

PEER-REVIEWED ARTICLE

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Microbiological Quality of Bottled Water Obtained from Mexican Small Water Purification Plants: A Pilot Study, Carried Out in Morelia (Central Mexico)

ABSTRACT

The aim of this work was to begin to determine the microbiological quality of bottled water samples obtained from small purification plants located in Morelia, Michoacan, Mexico. Various microorganisms were taken into account, including nontuberculous mycobacteria (NTM) species. All 20 samples analyzed were positive for aerobic mesophilic bacteria. Eleven (55%), 6 (30%), and 2 (10%) water samples were positive for total coliforms, fecal coliforms, and *Escherichia coli*, respectively. In total, 18 (90%) of the water samples exceeded the maximum allowed limit stipulated by Mexico's official guidelines, establishing that purified water must not exceed the limit of 2 log CFU/mL (100 CFU/mL) of aerobic mesophilic bacteria and the presence of total coliforms must not be detectable in any 100 mL (<1.1 most probable number/100 mL) of sample. Five (25%) of the purified water samples were positive for NTM. The findings clearly showed that most of the purified bottled water samples had unsatisfactory microbiological quality and some harbored NTM associated with human illness. Therefore,

the study as a pilot points to a need for Mexican health authorities to perform frequent monitoring of purified water producers to verify compliance with standards regarding microbial safety.

INTRODUCTION

Bottled water consumption has been growing steadily worldwide for the past few decades (1). North America contains two of the three largest individual bottled water markets, the United States and Mexico; together, they represented 22.2% of the world's packaged water market in 2018 (32). Mexico alone accounted for 9% of the global volume, with nearly 9.5 billion gal in 2018. Mexico leads the world in bottled water per capita consumption. Average intake in Mexico went from 64.5 gal in 2013 to 72.4 gal 5 years later (32). Because of the increase in demand, the water bottling industry has been booming throughout Mexico, with a large increase in the number of water purification plants (17). These plants are typically small businesses operating locally that disinfect, bottle, and distribute water, mostly in 20-L jugs. The 20-L jug size is the most frequently

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purchased (98%) size for Mexican households (18). People buy bottled water because they think that it is free from “impurities” and bacteria, and they consider it safer than tap water (1), but it is not necessarily so. Recently, we reported that a high percentage of bottled water produced by small water purification plants in Mexico City did not comply with Mexico’s official guidelines (7). In addition, bottled water has been implicated in outbreaks of illnesses in recent years. For example, in 2011, *Shigella sonnei* subgroup D from commercially bottled water was responsible for an outbreak of 21 cases of acute gastrointestinal illness in Florida (3). Craun et al. (9) reported 780 drinking water-associated disease outbreaks in the United States from 1971 to 2006. Of these outbreaks, 11 were associated with the use of commercially bottled water. Chemical contaminants in bottled water were identified in four outbreaks. Bacterial contaminants attributed to bottled water sources were identified in two outbreaks (non-O1 *Vibrio cholerae* and *S. sonnei*). No etiologic agents were identified in five outbreaks. With the report of these studies, it is evident that bottled water does not always meet the required microbiological quality standards.

We included nontuberculous mycobacteria (NTM) because they are pathogens that affect both immunocompromised and immunocompetent individuals (12). The incidence and prevalence of NTM diseases is increasing worldwide (35). Human subjects can inhale or ingest NTM in water, aerosols, or dust (35). Some NTM species can cause pulmonary disease; affect the skin, lymph nodes, or the gastrointestinal tract; and can produce disseminated disease

in severely immunocompromised individuals (12). Water has been reported as one of the most important reservoirs of NTM (11). Previous studies have shown that the mycobacterial strains recovered from NTM-infected patients can often be genetically matched to the mycobacterial strains found in the potable water used by those patients (14). The aim of this work was to assess the microbiological quality of bottled water samples obtained from small purification plants located in Morelia, Michoacan, Mexico, and to consider the occurrence of NTM in the samples to begin to gauge the safety of bottled water for human consumption in Mexico.

