Influencing factors in the occurrence of injured coliforms in the drinking water distribution system in the city of La Plata, Argentina

María Alejandra Córdoba, Valeria Fernanda Del Coco, Marta Cecilia Minvielle and Juan Ángel Basualdo

ABSTRACT

The objective of this study was to evaluate the influencing factors in the occurrence of coliforms in the drinking water in La Plata (Argentina) from June 1999 to June 2001. A total of 180 samples were collected from Rio de La Plata (102 samples) and Puelche Aquifer (78 samples); 45 samples were collected for each of the four seasons. The membrane filter procedure was used for isolating bacteria, and each sample was tested for chlorine and pH. The highest percentage of samples positive for coliforms in the two tested media was obtained in summer while the highest percentage of negative samples was obtained in winter. No *Escherichia coli* was isolated. The percentage of injured coliforms fluctuated between 70 and 100%. The most frequently isolated bacteria was *Enterobacter cloacae* in summer, *Enterobacter agglomerans* in autumn and *Klebsiella oxytoca* in winter and spring. Significant correlations were observed between coliforms and the distance from the initial treatment point, and with the level of free chlorine. We conclude that drinking water contamination in La Plata occurs in the distribution system due to increased temperatures and reduced disinfectant levels, which result in bacterial regrowth.

Key words | Argentina, bacterial regrowth, disinfectant, drinking water, temperature

María Alejandra Córdoba*
Valeria Fernanda Del Coco*
Marta Cecilia Minvielle
Juan Ángel Basualdo (corresponding author)
Cátedra de Microbiología y Parasitología,
Facultad de Ciencias Médicas,
Universidad Nacional de La Plata,
60 y 120,
La Plata (1900),
Argentina
*Also at:
Comisión de Investigaciones Científicas de la Provincia de Buenos Aires,
Calle 526 e/10 y 11,
La Plata (1900),
Argentina
Tel.: +54 221 453 0974
Fax: +54 221 425 8987
E-mail: *jabasua@atlas.med.unlp.edu.ar*

INTRODUCTION

Water treatment consists of a series of processes intended to transform raw water into drinking water according to current water quality standards (OMS 1995). The main purpose of water treatment is to protect consumers from pathogens and impurities that may be present in water and that may be harmful to health (OMS 1998). An efficient treatment should provide consumers with water free from coliform bacteria regardless of the degree of original raw water contamination (Organismo Regulador Aguas Bonaerenses (ORAB) 2004). However, in many distribution systems, treated water undergoes an increase in bacterial numbers with increasing distance from the point of treatment. This increase has been called regrowth and is recognized worldwide as one of the greatest problems within distribution systems (LeChevallier *et al.* 1996). Volk & LeChevallier (1999) have shown that coliform regrowth depends on the existing complex interaction between the physical and chemical characteristics of drinking water, as well as engineering and operational parameters in the distribution system. Hallam *et al.* (2001) and Zacheus *et al.* (2001) determined that the most frequent causes for contamination inside the pipe network are bacterial regrowth, bacterial presence in the biofilm and/or a decrease in disinfectant in the distribution system.

Even though the microbiological quality of drinking water in most countries of the world is evaluated by the detection and count of total coliforms, some controversy exists. For example, the emergence of waterborne diseases

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in a community where the microbiological analysis of water showed an absence of coliforms (Dutka 1973) was thought to be due to inefficient methods for the control and evaluation of drinking water.

