

Research Article

Assessment of the Bacteriological Quality of the Drinking Water Consumed in a Condominium of Students

Martone-Rocha Solange*, Martins Baptista Euclides, Peternella Alzira dos Santos Francisca, Razzolini Maria Tereza Pepe

Faculdade de Saúde Pública, Universidade de São Paulo, SP, Brasil

***Corresponding author**

Martone-Rocha Solange

Email: martone@usp.br

Abstract: The quality control of drinking water draws attention mainly from the bacteriological standpoint. The purpose of this study was to verify the bacteriological quality of the water distributed in a condominium of students. To determine the presence of fecal bacteria indicators, *Pseudomonas aeruginosa* and *Aeromonas* spp, the membrane-filter technique was used. For detection of heterotrophic bacteria, the "Pour Plate" technique was applied. The results showed that 40.7% showed a concentration of heterotrophic bacteria greater than 500 CFU/mL; and were positive in 2.7% and 23% for *E. coli* and *Pseudomonas aeruginosa*, respectively. The presence of *Aeromonas* spp was observed in 16.3% of the samples. Thus, it has been confirmed that the water used in the condominium studied does not comply with the standards issued by the Brazilian legislation before cleaning. It is clear that sanitary measures should be taken concerning the water consumed in the referred condominium.

Keywords: *Aeromonas* spp, Drinking-water; *E. coli*, *Pseudomonas aeruginosa*, Public Health.

INTRODUCTION

In developing countries, biological contamination of drinking water is a major concern for public health authorities [23]. According to the World Health Organization, approximately 5% of all deaths in these countries are directly related to water diseases resulting from poor quality of drinking water and lack of hygiene and sanitation [27]. As proven by the number of global outbreaks of water-borne diseases over the last years, exposure to poor quality water poses serious risk to consumer's health [21].

Cairncross *et al.* [5] pointed out that the discussion is restricted to the water source and the distribution system, without taking into consideration the piping system inside the dwellings. Regarding to storage water tanks [10, 12], emphasized that the major issue involving deterioration of drinking water quality is the inadequate maintenance of the internal distribution system, which is nowadays recognized as the major factor that compromises. Thus, water contamination problems have led investigators to assess the quality of the water which runs inside the buildings and dwellings [7, 24,2 6].

The quality of the water running along the internal distribution system can deteriorate due to lack of maintenance, as the presence of slits and cracks in storage tanks allowing the entrance of larvae and small

insects into the system [25]. Research carried out by Copeland *et al.* [9] in a slum in the city of Fortaleza-Brazil showed that 58% of the samples collected from storage tanks presented thermotolerant coliforms.

In Brazil, Heller *et al.* [16] carried out an investigation based on a case-control study which aimed at identifying factors that lead to acute diarrheal disease (ADD) in children. The study concluded that one of the main factors that contributed to this outbreak was the way water was usually stored.

The purpose of the present study was to assess the quality of drinking water distributed in a condominium of students. This condominium, constructed in 1960's, consists of seven buildings totalizing 473 apartments attending approximately 1,400 students.

MATERIAL AND METHODS

Sample Collection

A total of 21 samples were collected from water tanks and 92 samples were collected from taps located inside the dwellings, totalizing 113 samples. Samples were collected twice month from March to July 2009. Volumes of 1L of water were collected according to the Standard Methods for Examination of Water and Wastewater [3] in sterile disposable bottles containing sodium thiosulfate (1.8%), chilled at 4°C for transportation and examined along a 24-hour period. In

the field, residual chlorine was measured in samples collected from water tanks by a colorimetric method using free-chlorine using a portable device.

Bacteriological Analyses

To determine the presence of *E. coli*, *Pseudomonas aeruginosa* and *Aeromonas* spp, a membrane filtration technique was employed, and "Pour Plate" technique was used for enumeration of heterotrophic bacteria. The bacteriological analyses *E. coli*, heterotrophic bacteria, *Pseudomonas aeruginosa* were according to the Standard Methods for Examination of Water and Wastewater [3], in duplicate.

Escherichia coli

After filtering 100 mL of the sample, the membranes were transferred to plates containing medium m-Endo Agar (Difco® Detroit, MI, USA) and incubated at 35°C ± 0.5°C/24h ± 2 hours. For determining *E. coli*, each typical colony (green metallic sheen) yielded in m-Endo Agar for coliforms bacteria was transferred to EC-MUG broth (Difco®, Detroit, MI, USA) by examining positive tubes for fluorescence using a long-wavelength (366nm) ultraviolet lamp after incubation for 24 hours at 35°C ± 0.5°C. The samples which yielded fluorescence when exposed to UV light were considered positive for *E. coli*. The results were expressed in CFU/100mL.

