

## Microbiological criteria of tap water passed through some storage water tanks in Greater Cairo

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### ABSTRACT

Shortage in drinking water supply led to an increase of pumping water to storage tanks and its redistribution to the consumers. The water quality may be changed according to the storage condition and to the deficiency in cleaning of those tanks. Water samples were taken from the tanks of Helwan and El-Giza governorates (Cairo, Egypt) and examined to evaluate microbial water quality. The results revealed that all water samples were free from total coliforms, faecal streptococci, sulphite reducing bacteria (clostridia), salmonellae groups and total vibrios. The

total bacterial counts at 37°C and 22°C were 32 to 185 and 11 to 135 cfu·100ml<sup>-1</sup>, respectively. The total yeast and fungi counts were 9 to 66 and 4 to 35 cfu·100ml<sup>-1</sup>, respectively. Total counts of staphylococci (from 2 to 17 cfu·100ml<sup>-1</sup>), *Pseudomonas aeruginosa* (from 33 to 58 cfu·100ml<sup>-1</sup>), and coliphage (from 7 to 13 cfu·100ml<sup>-1</sup>) were also estimated. The free-living amoebae were detected in 75% of the examined storage water tank samples. According to the Egyptian standard for drinking water, most of the examined water samples were not acceptable for human consumption.

### INTRODUCTION

Water contamination, at its source and during household storage, is a major cause of enterically transmitted infections in developing countries (Molbak et al. 1989; Sandiford et al. 1989). The optimal approach for preventing water sources from contamination (construction of water disinfection, delivery systems and sewage treatment facilities) is expensive and time consuming. Moreover, the levels of contamination depend on a number of factors including the site of storage, type of container (galvanized cast iron, stainless steel and black polyethylene) and handling practices (the most common way of disinfecting water tank is by its chlorination). The most commonly used disinfectant is a highly concentrated calcium hypochlorite which is mixed with water and liberates 60 to 70% of its volume as chlorine (WHO 2009). Some studies (Molbak et al. 1989) stated that during its storage the drinking water was more contaminated than tap or piped water, indicating that interventions must be applied in order to decrease contamination of water at source and during household storage. The restriction on regular supply may lead to growth of microorganisms within the pipeline water systems during the phase when water is not flowing. Consequently, the water contamination appears to be higher than in areas very distant

from the sources. With the increase in incidence of reported water related diseases (even in developed countries (Craun et al. 2006)) the implementation of locally appropriate and culturally acceptable point-of-use disinfection is an urgent need to ensure a safe, reliable year-round supply. Safe hygienic household storage practices in developing countries have been established in other parts of the world (Quick et al. 1999).

The microbiological quality of drinking water can be reduced if pathogenic microbes penetrate water treatment process or if the conditions in the distribution network allow a pathogen contamination and enhance microbial growth (Miettinen et al. 1997). Moreover, the variation in the water quality is also correlated with the age of water (Momba et al. 2000). High levels of heterotrophic plate counts (HPC) were reported in water samples collected from a dead end region characterized by low flow conditions (Carter et al. 2000) and in parts of the pipelines with prolonged water retention time and in storage units (Tokajlan and Hashwa 2003).

The storage of drinking water mainly resulted in the re-growth of total bacterial count. Nevertheless, the persistence of pathogenic indicator microorganisms was observed on the walls of household containers (polyethylene and galvanized steel types), which may further deteriorate the water quality (Momba and Kaleni 2002).

Free-living amoebae are ubiquitous protozoa occurring in all types of environments throughout the world. They have been detected in many man-made water systems including drinking water distribution systems (Hoffmann and Michel 2001). Some species of the free-living soil and water amoebae (e.g. *Naegleria fowleri*, *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Sappinia dipoidia*) are recognized as etiologic of mostly amoebic encephalitis in humans and animals (Schuster and Visvesvara 2004).

The objective of this study was to quantify the microbiological quality of tap water after its passage through some water storage tanks in Greater Cairo (Helwan and El-Giza) in order to propose changes in the Egyptian regulation of the standards of the quality of water for drinking.

## MATERIAL AND METHODS

We examined 6 water tanks made of galvanized steel. They were from 3 to 5m<sup>3</sup> capacity and each tank served about 90-150 inhabitants. The tanks were regularly disinfected with 50-70% chlorine for 2h every 2-3 months. Water tanks were fed with finished potable water coming from conventional drinking water treatment plant with 0.5-1.0ppm residual chlorine.

Twenty four water samples of storage water tanks were collected from Helwan (12 samples) and El-Giza (12 samples). Sodium thiosulphate crystals (18mg·l<sup>-1</sup>) were added to the sampling bottles (two liters volume each) in order to eliminate residual chlorine from chlorinated drinking water.