There is strong evidence supporting the idea that indicator bacteria can be injured (McFeters *et al.* 1986*a*,*b*; McFeters 1989, 1990). Injury can be defined as reversible damage to the bacteria as a consequence of partial or inappropriate disinfection due to the chlorination process, pH alterations, extreme temperatures, the presence of metals and other toxic substances and/or solar radiation (McFeters *et al.* 1986*b*). The injured coliforms present in treated water are able to maintain their viability, but display metabolic damage determined by the strain resistance and final chlorine concentration (Singh *et al.* 1986; Walsh & Bissonnette 1987, 1989). When this metabolic damage is repairable, it is called a sublethal injury, a phenomenon that involves a decrease in oxygen uptake and a delay in the latent stage. If the injured bacteria cannot find a suitable culture medium for metabolic recovery, they will be unable to grow. Consequently, the culture media typically used for the detection of coliforms do not guarantee the isolation of injured bacteria (McFeters *et al.* 1982), and a great number of these bacteria in the water may be undetected (LeChevallier & McFeters 1985; Basualdo *et al.* 2001). These false negatives may lead to an inaccurate definition and acceptance of the microbiological quality of the drinking water.

Prior studies by our group have found injured coliforms in drinking water in the city of La Plata (Basualdo *et al.* 2001; Córdoba *et al.* 2001). The objective of the present study was to evaluate the factors influencing the occurrence of injured coliforms and the microbiological quality of the drinking water distribution system in La Plata, from June 1999 to June 2001.

MATERIALS AND METHODS

Distribution system

La Plata city, the capital of the Province of Buenos Aires, has two separate sources of drinking water. Together, these two systems provide water for a total of approximately 454,000 consumers. One of these sources is Río de La Plata, and the water is treated at a treatment plant in Donato Gerardi by conventional decontamination followed by a final chlorination stage. Water obtained by this process is stored at the Bosque reservoir station. The second source of drinking water comes from Puelche Aquifer and the water is stored at Saavedra and San Martin's Parks reservoirs. This groundwater is chlorinated before entering the distribution system. A network of main pipes with some rechlorination stations transport the water from Bosque and Saavedra and San Martin's Parks' reservoirs to the suburbs.

Drinking water production rates are about 3,600,000 m^3 per day for the Donato Gerardi treatment plant and 3,920,000 m^3 per day for Puelche Aquifer (data provided by Organismo Regulador de Aguas Bonaerenses).

Sample collection

The sampling program covered a 24-month period, from June 1999 to June 2001. In order to study the evolution of water quality in La Plata's distribution system, a total of 180 samples of water were collected: 102 from Rio de La Plata and 78 from Puelche Aquifer. The sampling points were randomly selected at the immediate outlet of each reservoir (Bosque, Saavedra and San Martin Parks) and along the pipeline up to 10 km after the reservoir station. Samples were collected weekly prior to storage of the water in household reservoirs. A total of 45 samples for each season of the year was collected (OMS 1995). The samples destined for microbiological study were collected in sterile bags (Nasco's Whirlpak™; Network International Technologies, Incorporated, Buenos Aires, Argentina) containing sodium thiosulfate. They were taken to the bacteriology laboratory and analyzed within 4 h after collection. Samples were refrigerated at 10°C from collection to processing in the laboratory.

Microbiological and physicochemical analysis

The following data were obtained from each of the collected samples: the number of positive samples for coliforms, the number of isolated colonies and identified species, and the distance (in kilometers) between the reservoir station and each sampling point. Methods of analysis were based on *Standard Methods* (1998) and all microbiological analyses were processed in duplicate.

A membrane filter procedure was used for isolating total coliforms in drinking water (*Standard Methods* 1998). A 100 ml sample was filtered through a 0.45 μm membrane (Future Medical Technologies International, Inc., Riviera Beach, FL, USA) that was placed onto a filter pad soaked in m-Endo broth (Future Medical Technologies International, Inc.), incubated at 35°C for 24 h. Pink or red colonies with a typical metallic-green sheen and domed appearance were counted as presumptive total coliforms and confirmed through identification.

Fecal coliforms were determined by the membrane filter procedure (*Standard Methods* 1998). A 100 ml sample was filtered through a 0.45 μm membrane and the membrane was placed onto filter pads soaked in m-FC broth incubated at 35°C for 4 h, followed by 18 h at 44.5°C.

