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Drinking water microbiological survey of the Northwestern State of Sinaloa, Mexico

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ABSTRACT

A potable water survey, in two important municipalities of the state of Sinaloa, Mexico was conducted. Culiacan, capital city of Sinaloa and its neighboring municipality, Navolato were selected to enumerate *Aeromonas hydrophila*, *Escherichia coli*, fecal and total coliforms, *Pseudomonas aeruginosa*, and Heterotrophic plate count bacteria from 100 households' taps. Manganese; residual chlorine; pH; temperature and turbidity were also examined. Overall, *Aeromonas hydrophila* was not detected in any of the samples, 3% contained *Escherichia coli*, 28% had fecal and 46 total coliforms, *P. aeruginosa* was present in 15% of the samples. HPC bacteria were found in all of the samples but 43% had numbers greater than 500 CFU per ml. The average numbers obtained for the physico-chemical parameters were 0.15 mg/L; 0.32 mg/L; 6.5; 28.7°C and 2.92 NTU for manganese, residual chlorine, pH, temperature and turbidity, respectively. The findings of the current study demonstrate that potable water from both municipalities can harbor substantial numbers of indicator and opportunistic pathogens suggesting that additional treatment in the household may be needed.

Key words | bacteria, opportunistic pathogens, potable water

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INTRODUCTION

The purpose of a water supply distribution system is to deliver safe potable water which is also adequate in quantity and acceptable in terms of taste, odor, and appearance. A significant proportion of the world's population use potable water for drinking, cooking and personal and home hygiene (WHO 2004). However, in the past decade there has been a growing concern among the general public with respect to the safety and aesthetic qualities (taste and odour) of potable water supplies (Geldreich 1996). Potable water released into the distribution system becomes altered during its passage through pipes, open reservoirs, standpipes and storage tanks. Transient negative pressure and pipeline leak events provide a potential portal for the entry of groundwater into treated drinking water; and permit fecal indicators and microbial pathogens present in the soil and water exterior to enter the distribution system (LeChevallier *et al.* 2003). A wide variety of bacteria such as those known as opportunistic pathogens (*Flavobacterium*, *Pseudomonas* and *Mycobacterium*) can be incorporated into the piped potable water, colonizing the surface's pipelines with high numbers of biofilm-forming bacteria (Rusin *et al.* 1997*a*; Codony *et al.* 2005).

Bacteria may enter the distribution system through the failure to disinfect water or maintain a proper disinfection residual; low pipeline water pressure; intermittent service; excessive network leakages; corrosion of parts; and inadequate sewage disposal (Lee & Schwab 2005).

Every effort should be made to achieve a drinking water quality free of microbial risk (Craun *et al.* 2005). Failure to do so may expose the general population to water-related microorganisms and the potential health consequences are such that its control must always be of paramount importance and must never be compromised.

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In developing countries, rural areas face fast growing population densities, affecting the quality of life and provoking the migration of people to cities in search of better living conditions. Migration increases city-urbanization which in turns affects both water availability and quality (Lee & Schwab 2005). The demand for water in these communities has placed emphasis on storing water, by the public, into private water storage tanks encouraging stagnancy of water and growth of microorganisms (LeChevallier *et al.* 1996).

In Mexico, southern communities remain without the infrastructure for water and sewage. People often collect and store water in large containers, increasing contamination events. Sanchez-Perez *et al.* (2000) reported that children consuming poor drinking water quality in Chiapas' high level poverty areas had higher parasitic prevalence. In contrary, almost 100% of the urban population from Mexican north-west municipalities has access to potable water. Culiacan, capital city of the state of Sinaloa and its neighboring municipality Navolato has an urban population reaching almost 900, 000 inhabitants (INEGI 2000). However, the existing distribution systems are operating intermittently and at a fraction of their capacity, implying possible consequences to the microbiological quality of the drinking water (Candil-Ruiz *et al.* 1990; Chaidez *et al.* 1999).

Considering that most of the households in the Culiacan and Navolato municipalities use water that originates from a public distribution system, and that water may change its quality as it travels through the pipeline network, two aims are to be considered in this study: 1) To determine the frequency of contamination as tested for with indicators of bacterial and physico-chemical water quality at the tap in Culiacan and Navolato's residents households; and 2) To characterize the bacterial organisms delivered from the tap.

MATERIAL AND METHODS

Sample collection

Households' tap-water samples from both municipalities Culiacan and Navolato were collected in the summer of 2004. Residences located within the urban area were chosen as the only criteria to participate in the study. Potable water in both cities is obtained by extracting river water from wells along the Humaya, Tamazula and Culiacan river shores. Water is stored in municipal reservoirs and treated by flocculation, sedimentation, filtration and the final product is chlorinated (1 – 2 mg/L).