MATERIALS AND METHODS

Area of study and water collection

The selected area for this pilot study was Morelia, a city located in the north central part of the state of Michoacan in central Mexico (Fig. 1). Morelia is the capital and largest city of the state, representing a large urban area of ~1,200 km² with 784,776 registered inhabitants (16) and an estimated average of 2,600,000 national and international visitors per year (34). From November 2018 to March 2019, a total of 20 purified water samples sold in polycarbonate 20-L bottles were purchased from 20 small water purification plants throughout Morelia City (Fig. 1). The 20 plants evaluated represent 43% of the total of the 46 purification plants found in Morelia.

Chemical and microbiological analysis

Each 20-L bottle containing purified water was vigorously shaken, and the jug mouth was disinfected with 70%

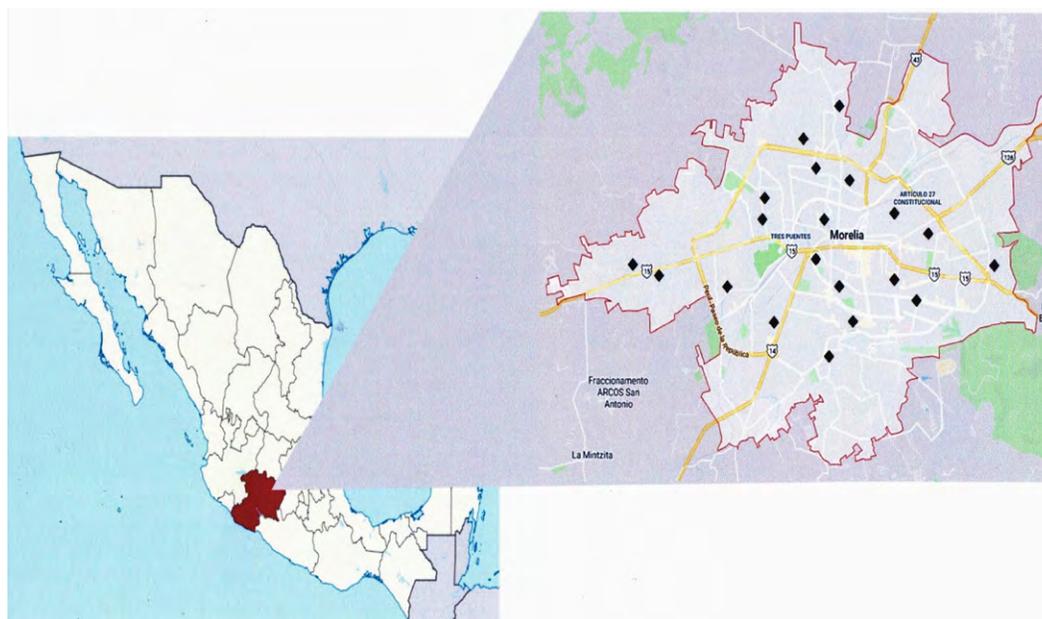


FIGURE 1. Localization of sites of Morelia, Michoacan, Mexico, where the purified water samples (•) were collected.