Total injured and injured fecal coliforms were determined by the membrane filter procedure (*Standard Methods* 1998). A 100 ml sample was filtered through a 0.45 μm membrane and the membrane placed onto mT7 agar. Total injured coliforms were assayed by growth on mT7 agar for 22–24 h at 35°C, while the damaged thermoresistant fecal coliforms were given an 8-h adaptation period at 37°C, followed by incubation for 12 h at 44.5°C. All yellow colonies were recorded as presumptive total injured coliforms, while blue colonies were recorded as presumptive injured fecal coliforms. Confirmation was carried out by identification using standard bacteriological testing (Holt *et al.* 1994) as well as three commercial test kits, Api 20 E (BioMérieux Products, Marcy l'Étiole, France), Biotype 100 (BioMérieux) and Sensident EM-Ident E (Merck, Darmstadt, Germany).

The samples were tested for residual disinfectant and pH. These parameters were measured in the sampling site. Free chlorine was determined by a Microquant 14826 commercial kit (D P D; Merck) and pH was measured by indicator strips with a 0–14 range (Merck).

Statistical analysis

Frequency, position and variation measures were applied to the descriptive statistical analysis. Statistical comparisons were made by using the paired *t* test. Pearson's correlation coefficient was applied to the analytical study of correlation verification. SPSS software, version 11.5 (SPSS Inc., Chicago, IL, USA), was used throughout. All statistical calculations were based on 95% confidence intervals.

RESULTS

Coliform bacteria were detected in all four seasons but *Escherichia coli* was not isolated. With regards to the culture medium used for bacteria isolation, the highest percentage of positive samples for coliforms in the two tested media was obtained in summer where the average temperature was 21.6°C, while the highest percentage of negative samples was obtained in winter where the average temperature was 10.6°C (Figure 1). In every season, a high percentage of samples was positive only in m-T7 agar.

In each season, in groundwater, the average number of coliform colony-forming units (CFU) in 100 ml, detected in m-Endo, ranged between 0 and 1; these values ranged between 2 and 8 in m-T7 (Table 1). The percentage of injured coliforms fluctuated between 80–100%. In the samples from surface water, the average number of CFU

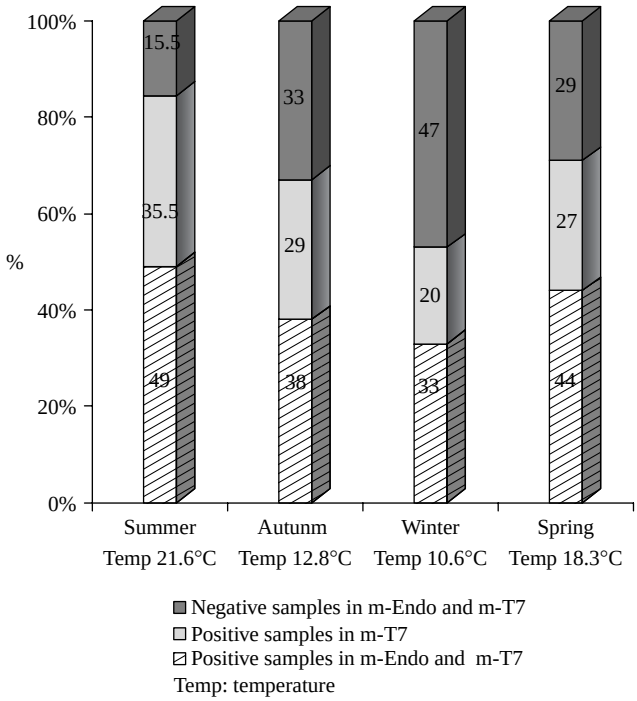

Figure 1 | Detection of total coliforms in 180 samples of drinking water from the distribution network in the city of La Plata by season, 2001.