Heterotrophic Plate Count (HPC)

This technique consists of inoculating 0.1 and 1.0 mL of the sample using plate count agar (PCA). The plates were then incubated at 35°C ± 0.5°C/48 hours, and colony forming units were counted. The results were expressed in CFU/mL

Pseudomonas aeruginosa

After filtering 100 mL of the sample, the membranes were transferred to a Petri dish containing M-PAC medium (Difco®, Detroit, MI, USA). They were then incubated at 42°C ± 0.5°C/24 hours. The typical colonies (brownish to greenish-black center) were transferred to plates containing Agar milk (Difco®, Detroit, MI, USA), in order to check the presence of *Pseudomonas aeruginosa*. After incubation, casein hydrolysis was evidenced by a clear halo around the colonies and by production of a green pigment which diffuses the

culture medium, was considered positive for *Pseudomonas aeruginosa*. The results were expressed in CFU/100mL.

Aeromonas spp

Determination of these bacteria was performed using ampicillin dextrin agar (ADA) [15]. Volumes of 500 mL were concentrated by membrane filtration. The membranes were then transferred to alkaline peptone water (APW) and incubated for 24 h at 35°C ± 0.5°C. A loopful from the enriched broth was streaked in ADA plates and incubated for 24h at 30°C ± 0.5°C. Convex, yellow-colored, 2-3mm-diameter typical colonies were screened in Kligler iron agar (24h at 35°C ± 0.5°C). Reaction was positive for cytochrome oxidase, indole and gas production. Lactose acidification and glucose fermentation were also performed. The results were expressed as Absence (A) or Presence (P) of *Aeromonas* spp.

RESULTS

Along the period a total of 113 samples were analyzed. The results showed that 2.7% (3/113) and 23% (26/113) of the samples were positive for *E. coli* and *Pseudomonas aeruginosa*, respectively. Concentration of heterotrophic bacteria was higher than 500 CFU/mL in 40.7% (46/113) of the samples, which means that the water quality is not in compliance with the Brazilian legislation which establishes as standard < 500 CFU/mL [4]. *Aeromonas* spp was detected in 16.3% (8/49) of the samples.

After two months of samples collection the responsible for the premises carried out a cleaning procedure in the tanks. This procedure consisted on draining the tanks and then scrubs all the tanks surfaces with a brush simultaneously removing residues. After that it was left to dry and then fill it up partially with water adding sodium hypochlorite (2L for 1000L). After two hours, the solution was drained through the taps and flush toilets. After all, the tank was filled with water which was ready to consume. Besides that there was a replacement of tanks lids.

The collection kept on and the results are reported as before and after maintenance procedure.

Table 1: Total number and percentage of samples collected the water tanks and taps, from March to July 2009

Organism	Before Cleaning		After Cleaning	
	No. Positive samples	%	No. Positive samples	%
Heterotrophic bacteria (*) (CFU/mL)	37(52)	71.1	9(61)	14.8
<i>E. coli</i> (CFU/mL)	3(52)	5.8	0(61)	0
<i>Pseudomonas aeruginosa</i> (CFU/mL)	25(52)	48.1	1(61)	1.6
<i>Aeromonas</i> spp (P/A)	7(20)	35.0	1(29)	3.5

(*) = > 500 CFU/ML, P/A = Presence and Absence

Before Cleaning

A total of 52 samples of tap water and water tanks were evaluated before the cleaning procedure; 71.1% (37/52) were positive for heterotrophic bacteria (>500 CFU/mL); 5.8% (3/52) were positive for *E. coli*; and 48.1% (25/52) were positive for *Pseudomonas aeruginosa*. Out of 20 samples analyzed for *Aeromonas* spp, seven were positive (35%) (Table 1).

Out of 45 samples collected from taps, 78.8% (35/45) showed a high concentration of heterotrophic bacteria (> 500 CFU/mL) and 6.7% (3/45) were positive for *E. coli* (<1 to 2 CFU/100 mL) (Table 2 and 3). The values of residual chlorine measured in samples from taps and tanks before cleaning procedure were lower than 0.2 mg/L in 64.3% (18/28) of the samples.

The results show that the majority of tap water samples do not comply with the Brazilian Regulation which standards absence of *E. coli* and 500CFU/mL as maximum concentration for heterotrophic bacteria and it is mandatory that the minimal concentration of residual chlorine is 0.2 mg/L (Brasil 2011).

