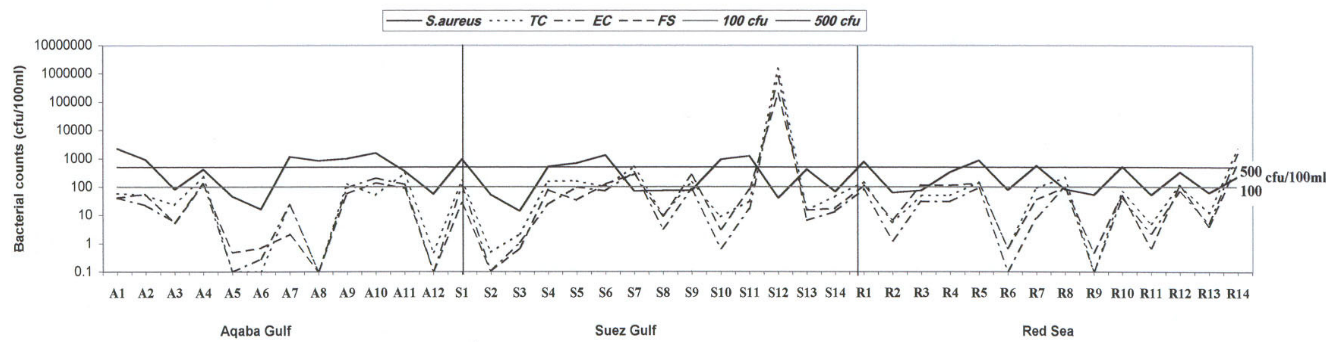

Fig.4. Annual means of bacterial counts (cfu/100ml) recorded in surface coastal waters of Aqaba Gulf (A), Suez Gulf (S) and Red sea (R) during 2003.

S.aureus: *Staphylococcus aureas* bacteria | Tc: total coliforms | EC: *E. coli* bacteria | FS: fecal streptoccoci bacteria

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