Table 1 | Detection of total injured coliforms in the four seasons of the year in the drinking water distribution network in the city of La Plata, 2001

Source of drinking water	Total number of samples	Season	Coliforms/100 ml Mean (standard deviation) m-Endo	m-T7	*p* value	% Injury*
Surface water		Summer	3 (±4.2)	9 (±9)	<0.01	67
Río de	102	Autumn	2 (±3.2)	7 (±6)	<0.01	71
La Plata		Winter	1 (±1.2)	5 (±3.5)	<0.01	80
		Spring	1.5 (±2.1)	7 (±5.4)	<0.01	79
Ground water		Summer	1 (±1.4)	8 (±9)	<0.01	87
Puelche	78	Autumn	0	2 (±1.3)	–	100
Aquifer		Winter	0.4 (±0.7)	2 (±1.2)	<0.01	80
		Spring	0	2 (±1)	–	100

*% injury = [(m-T7 count − m-Endo count)/m-T7 count] × 100.
CFU: colony-forming units.

in m-Endo was between 1 and 3; these values increased to 5–9 when m-T7 was used. The percentage of injured bacteria varied from 70 to 79%.

There was a statistically significant difference between the occurrence of coliform bacteria in the drinking water distribution system and the season ($p = 0.00002$) (Figure 2). The most frequently isolated species was *Enterobacter cloacae* in summer, *Enterobacter agglomerans* in autumn and *Klebsiella oxytoca* in winter and spring. The least frequently isolated species was *Enterobacter aerogenes* in summer and autumn, and *E. agglomerans* in winter and spring.

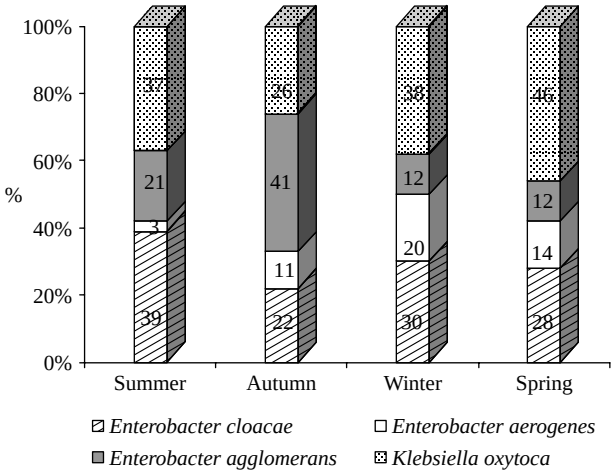

Figure 2 | Percentage distribution of the total coliform species isolated in the drinking water distribution network in the city of La Plata by season, 2001.

When relating CFU counts in m-Endo and m-T7 to the distance (in kilometers) from the source of the sample to the initial treatment point, a statistically significant correlation was observed in both cases ($r = 0.49$ and 0.65, respectively). Free chlorine values in samples from both superficial and underground waters were between 0 and 0.50 mg l^{-1}; pH values for the samples in the system ranged between 5 and 7.

The relationship between coliforms detected in m-Endo and m-T7 and residual free chlorine is showed in Figure 3. When free chlorine values in the distribution system are below recommended values (<0.25 mg l^{-1}) (OMS 1998), the percentage of positive samples in m-Endo exceeded 40%. When the medium used was m-T7, the percentage of positive samples remained above 20%, even in those cases where the residual free chlorine was over 0.25 mg l^{-1}.

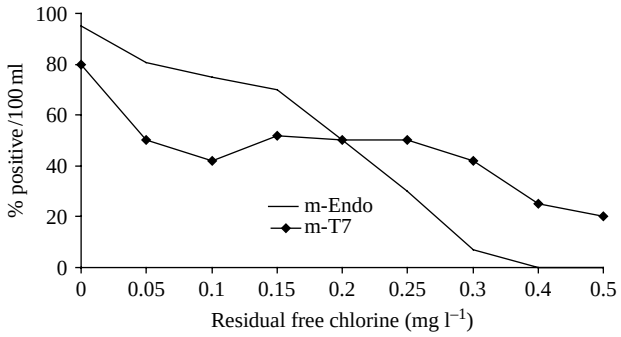

Figure 3 | Relationship between the detection of coliforms in m-Endo and m-T7 and residual free chlorine in La Plata, 2001.