A randomized sampling was carried out in each of the cities, with the aid of the city map; the units of sampling were selected by counting the city-blocks (colonias), then neighborhood (manzanas) and finally selecting a total of 100 households for bacterial and physicochemical tap water evaluations.

Bacteriological analyses

Water samples were collected in sterile one-litre plastic bottles. In order to assess the types of bacteria the consumer actually ingests from the tap, the taps were sanitized before sample collection. First-draw water samples were collected aseptically in sterile 1-litre plastic bottles from the tap. Each bottle contained 2 ml of 10% sterile sodium thiosulphate to neutralize any residual chlorine. Samples were stored in a plastic cooler and packed with ice for transport to the laboratory for immediate processing. All bacteriological analyses were carried out according to the *Standard Method for the Examination of Water and Wastewater* (American Public Health Association (APHA) 1998) unless otherwise indicated. *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *E. coli* and total and fecal coliforms were enumerated by the membrane filtration method (0.45 μm, Gelman Science, Ann Arbor, MI) using the *Aeromonas* agar, *Pseudomonas* agar base (Oxoid, Hampshire, Eng) and ECC agar (Chromoagar, Paris, France), respectively. Recently, a number of enumeration methods have been developed in which the activity of the enzyme β-glucuronidase, a characteristic *E. coli* enzyme, is used to signal the presence of *E. coli*. Chromogenic substrates, such as those incorporated in the commercial product CHROMagarECC, developed by CHROMagar (Paris, France), simultaneously detects coliforms and *E. coli* (Alonso *et al.* 1999)

A. hydrophila and *P. aeruginosa* samples were incubated at 37°C for 36 h, total coliforms were incubated at 37°C and *E. coli* and fecal coliforms at 44.5°C for 24 h. Heterotrophic plate count (HPC) bacteria were enumerated using the spread plate method using R2A agar (Difco Laboratories, Detroit, MI, USA), and incubated at 37°C for 24 h.

The API20E biochemical strips (Biomereux Vitek, Hazelwood, MO) were utilized for the identification of fermentative bacteria.

Physico-chemical analyses

Physico-chemical analyses were performed according the APHA (1998). All water samples were tested for temperature, turbidity, pH and residual chlorine.

Statistical methods

Water quality data were analyzed by the Pearson correlation and the linear regression with EXCEL computer software (Microsoft Co., Redmond, WA, USA).

RESULTS

HPC bacteria exceeded 500 CFU in 43% in all of the samples tested (Table 1). *P. aeruginosa* was found in 15% of the samples from both municipalities (Table 1). Fecal coliforms and total coliforms were detected in 28% and 46% of the samples collected from Culiacan and Navolato's households, respectively. *E. coli* was identified in 1.3% and 7.4% of the samples collected from Culiacan and Navolato's households, respectively. Coliforms are common in intermittent water pressure pipes and may also be present in storage tanks (Rabi & Abo-Shehada 1995). It has been previously noted that coliform bacteria (including *E. coli*) will grow in distribution systems (Toner *et al.* 2005).

The greatest average concentration of HPC bacteria were observed in water obtained from Culiacan taps (Table 2). The maximum value was 5320 CFU/ml versus the value obtained from Navolato's tap (3930 CFU/ml). No trend in HPC bacterial numbers among samples was seen during the four months sampling period. The concentration appeared to vary randomly for any one home. No *Aeromonas hydrophila* were detected in any of the samples. *P. aeruginosa* average concentration was different in Culiacan and Navolato's tap (4 and 36 CFU/ml, respectively).

A summary of the physical/chemical data for the water samples is shown in Table 3. The average in manganese concentration was different in Culiacan and Navolato's tap (0.0648 and 0.3731 mg/L, respectively).The pH values were similar in all of the water samples. The average pH ranged from 6.5 to 7.2, the average temperature was between 28.7 and 29.3°C. and the average chlorine ranged from 0.06 to 0.32. The average turbidity in all tap water samples ranged from 1.04 to 2.92 NTU.

Table 1 | Percentage bacterial occurrence in potable water

Organisms	Culiacan (73)[d]	Navolato (27)[d]	Total[e]
Aeromonas hydrophila[a]	0	0	0
Pseudomonas aeruginosa[b]	19.1	0.27	15
Fecal coliforms[b]	9.5	77.7	28
Total coliforms[b]	17.8	88.8	46
Escherichia coli[b]	1.3	7.4	3
HPC[c]	49.3	29.6	43

[a] Per 500 ml sample volumes
[b] Per 100 ml sample volumes
[c] HPC greater than 500 CFU per ml
[d] Number of samples
[e] Overall percentage