100ml of water were filtrated from each bottled water sample and were analyzed for detection of total staphylococci, *Pseudomonas aeruginosa* and salmonellae groups by using membrane filters (0.45µm pore size and 47mm diameter) and the filters were transferred onto the surface plate of mannitol salt agar, PA agar and bismuth sulphite agar, respectively (APHA 2005).

Detection of total yeast, fungi, and total vibrios were carried out using membrane filter technique. A 100ml of each type of bottled water samples were separately filtrated through a nitrocellulose membrane filter (0.45µm pore size and 47mm diameter). The membrane was transferred onto selective media (Candida agar, Malt yeast extract agar and Thiosulphate citrate bile salt sucrose agar, respectively). Yeast, salmonellae and total vibrios were detected and identified according to El-Taweel and Shaban (2001).

Detection and enumeration of sulphite reducing clostridia was carried out according to Fewtrell et al. (1997).

Classical bacterial indicators such as total bacterial count, total coliform, faecal coliform and faecal streptococci were enumerated by using MPN methods according to APHA (2005).

Enumeration of coliphage was carried out by filtration of 100ml sample through 0.45µm pore size and 47mm diameter filter, then 3ml from concentrated water samples were mixed with 0.1 to 0.5ml *E. coli* ATCC 13706 and trypticase soy agar according to APHA (2005).

Free-living amoebae were detected in water samples according to the method of Ali and Al-Herrawy (2001). Briefly, water samples (10 litres each) were filtrated through cellulose nitrate membrane filters (1.2µm pore size and 142mm diameter). The filter holder was washed with 10ml of sterile distilled water and the membrane was inverted face to face on the surface of non-nutrient (NN) agar plates previously seeded with 0.1ml living *Escherichia coli*. The inoculated plates were incubated at 22°C for 7 days with daily microscopic examination for the presence of any amoebic growth (Al-Herrawy 1992).

## RESULTS AND DISCUSSION

The tanks water samples of the Helwan and El-Giza governorates were examined during 2008 to evaluate their microbiological parameters. Results of the total bacterial viable count·ml<sup>-1</sup> at 22°C and 37°C incubation temperatures and total mold (yeast and fungi)·100ml<sup>-1</sup> as well as total count of free-living amoebae pfu·l<sup>-1</sup> are in Table 1. Although all samples were free from bacterial indicators (i.e. total coliforms, faecal streptococci), salmonellae and vibrios groups as well as sulphite reducing clostridia, the range of total bacterial count of all tested samples incubated at 37°C (from 32 to 185 cfu·ml<sup>-1</sup>) was higher than that of incubated at 22°C (from 11 to 135 cfu·ml<sup>-1</sup>). Moreover, El-Giza samples recorded the average values (83.6 and 6.3·ml<sup>-1</sup>, respectively) of total bacterial count at 37°C and 22°C incubation temperature were higher than Helwan samples (62.4 and 30.8·ml<sup>-1</sup>, respectively), whereas the vice versa was true for the average value of total yeast and fungi count. In the present study some water samples were accepted following the Egyptian Standards (2007) for drinking water (total bacterial count must be less than 50·ml<sup>-1</sup> at 22°C and 37°C), while in the European standard total bacterial count at 22°C must be less than 100·ml<sup>-1</sup> (Barrell et al. 2000; European Union 1998).

According to the Egyptian Standards 2007, the examined drinking water samples in the present study were not fit for human consumption because the examined water samples contained some microbial pathogens. This may be due to some factors contributing to greater risks of microbial contamination of stored water such as high temperatures, increased storage time, high levels of airborne particles (dust storms), and some animals (birds, insects, others) that might gain access to the tanks what could lead to increase in the microbial contamination (Donald et al. 2006; Hinton and Holser 2009).

Although Shaban (1993) has found that 70 out of 72 samples of El-Giza storage tanks water were coliform free, Samhan (1998) detected coliforms in 13 out of 60 samples of storage tanks water, whereas faecal streptococci were present in 25 out of 60 storage tank water samples of El-Giza site in Greater Cairo. The log total viable bacterial count at 22°C and 37°C ranged from 0.30 to 3.78 and from 0.6 to 4.0 cfu·ml<sup>-1</sup>, respectively.