Fifty percent (91/180) of all analyzed samples showed free chlorine values of 0.25–0.5 mg l^{-1}. Two percent (2/91) of these samples showed three or more CFU/100 ml in m-Endo, while these percentages were increased to 42% (38/91) when m-T7 was used. There was a statistically significant difference between the number of coliform bacteria and the culture medium used for bacteria isolation ($p < 0.001$).

Correlation analyses for sample source, free chlorine, pH and presence of coliforms are shown in Table 2. Significant negative correlations were found between the source of the sample and free chlorine and between free chlorine and the number of coliforms in m-Endo and m-T7.

DISCUSSION

Results obtained show that the occurrence of coliforms in La Plata's distribution system was higher in summer, regardless of the medium used for their isolation, while the lowest percentage of positive samples occurred in winter. These results are in agreement with those obtained by Neden *et al.* (1992) and LeChevallier *et al.* (1996), who also correlated the occurrence of coliforms with high temperatures. The highest number of coliforms was obtained in summer for both systems, suggesting that temperature is clearly a factor related to bacterial regrowth. Another factor to consider is higher water intake during this period because the amount of water consumed is higher in summer. This may increase the removal of coliforms present in the biofilm.

Table 2 | Correlation between source of sample, residual free chlorine, pH and presence of total coliforms in drinking water in La Plata, 2001

First variable	Second variable	Correlation coefficient	P
Source of the sample*	pH	−0.007	<0.01
	Free chlorine	−0.912	<0.01
	Coliforms m-Endo	0.49	<0.01
	Coliforms m-T7	0.65	<0.01
Free chlorine	pH	−0.073	<0.01
	Coliforms m-Endo	−0.539	0.005
	Coliforms m-T7	−0.546	<0.01
pH	Coliforms m-Endo	−0.083	0.471
	Coliforms m-T7	−0.065	0.406

*Source of the sample is a parameter to indicate sample site as a measure of distance from the initial treatment point (km).

In all cases, the number of coliforms was always higher in m-T7 agar. *E. cloacae*, *E. agglomerans* and *K. oxytoca* were the principal species implicated in regrowth within La Plata's drinking water distribution system. The regrowth of these species can decrease water quality and generate public health problems (Basualdo *et al.* 2001; Córdoba *et al.* 2001).

Given that the samples were not taken from the water treatment plant, and according to the data supplied by the private company in charge of administering drinking water in La Plata, the microbiological quality of the water entering the distribution system would comply with the tolerable limits established by ORAB. Since the samples were obtained before entering household reservoirs (Bosque, Saavedra and San Martin's Parks), water contamination may occur inside the network pipe. In view of this regrowth, frequent water analysis with more sensitive media, such as m-T7, is advisable. In this way, all coliforms present in the distribution system can be isolated. Furthermore, the sites where the occurrence of coliforms begins can be detected and preventive measures taken at an early stage.

The positive correlation of coliform CFU obtained in both media with the distance between the sampling site and the initial treatment point implies that the bacterial number increases as water moves through the distribution system. Niquette *et al.* (2001) observed similar results in Brussels' distribution system.

LeChevallier *et al.* (1991) reported that temperature and residual free chlorine can be used to predict 84% of the variations in the density of heterotrophic bacteria. By means of a linear regression model, they observed that 1 mg l^{-1} residual free chlorine was needed to limit the bacterial levels to under 500 CFU/ml. The percentage of positive samples in m-Endo was shown to exceed 40% in La Plata's drinking water distribution system when residual free chlorine was below 0.25 mg l^{-1}. This shows that the reduced amount of disinfectant is one of the factors related to bacterial survival in drinking water. These results are in agreement with those reported by LeChevallier *et al.* (1991, 1996).