Table 2 | Average, maximum and minimum values for the bacterial occurrence

	Culiacan (73)[e]			Navolato (27)[e]		
	Avg[a]	Min[a]	Max[a]	Avg[a]	Min[a]	Max[a]
Aeromonas hydrophila[b]	0	0	0	0	0	0
Pseudomonas aeruginosa[c]	4	1	125	36	<1	975
Fecal coliforms[c]	1	1	2	30	1	740
Total coliforms[c]	1	1	27	37	1	890
Escherichia coli[c]	<1	<1	1	7	1	180
HPC[d]	888	20	5320	490	1	3930

[a] Avg, Average; Min, Minimum; Max, Maximum
[b] Per 500 ml sample volumes
[c] Per 100 ml sample volumes
[d] HPC; Heterotrophic Plate Count
[e] Number of samples

Table 3 | Average, maximum and minimum values for the physico-chemical parameters in analyzed water

	Culiacan (73)[d]			**Navolato (27)**[d]		
	Avg[a]	**Min**[a]	**Max**[a]	**Avg**[a]	**Min**[a]	**Max**[a]
Manganese[b]	0.0648	0.0001	0.7823	0.3731	0.0094	0.7246
pH	6.5	6	7	7.2	6.5	7.5
Temperature	28.7	26	32	29.3	26	32
Total Chlorine[b]	0.32	0.01	1.07	0.06	0.01	0.17
Turbidity[c]	2.92	0.56	24.9	1.04	0.37	1.92

[a] Avg, Average; Min, Minimum; Max, Maximum
[b] mg/L, miligrams per litre
[c] NTU, nephelometric units
[d] Number of samples

Correlation and regression analyses were performed on bacteriological and physico-chemical results between the two municipalities. Statistical differences were observed on total and fecal coliforms and HPC in Culiacan and Navolato ($P = 0.05$) (data not shown).

DISCUSSION

Our study indicates that natural ageing and corrosion of the pipelines may comprise the water disinfectant residual, creating conditions that favour the growth of biofilm-forming bacteria. Bacteria metabolic activity over the precipitation of Manganese within the distribution system might contribute to high numbers of bacterial organisms at the tap (Toner *et al.* 2005).

P. aeruginosa was the most prevalent opportunistic pathogen isolated from the water samples. Predominantly it is defined as a nosocomial pathogen, but it is present in many environments including biofilms in the water distribution system (Geldreich 1996). Over 19% of the samples resulted positive for the presence *P. aeruginosa*.

Its ability to multiply at low substrate concentration enables its growth in surface water pipelines.

A. hydrophila was not isolated in this study. It has been suggested that heterotrophic bacteria might be inhibitory to *A. hydrophila* (Hunter 1993). *Aeromonas hydrophila* appears on the Contaminant Candidate List (CCL) as an organism to be considered for regulation and it will be monitored as an unregulated contaminant by selected utilities in the United States in the near future (Embrey *et al.* 2002).

The use of certain bacteria as indicators of the potential presence of pathogenic microorganisms in treated waters is the standard means of assessing its microbiological quality. In the past, fecal coliforms, or thermotolerant coliforms were used as the definitive indicator of fecal pollution for most drinking and freshwaters (Toranzos *et al.* 2002). In environmental samples, this functionally defined class of bacteria contains appreciable fractions of bacteria of non-fecal origin (Leclerc *et al.* 2001), and thus their utility as a surrogate for fecal contamination has been questioned (Doyle & Erickson 2006). *E. coli* is considered to be superior to fecal coliforms because it is the only fecal coliform bacteria of true fecal origin; it is present in large numbers (approximately 10^9 CFU/g, depending on the animal source) in the feces of warm-blooded animals; it survives longer than some bacterial pathogens, yet is resistant to regrowth outside of the host under typical environmental conditions; and it can be detected and quantified simply and affordably (Gleeson & Gray 1997; Edberg *et al.* 2000; Leclerc *et al.* 2001; Vasudevan *et al.* 2003).

HPC bacteria, as a group, do not present a risk to water consumers (WHO 2004). High numbers in a distribution system may represent water-related quality problem (Rusin *et al.* 1997*b*; WHO 2004). In summary, this study has shown that treated water supplies in north-west Mexico can harbor substantial numbers of indicator and opportunistic pathogens.

Assuming the water leaving the treatment plant meets bacteriological standards, the presence of coliform indicator bacteria in tap water samples suggests that the water is becoming contaminated in the journey through the distribution and plumbing systems. These data further suggest that additional treatment in the household before consumption may reduce exposure to potential disease causing organisms.

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REFERENCES

Alonso, J. L., Soriano, A., Carbajo, O., Amoros, I. & Garelick, H. 1999 Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5°C. *Appl. Environ. Microbiol.* **65**, 3746–3749.