ethanol solution before taking the water sample. The pH and chlorine residual concentrations of all water samples were determined by using a pH meter (model pH 209, HANNA Instruments, Sarreola di Rubano-PD, Italy) and the N,N-diethyl-p-phenylene-diamine method (25), respectively. Each sample was tested for the presence of aerobic mesophilic bacteria (AMB), total coliforms (TC), and fecal coliforms (FC), following methods approved by the Mexican official guidelines NOM-201-SSA1-2015 (25) and NOM-210-SSA1-2014 (26). For the isolation of *Escherichia coli*, for each *E. coli* tube positive for FC, a loopful was transferred to tubes containing *E. coli* broth with 4-methylumbelliferyl- β -D-glucuronide and incubated for 24 h at $35 \pm 0.5^\circ\text{C}$. Each tube was examined for growth (turbidity, gas) and determined for fluorescence production. From the positive tubes, a loopful of broth was removed and streaked on an eosin methylene blue agar plate that was incubated for 24 h at $35 \pm 0.5^\circ\text{C}$. Two to three *E. coli*-like colonies that grew were then selected and biochemically characterized with the IMViC test (indole test, methyl red test, Voges-Proskauer test, and citrate test). All *E. coli* strains isolated from eosin methylene blue plates were analyzed using two multiplex PCR assays to identify different diarrheagenic *E. coli* pathotypes (6, 22). All data were analyzed according to guideline NOM-041-SSA1-1993 (041 Guideline) (24). This guideline establishes that purified water must have a pH between 6.5 and 8.5, a free of residual chlorine concentration of up to 0.1 ppm, must not exceed the limit of 2 log CFU/mL (100 CFU/mL) of aerobic mesophilic bacteria (AMB), and the presence of TC must not be detectable in any 100 mL (<1.1 most probable number [MPN]/100 mL) of sample.

Isolation and identification of mycobacteria

Five hundred milliliters of water from each jug was filtered using the CORNING sterile filtration system with a 0.22- μm membrane. Subsequently, the membrane was placed onto Middlebrook 7H10 agar plates (Difco, BD) supplemented with albumin dextrose catalase (BD BBL prepared media), cycloheximide (500 $\mu\text{g}/\text{mL}$), and the PANTA cocktail (BD BBL: 40 U/mL polymyxin B, 4 $\mu\text{g}/\text{mL}$ amphotericin B, 16 $\mu\text{g}/\text{mL}$ nalidixic acid, 4 $\mu\text{g}/\text{mL}$ trimethoprim, and 4 $\mu\text{g}/\text{mL}$ azlocillin). Plates were incubated at 35°C and were examined daily for the first 8 days and thereafter once a week for 2 months. Once bacterial growth had been observed on the Middlebrook 7H10 agar, the number of CFU/500 mL was directly determined for each plate, and the identification of acid-fast bacilli was carried out by Ziehl-Neelsen stain. Isolates belonging to the genus *Mycobacterium* and to the *M. tuberculosis* complex were identified by two PCR assays described previously (8). NTM species were identified by three methods: (i) PCR restriction enzyme pattern analysis of the 65-kDa heat shock protein gene *hsp65*, as described by Telenti et al. (37); (ii) sequencing of the hypervariable region 2 of the 16S rRNA gene (20); and (iii) sequencing

of a fragment (723 bp) of the *rpoB* gene (2). Nucleotide sequences were compared with known sequences in the GenBank database by using the Blastn algorithm. Species identifications were based on the 100% similarity cut-off for the 16S rRNA gene and $\geq 97\%$ for the *rpoB* gene.

RESULTS

Chemical and microbiological quality of purified water

The pH range of the water samples was found to be between 6.8 and 7.8, and the chlorine concentration was <0.1 ppm. All samples were thus found to be within the chemical standards range for pH recommended by Mexico's official guidelines for purified bottled water. Regarding the microbiological quality, all 20 samples analyzed were positive for AMB (Table 1). Concentrations of AMB ranged from 1.12 to 6.45 log CFU/mL. In total, 11 (55%), 6 (30%), and 2 (10%) water samples were positive for TC, FC, and *E. coli*, respectively (Table 1). In the positive samples, TC and FC concentration ranged from 1.1 to >8 MPN/100 mL and from 1.1 to 4.6 MPN/100 mL for *E. coli*. None of the six *E. coli* strains isolated from two water samples belonged to diarrheagenic pathotypes. In total, 18 (90%) of the water samples were found to fall outside Mexico's accepted official guidelines: 7 (35%) water samples exceeded the maximum allowed limit for AMB, 5 (25%) for TC, and 6 (30%) samples exceeded both indicators (AMB plus TC; Table 2).