Analysis of the samples for free chlorine, pH and the presence of coliforms in both media revealed a significant negative correlation between coliform bacteria and residual

free chlorine. The negative correlation implies that bacterial number increases as free chlorine decreases. The increase observed in La Plata's drinking water distribution system can be ascribed to a decrease in the levels of residual free chlorine. The highest counts obtained in surface and ground water could be related to the impossibility of maintaining an effective concentration of residual free chlorine in all sectors of the system. LeChevallier *et al.* (1996) showed that a residual free chlorine concentration of $0.5\,mg\,l^{-1}$, maintained in all sectors of New Jersey's distribution system (United States), was necessary to obtain the lowest levels of total coliforms.

Several authors have demonstrated that bacteria present in the biofilm can develop even in the presence of free chlorine. Le Chevallier *et al.* (1988) observed that the maintenance of $1\,mg\,l^{-1}$ of residual free chlorine was insufficient to control the occurrence of coliforms. This was later corroborated by Lu *et al.* (1999) and Zacheus *et al.* (2001). Coliforms isolated in our study could come from biofilms. The inadequate maintenance of free chlorine throughout the distribution system could enhance bacterial growth.

Although the results of this study are in agreement with those obtained in other parts of the world, it is important to emphasize that each distribution system is unique. The determination of the factors involved in regrowth depends on the data obtained during the monitoring of water quality. Therefore, we recommend that the detection of injured coliforms be added to the routine analysis of water in the distribution system, to ensure the quality of the drinking water received by the consumer. In addition, some improvements should be made to limit injured bacteria, and an adequate concentration of free residual chlorine should be maintained in the drinking water within the entire distribution system.

CONCLUSIONS

According to the results obtained in the present study, it can be inferred that La Plata's drinking water contamination is produced within the distribution system by bacterial regrowth due to temperature increases and disinfectant decreases. This bacterial regrowth included coliforms, but not *Escherichia coli*.

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REFERENCES

Basualdo, J. A., Córdoba, M. A., De Luca, M. M., Rocía, I. L., Pezzani, B. C., Vay, C., Ageron, E. & Grimont, P. 2001 Aislamiento y caracterización de coliformes injuriados en la red de distribución de agua de bebida de la ciudad de La Plata, Buenos Aires, Argentina. *Rev. Argent. Microbiol.* **33**, 9–14.

Córdoba, M. A., Roccia, I. L., De Luca, M. M., Pezzani, B. C. & Basualdo, J. A. 2001 Resistance to antibiotics in injured bacteria isolated from drinking water in the city of La Plata, Argentina. *Microbiol. Immunol.* **45**(5), 383–386.

Dutka, B. J. 1973 Coliforms are an inadequate index of water quality. *J. Environ. Health* **36**, 39–46.

Hallam, N. B., West, J. R., Forster, C. F. & Simms, J. 2001 The potential for biofilm growth in water distribution systems. *Water Res.* **53**(17), 4063–4071.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. 1994 *Bergey's Manual of Determinative Bacteriology*, 9th edition. Williams & Wilkins, Baltimore, MD.

LeChevallier, M. W. & McFeters, G. A. 1985 Enumeration of injured coliforms in drinking water. *J. AWWA* **77**, 81–87.

LeChevallier, M. W., Cawton, C. D. & Lee, R. G. 1988 Factors promoting survival of bacteria in chlorinated water supplies. *Appl. Environ. Microbiol.* **54**(3), 649–654.

LeChevallier, M. W., Schulz, W. & Lee, R. G. 1991 Bacterial nutrients in drinking water. *Appl. Environ. Microbiol.* **57**(3), 857–862.

LeChevallier, M. W., Welch, N. J. & Smith, D. B. 1996 Full-scale studies of factors related to coliform regrowth in drinking water. *Appl. Environ. Microbiol.* **62**(7), 2201–2211.