Candil-Ruiz, A., Uribe, M., Diaz, S. P., De Leon, R., Naranjo, J. & Gerba, C. P. 1990. Calidad microbiologica del agua de la zona centro de Sinaloa. Sociedad Mexicana de Ingenieria Sanitaria y Ambiental, A.C. VII Congreso Nacional. 19–21 Septiembre.

Chaidez, C., Candil, A. & Gerba, C. P. 1999 Microbiological survey of private roof water tanks in Culiacan, México. *J. Environ. Health Sci.* **34**, 1967–1978.

Codony, F., Morato, J. & Mas, J. 2005 Role of discontinuous chlorination on microbial production by drinking water biofilms. *Water Res.* **39**, 1896–1906.

Craun, G. F., Calderon, R. L. & Craun, M. F. 2005 Outbreaks associated with recreational water in the United States. *Int. J. Environ. Health Res.* **15**, 243–262.

Doyle, M. P. & Erickson, M. C. 2006 The fecal coliform assay, the results of which have let to numerous misinterpretations over the years, may have outlived its usefulness. *Microbe* **4**, 162–163.

Edberg, S. C., Rice, E. W., Karlin, R. J. & Allen, M. J. 2000 *E. coli*: the best biological drinking water indicator for public health protection. *J. Appl. Microbiol.* **88**, 106S–116S.

Embrey, M. A., Parkin, R. T. & Balbus, J. M. 2002 *Handbook of CCL Microbes in Drinking Water*. American Water Works Association, Denver, USA.

Geldreich, E. E. 1996 Characterizing microbial quality of water supply. In *Microbial Quality of Water Supply in Distribution Systems* (ed. E. E. Geldreich). CRC Inc., Boca Raton, FL, USA, p. 236.

Gleeson, C. & Gray, N. 1997 *The Coliform Index and Waterborne Disease*. E & FN Spon, London, UK.

Hunter, P. R. 1993 The microbiology of bottled mineral waters. *J. Appl. Bacteriol.* **74**, 345–352.

Instituto Nacional de Estadística, Geografia e Informatica (INEGI) 2000 XII Censo general de población y vivienda. Available at: www.inegi.gob.mx.

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

LeChevallier, M. W., Gullick, R. W., Karim, M. R., Friedman, M. & Funk, J. E. 2003 The potential for health risks from intrusion of contaminants into the distribution system from pressure transients. *J. Water Health* **1**, 3–14.

Leclerc, H., Mossel, D. A. A., Edberg, S. C. & Struijk, C. B. 2001 Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. *Ann. Rev. Microbiol.* **55**, 201–234.

Lee, E. L. & Schwab, K. J. 2005 Deficiencies in drinking water distribution systems in developing countries. *J. Water Health* **3**, 109–127.

Rabi, A. Z. & Abo-Shehada, M. N. 1995 Sanitary survey of private drinking water reservoirs in northern Jordan. *Int. J. Environ. Health Res.* **5**, 255–261.

Rusin, P. A., Rose, J. B., Haas, C. H. & Gerba, C. P. 1997*a* Risk assessment of opportunistic bacterial pathogens in drinking water. *Rev. Environ. Contamin. Toxicol.* **152**, 57–83.

Rusin, P. A., Rose, J. B. & Gerba, C. P. 1997*b* Health significance of pigmented bacteria in drinking water. *Water Sci. Technol.* **32**, 21–27.

Sanchez-Perez, H. J., Vargas-Morales, M. G. & Mendez-Sanchez, J. D. 2000 Calidad bacteriologica del agua para consumo humano en zonas de alta marginacion de Chiapas. *Salud Publica de México* **42**, 397–406.

Standard Methods for the Examination of Water and Wastewater 1998 18th edition. American Public Health Association/Mary Ann H. Franson/Washington, DC.

Toner, B., Fakra, S., Villalobos, M., Warwick, T. & Sposito, G. 2005 Spatially resolved characterization of biogenic manganese oxide production within a bacterial biofilm. *Appl. Environ. Microbiol.* **71**, 1300–1310.

Toranzos, G. A., McFetters, G. & Borrego, J. J. 2002 Detection of microorganisms in environmental freshwaters and drinking water. In *Manual of Environmental Microbiology* (ed. C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney & L. D. Stetzenbach). ASM Press, Washington, DC, USA.

Vasudevan, P., Annamalai, T., Sartori, L., Hoagland, T. & Venkitanarayanan, K. 2003 Behavior of enteroaggregative *Escherichia coli* in bottled spring and mineral water. *J. Food Protec.* **66**, 497–500.

WHO 2004 *Guidelines for drinking-water quality*, 3rd edition, Vol. **1**, *Recommendations*. Geneva, World Health Organization, available at: http://www.who.int/water_sanitation_health/dwq/gdwq3/en/index.html

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