However, *Pseudomonas aeruginosa* and *Aeromonas* spp are not ruled by Brazilian legislation results show they are present in considerable frequency in tap water samples. *Pseudomonas aeruginosa* was present in

53.3% (24/45) of the samples, while *Aeromonas* was present in 31.3% (5/16) (Table 2).

Out of seven samples from tanks 28.6% (2/7) exhibited concentration of heterotrophic bacteria higher than 500 CFU/mL (Table 1) while *Pseudomonas aeruginosa* was present in 14.3% (1/7) with concentration ranged from < 1 to 2 CFU/100 mL. *Aeromonas* was detected in 50% (2/4) of the samples.

After cleaning

After the cleaning procedure, a total of 61 samples were analyzed. Out of 47 samples collected from taps, 12.8% (6/47) and 2.1% (1/47) were positive for heterotrophic bacteria and for *Pseudomonas aeruginosa*, respectively (Table 2). Concentration of heterotrophic bacteria ranged from <1 to 800 CFU/100 ml (Table 4). *Aeromonas* was absent in all samples collected from taps.

Heterotrophic bacteria (> 500 CFU/mL) were detected in 21.4% (3/14) of samples collected from tanks (Table 2 and 3), whereas *Aeromonas* was present in 14.2% (1/7) of such samples (Table 3).

Distinctly that observed before the maintenance procedure, here all samples analyzed the values of residual chlorine were in accordance with Brazilian legislation for drinking water (0.2 mg/L).

Table 2: Number of samples positive and percentage for heterotrophic bacteria, *E. coli*, *Pseudomonas aeruginosa* and *Aeromonas* spp collected from taps and water tanks before and after cleaning procedure.

Organism	Before Cleaning				After Cleaning			
	No. Positive samples	%						
Heterotrophic bacteria (*) (CFU/mL)	35(n=45)	78.8	2(n=7)	28.6	6(n=47)	12.8	3(n=14)	21.4
<i>E. coli</i> (CFU/mL)	3(n=45)	6.7	0(n=7)	0	0(n=47)	0	0(n=14)	0
<i>Pseudomonas aeruginosa</i> (CFU/mL)	24(n=45)	53.3	1(n=7)	14.3	1(n=47)	2.1	0(n=14)	0
<i>Aeromonas</i> spp (P/A)	5(n=16)	31.3	2(n=4)	50.0	0(n=22)	0	1(n=7)	14.3

(*) = > 500 CFU/mL, P/A = Presence and Absence

Table 3: Concentration of heterotrophic bacteria, *E. coli*, *Pseudomonas aeruginosa* in samples collected from water tanks and taps

Organism	Water Tanks		Taps	
	Before Cleaning	After Cleaning	Before Cleaning	After Cleaning
CFU 100 mL				
<i>E. coli</i>	<1	<1	<1 to 2	<1
<i>Pseudomonas aeruginosa</i>	<1 to 2	<1	<1 to 3	<1 to 7
CFU/ mL				
Heterotrophic bacteria	Before	After	Before	After
	<1 to >1600	<1 to 800	<1 to >1600	<1 to 800

DISCUSSION

As a standard procedure, water intended for human consumption is distributed to consumers after treatment. Nevertheless, the quality of treated water can deteriorate during distribution due to contamination and inadequate storage conditions [22].

Several studies as [1,7,13,17,18,22] reported the presence of microorganisms in treated water such as *Pseudomonas aeruginosa* and *Aeromonas* spp. Nevertheless, so far only few studies seeking to elucidate the presence of microorganisms in water reservoirs have been developed.

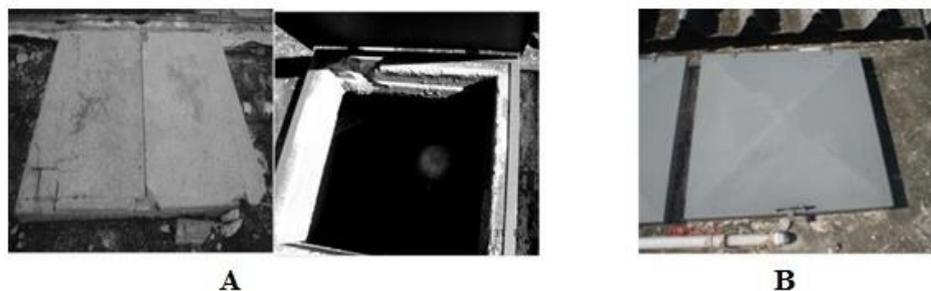


Fig. 1: Water storage tanks before the cleaning procedure (A) and after washing and exchanging of lids (B)

Amaral *et al.* [2] expressed concern about contamination of water storage tanks by animal excrements due to inadequate sealing. They also stated that deterioration of the microbiological quality of water is related to lack of regular cleaning of the internal water system.