Mycobacteria isolation and identification

NTM were isolated from 5 (25%) of the 20 water samples, recovering a total of five isolates. Concentrations of mycobacteria ranged from 3 to 25 CFU/500 mL. According to three molecular methods (PCR restriction enzyme pattern analysis of the gene *hsp65* and the sequencing of the 16S rRNA and *rpoB* genes), all NTM identified in this study belonged to the *Mycobacterium fortuitum* complex: *Mycobacterium porcinum* ($n = 2$), *M. fortuitum* ($n = 1$), *Mycobacterium conceptionense* ($n = 1$), and *Mycobacterium* sp. ($n = 1$).

DISCUSSION

In this work, the microbiological quality of purified bottled water obtained from small water purification plants was evaluated, and 90% of purified bottled water samples did not comply with the 041 Guideline. These results are similar to those published by Cerna-Cortes et al. (7) in Mexico City and Pant et al. (29) in Dharan, Nepal, who found that 72.9 and 87.5%, respectively, of bottled water samples analyzed did not comply with the World Health Organization microbiological criteria for drinking water (<100 CFU/mL of AMB and absence of coliforms) (39, 40). However, our results were higher than those reported by Abd El-Salam et al. (1) in Egypt and Halage et al. (13) in Kampala, Uganda, who reported that 54.8 and 15%, respectively, of bottled water samples evaluated exceeded the World Health Organization guidelines.

TABLE 1. Quantity and frequencies of aerobic-mesophilic bacteria (AMB), total coliforms (TC), fecal coliforms (FC), and *E. coli* in bottled purified water samples^a

Microorganism Group	Minimum	Median	Maximum	Frequency (%)
AMB	1.12	3.18	6.45	20 (100)
TC	<1.1	1.1	>8	11 (55)
FC	<1.1	<1.1	>8	6 (30)
<i>E. coli</i>	<1.1	<1.1	4.6	2 (10)

^a*n* = 20. Minimum, median, and maximum values are in log CFU/mL for AMB and in MPN/100 mL for TC, FC, and *E. coli*.

TABLE 2. Microbiological indicators for which bottled purified water samples^a were outside the 041 Guideline

Microorganism group	No. of samples outside of the 041 Guideline ^{b*} (%)
Only AMB	7 (35)
Only TC	5 (25)
Both AMB and TC	6 (30)
Total	18 (90)

^a*n* = 20.

^{b*}Guideline that establishes that purified water bottled must not exceed the limit of 2 log CFU/mL (100 CFU/mL) of AMB and the presence of TC must not be detectable in any 100 mL (<1.1 MPN/100 mL).

In this study, all water samples contained AMB, and 13 (65%) of them were outside the 041 Guideline: 7 (35%) samples exceeded the maximum allowed limit for only AMB and 6 (30%) samples exceeded both indicators (AMB plus TC; Table 2). These results were higher (0 to 33.3%) than those reported by Abd El-Salam et al. (1) in Egypt, Igbeneghu and Lamikanra (15) in Nigeria, Osei et al. (28) in Ghana, and Venkatesan et al. (38) in India. In general, a high concentration of AMB can be taken as an indication of deterioration in water quality and as a sign of the presence of human pathogenic microorganisms (23, 39). A high level of AMB in water indicates the potential presence of (opportunistic) pathogens such as *Aeromonas*, *Klebsiella*, and *Pseudomonas* (39) that can spread disease to humans (23) and importantly to immune-compromised persons whose risk of severe infections is high (15).