**Table 1. Microbiological criteria of Helwan and El-Giza storage water tank samples in 2008.**

Sample sites	Total bacterial count·100ml <sup>-1</sup> at:		Additional indicators (CFU)·100ml <sup>-1</sup>						
	37°C	22°C	Total staphylococci	<i>Pseudomonas aeruginosa</i>	Yeast	Fungi	Coliphage (PFU)	FLA (PFU)·l <sup>-1</sup>	
<b>Helwan</b>	1	34	32	2	34	9	4	7	0
	2	37	24	2	37	11	6	9	0
	3	92	81	2	41	24	12	7	2
	4	32	11	3	44	13	6	8	0
	5	44	29	2	36	12	7	8	2
	6	84	51	3	33	23	11	10	4
	7	111	68	5	39	55	28	11	1
	8	121	77	6	55	66	35	13	7
	9	54	42	4	37	12	5	7	1
	10	35	18	2	33	11	7	7	0
	11	48	36	2	35	10	6	7	1
	12	56	48	3	43	15	4	8	1
<b>Average</b>		<b>62.4</b>	<b>30.8</b>	<b>3</b>	<b>38.9</b>	<b>20.9</b>	<b>10.9</b>	<b>8.5</b>	<b>1.6</b>
<b>El-Giza</b>	1	44	35	2	36	14	5	7	0
	2	86	62	2	34	46	11	7	1
	3	77	59	4	46	22	4	9	1
	4	53	44	2	48	11	7	8	1
	5	103	75	9	38	23	9	9	5
	6	81	73	3	33	18	5	8	1
	7	64	49	3	39	14	13	7	1
	8	185	135	17	58	59	34	13	7
	9	39	24	2	34	9	8	7	0
	10	44	41	3	33	10	4	9	1
	11	115	87	2	41	62	32	12	7
	12	112	81	2	43	55	29	11	6
<b>Average</b>		<b>83.6</b>	<b>63.8</b>	<b>4.3</b>	<b>40.1</b>	<b>28.6</b>	<b>13.4</b>	<b>9</b>	<b>2.6</b>

PFU - plaques forming unit

FLA - free-living amoeba

The coliform group, faecal streptococci, vibrios groups, salmonellae groups, and sulphite reducing clostridia were absent from all samples

The total yeast and fungi count in the present study ranged from 4 to 35 cfu·100ml<sup>-1</sup> and from 2 to 17 cfu·100ml<sup>-1</sup>, respectively, with the highest average values in El-Giza site being 28.6 and 13.4 cfu·100ml<sup>-1</sup>, respectively. In similar studies, Samhan (1998) found that yeasts were detected in 11.7% of all tested storage tank water samples of Greater Cairo. The contamination of storage tank water may result from rodent hides, wastes of birds, airborne or re-growth in the network systems. Also, Nagy and Olson (1982) found more filamentous fungi in chlorinated than in un-chlorinated supplies. These organisms have been associated with taste and odor complaints.

In the present study, although all samples were free from bacterial pollution indicators, the presence of total staphylococci, *Pseudomonas aeruginosa*, and coliphage were detected. Pollution with organic material, high levels of airborne particles (dust storms), some animals (birds, insects and others) that reached to the tanks may cause an increase in the microbial contamination (Doggett 2000; Donald et al. 2006; Hinton and Holser 2009). Moreover, total staphylococci, *Pseudomonas aeruginosa*, and coliphage ranged from 2 to 17 cfu·100ml<sup>-1</sup>, from 33 to 58 cfu·100ml<sup>-1</sup> and from 7 to 13 pfu·100ml<sup>-1</sup>, respectively. These results agree with those obtained by Hinton and Holser (2009), who reported that *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been found in some water tanks and used as an indicator for drinking water. In addition, *Candida albicans* and *Staphylococcus* sp. can be considered as complementary tests for the evaluation of water pollution (Evans 1977; WHO 2004). Also, coliphage was used by El-Abagy et al. (1988) as a more specific index of faecal pollution. European standard for drinking water established that pathogenic staphylococci and *Pseudomonas aeruginosa* must be always absent from 250ml of drinking water (European Union 1998; WHO 2006), while Egyptian Standards (2007) for potable water stated that these pathogenic bacteria must be absent from 100ml.

Our results showed that free-living amoebae were detected in 66.7 and 83.3% of tested storage water tank samples collected from Helwan and El-Giza governorates, respectively. Free-living amoeba have been detected by other workers in drinking water distribution systems (Hoffmann and Michel 2001; Michel et al. 1998). Most of these amoebae are extremely resistant to conventional disinfection processes used in drinking water (Loret et al. 2008). Risk related to free-living amoeba in drinking water arises from the presence of some highly pathogenic species, able to cause meningoencephalitis, keratitis and skin lesion (Schuster and Visvesvara 2003). In addition, it is well established that some pathogenic microorganisms (like *Listeria*, *Salmonella*, *Legionella*, *Simkania* and *Mycobacterium*) have developed mechanisms allowing them not only to survive, but also to rapidly grow and proliferate inside free-living amoebae (Loret et al. 2008).

It could be concluded that the improvement of the storage tank material and cleaning of the tanks as well as

proper washing by suitable disinfectants, must be applied in order to produce microbiologically accepted stored tap water.

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