Studies carried out by Chen *et al.* [7] and Lantenshlager *et al.* [18] show that the period which drinking water is stagnated in taps can lead to bacterial growth. This fact also could be observed in our study in terms of high concentration of heterotrophic bacteria and *Pseudomonas aeruginosa* for this reason Brazilian legislation demand minimum residual chlorine in drinking water. It was observed that after cleaning procedure residual chlorine was higher in tap water samples resulting in a decrease of heterotrophic bacteria concentration as well as *Pseudomonas aeruginosa*.

Grenfell *et al.* [13] also found the same kind of microorganisms in water tanks on cruise ships. In our study we observed that after cleaning and replacement of the lids of tanks, the presence of *E. coli* was not detected (Table 2). This fact confirms that when the maintenance adequately performed the presence of contaminants can be reduced (Fig. 1 b).

Other aspect we have to consider is that bacterial growth depends on the type and age of the internal distribution system. Corrosion can develop cracks and crevices on the piping surface that housing heterotrophic bacteria [6, 18]. In addition to the fact that

The presence of microorganisms detected by the present study (Table 2) indicates fecal contamination. The same was verified by Koksal *et al.* [17] in samples collected from taps. The contamination arising from sewage was discarded because these premises are located in a University campus which counts on a well established sewage connection.

Thus, the fecal contamination can be present due to the flaws and cracks observed in the tanks. These conditions allow the entry of animal excreta (poultry and small mammals) in water tanks because of the inadequate sealing. Figure 1(a) show the condition of these tanks before the maintenance procedure.

lack of maintenance of water tanks results in biofilm formation in the internal system, which can also lead deterioration of the water quality.

Considering that the student's premises were built more than 50 years ago, most probably the conditions of the whole building pipeline leave much to be desired.

According to the WHO [27], high concentrations of heterotrophic bacteria can be found in stagnant water throughout the distribution system, that is, both in water tanks and along the pipeline. This observation has been confirmed by the results obtained in our evaluation. Regular monitoring of heterotrophic bacteria is essential not only to control water microbiological quality but also to minimize human exposure to pathogenic microorganisms [8,11,19].

Biofilm formation could also explain the presence of both heterotrophic bacteria in water tanks and taps (Tables 1) even after the cleaning procedure, and could also be associated with the detachment of biofilm within the domestic distribution system [7].

The presence of *Pseudomonas aeruginosa* and *Aeromonas* spp has been largely studied when it comes to drinking water, as these bacteria represent a risk to the human health [6]. In addition, they can be associated with biofilms, thus minimizing the action of disinfectants. Nevertheless, so far research on these bacteria are not mandatory on the routine for evaluating the quality of drinking water. Razzolini *et al.* [20]

highlighted the importance of investigating the occurrence of *Aeromonas* in fresh water systems; particularly in storage tanks wherein the concentration of residual chlorine is generally low. These bacteria were found particularly in samples before the cleaning procedure.

Pseudomonas aeruginosa is the microorganism most frequently found in biofilm formed in water distribution systems [14]. The presence of such microorganism is a reason for great concern because of the risk it represents to human health [6, 7] stated that aging and corrosion of the water pipeline favor the development of microorganisms, including *Pseudomonas aeruginosa*, even after cleaning and disinfecting the tanks (Tables 3).

The present study confirms the importance of monitoring and assessing the quality of water reservoirs in hospitals, health centers, schools and other public institutions, as storage tanks can be considered the most vulnerable part of the whole water system.

CONCLUSION

- According to the results of the present study, the quality of the water consumed in the condominium studied is inadequate most of the time being more evident before maintenance procedure.
- We could also observe that after cleaning there was a decrease in the concentration of heterotrophic bacteria.
- But we also observed the presence of *Aeromonas* spp even after the cleaning procedure and replacement of tank lids. No doubt the cleaning provided an improvement of the water quality and a reduction in the entry of waste that can lead to water deterioration.
- Another factor that could explain the improvement in water quality is that after cleaning the concentration of residual chlorine was above 0.2 mg/L.
- The present study confirms the importance of maintenance of the internal system, as storage tanks are considered the most vulnerable part of the whole internal water system.

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