Lu, W., Kiene, L. & Levi, Y. 1999 Chlorine demand in water distribution systems. *Water Res.* **33**, 827–835.

McFeters, G. A. 1989 Detection and significance of injured indicator and pathogenic bacteria in water. In *Injured Index and Pathogenic Bacteria: Occurrence and Detection in Foods, Feeds, and Water* (ed. B. Ray), pp. 179–210. CRC Press, Boca Raton, FL.

McFeters, G. A. 1990 Enumeration, occurrence, and significance of injured indicator bacteria in drinking water. In *Drinking Water Microbiology: Progress and Recent Developments* (ed. G. A. McFeters), pp. 179–210. Springer-Verlag, New York.

McFeters, G. A., Cameron, S. C. & LeChevallier, M. W. 1982 Influence of diluents, media and membrane filters on detection of injured waterborne coliform bacteria. *Appl. Environ. Microbiol.* **43**(1), 97–103.

McFeters, G. A., Kippin, J. S. & LeChevallier, M. W. 1986 Injured coliforms in drinking water. *Appl. Environ. Microbiol.* **51**(1), 1–5.

McFeters, G. A., LeChevallier, M. W., Singh, A. & Kippin, J. S. 1986 Health significance and occurrence of injured bacteria in drinking water. *Water Sci. Technol.* **18**(10), 227–231.

Neden, D. G., Jones, R. J., Smith, J. R., Kirmeyer, G. J. & Foust, G. W. 1992 Comparing chlorination and chloramination for controlling bacterial regrowth. *J. AWWA* **84**, 80–88.

Niquette, P., Servais, P. & Savoir, R. 2001 Bacterial dynamics in the drinking water distribution system of Brussels. *Water Res.* **35**(3), 675–682.

Organismo Regulador de Aguas Bonaerenses (ORAB) 2004 Ministerio de Infraestructura, vivienda y servicios Públicos. Gobierno de la Provincia de Buenos Aires. Marco legal: Código de aguas (ley no. 12.257). Available at: http://www.orab.mosp.gba.gov.ar/

Organización Mundial de la Salud (OMS) 1995 *Guías Para la Calidad del Agua Potable*, 2nd edition, Vol. 1 *Recomendaciones*. OMS, Ginebra.

Organización Mundial de la Salud (OMS) 1998 *Guías Para la Calidad del Agua Potable*, 2nd edition, Vol. 3 *Vigilancia y Control de Los Abastecimientos de Agua a Las Comunidades*. Interprint Ed., Valetta, Malta.

Singh, A., Yeager, R. & McFeters, G. A. 1986 Assessment of *in vivo* revival, growth and pathogenicity of *Escherichia coli* strains after copper and chlorine induced injury. *Appl. Environ. Microbiol.* **52**(4), 832–837.

Standard Methods for the Examination of Water and Wastewater 1998 20th edition, American Public Health Association, Washington, DC.

Volk, C. J. & LeChevallier, M. W. 1999 Impacts of the reduction of nutrient levels on bacterial water quality in distribution systems. *Appl. Environ. Microbiol.* **65**(11), 4957–4966.

Walsh, S. M. & Bissonnette, G. K. 1987 Effect of chlorine injury on heat-labile enterotoxin production in enterotoxigenic *Escherichia coli*. *Can. J. Microbiol.* **33**(12), 1091–1096.

Walsh, S. M. & Bissonnette, G. K. 1989 Survival of chlorine-injured enterotoxigenic *Escherichia coli* in an *in vivo* water system. *Appl. Environ. Microbiol.* **55**(5), 1298–1300.

Zacheus, O. M., Lehtola, M. J., Korhonen, L. K. & Martikainen, P. J. 2001 Soft deposits, the key site for microbial growth in drinking water distribution networks. *Water Res.* **35**(7), 1757–1765.

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