Other bacterial groups commonly used to monitor water quality are TC of which FC are a subset and *E. coli* is the most important species. If one of these groups is detected, it means that a contamination of fecal origin has occurred, and some of these microorganisms can potentially cause disease (40). TC should be absent immediately after disinfection, and the presence of these organisms indicates

inadequate treatment (40). In our study, 55% of the water samples in total were positive for TC. Our results are higher than the results reported for Egypt, Nigeria, and Nepal (25 to 28.6% of samples) (1, 15, 29). Our results differ from those reported by Venkatesan et al. (38) in Chennai, India, who reported that none of the bottled water samples analyzed contained TC. Regarding FC and *E. coli*, we identified 30 and 10% of positive samples, respectively. Our results differ from the results reported for Egypt, Uganda, Nepal, and India, where none of the purified water samples were found to be positive for FC and *E. coli* (1, 13, 29, 38). A high concentration of bacteria in bottled water may occur through carriers such as introduced flakes of human skin (29). For this reason, we recommend that water plant workers be checked constantly to identify asymptomatic carriers of enteric bacterial pathogens. The high percentage of bottled water samples outside of the guidelines may be due to inefficient disinfection processes by the purification plants. The introduction of microorganisms might also occur during the processing or handling of water (29) or from a lack of stringent treatment of containers before filling with the purified water (27). Therefore, frequent monitoring by

Mexican health authorities is needed to verify compliance with standards regarding microbial safety.

In this study, NTM were identified in 25% of water samples. The results are similar to our previous results (29.7%) in bottled purified water in Mexico City (7), a large metropolitan area. All NTM species identified in the present study belonged to the *M. fortuitum* complex, composed of related species that share a close phylogenetic relationship and can cause pulmonary and extrapulmonary infections (5, 19). *M. fortuitum* has been frequently isolated from wild animals including reptiles, amphibians, and invertebrates, whereas *M. porcinum* is primarily recovered from swine with lymphadenitis (4). Recently, *M. conceptionense* has been isolated from aquarium fish (10). Thus, a variety of animals can be a permanent or transient source of NTM species. The NTM species identified in this study were detected in various environments, including natural waters, drinking water distribution systems, and soils. By contrast, both *M. fortuitum* and *M. porcinum* were isolated from potable water in a previous study carried out by us (30) in Mexico City. Control of NTM is challenging because, unlike traditional pathogens, they are native members of drinking water microbial communities. In addition, they can be resistant to disinfectant residuals and other biocides (such as copper ions) due to a thick, hydrophobic cell wall rich in mycolic acids that helps them form aggregates in liquid media (31). Moreover, NTM have the ability to form biofilms on different surfaces, resist high temperatures, and grow in marginal environments with low nutrient and oxygen content (11). It has been reported that *Mycobacterium avium* can survive attached to polyethylene terephthalate water bottles and that the cell adhesiveness to the polyethylene terephthalate wall increases with time (36). Furthermore, NTM are also highly resistant to chlorine and UV light radiation. Le Dantec et al. (21) reported that to obtain an inactivation level of 99.9% for mycobacteria it is necessary to use chlorine concentrations that are 100 to 330 times higher, depending

of the species, than the concentrations required to obtain the same inactivation level for *E. coli*. Also, Schiavano et al. (33) found that a dose of 192 mJ/cm² was needed to obtain complete inactivation of *M. avium* subsp. *hominissuis*, a dose that is much higher than the limit recommended by the international standards for UV disinfection of drinking water (16 to 40 mJ/cm²).

This study has some limitations. Although the number of water samples represents 43% of purification plants found in Morelia, the number of samples is still small. Therefore, a more representative sample across the population would need to be taken to assess the risk. In addition, the source and treatment process of bottled water contamination should be investigated more thoroughly. Further studies of final product quality in storage conditions are also required.

RECOMMENDATIONS

This pilot study showed that most of the purified bottled water analyzed had unsatisfactory microbiological quality, and some harbored NTM associated with illness. Thus, there is a need for pertinent authorities to intensify efforts in routine monitoring of the various activities in the purified bottled water industry, with the view to supplying safe and wholesome water to the public.

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