Research Note

Examination of Bottled Water for Nontuberculous Mycobacteria

ALAN E. HOLTZMAN,1* TIMOTHY W. ARONSON,1 NORMAN GLOVER,1 SEYMOUR FROMAN,1 GERARD N. STELMA, JR,2 SALLY N. SEBATA,1 MARK G. BOIAN,1 TIEN T. TRAN,1 and O. GEORGE W. BERLIN1

1Olive View–UCLA Education and Research Institute, Olive View–UCLA Medical Center, 14445 Olive View Dr., Sylmar, California 91342; and 2U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, USA

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ABSTRACT

The objective of this study was to examine bottled water for the presence of nontuberculous mycobacteria as a potential source of infection in AIDS patients. Twenty brands of bottled water commonly used in the Los Angeles area were tested for the presence of nontuberculous mycobacteria. The three brands most commonly used in the Los Angeles area were tested most frequently. Sixty-nine samples were filtered and the filters were treated using cetypyridinium chloride, sodium hydroxide, or oxalic acid (or a combination of these) as decontaminants to remove background flora. An aliquot of each sample was untreated. The filters were placed on selective Middlebrook 7H10 agar plates containing 500 μg of cycloheximide per ml. Plates were examined at 3 and 8 weeks. No acid-fast organisms were found. Although no nontuberculous mycobacteria were observed in any samples tested, before recommending the use of bottled water as an alternative to tap water by high-risk patients, the possible presence of other contaminants must be considered.

Key words: Nontuberculous mycobacteria, bottled water

With the increased public concern for environmentally induced health problems and the desire for a better-tasting product, the use of bottled rather than tap water for drinking has expanded in popularity in recent years. The consumption of bottled water in the U.S. has risen from 4 gal (1 gal = 3.785 liters) per person per year in 1984 to 9.7 gal per year in 1994 (1). The greatest consumption of bottled water in the U.S. is in California, accounting for a 30.4% market share (1). Most bottled waters consumed are noncarbonated domestic brands.

Regulations for marketed bottled water are established by the U.S. Food and Drug Administration. These encompass water sources, plant and equipment design, the sanitary nature of both facilities and operations, appropriate record keeping and proper sampling of product water (6). State and industry standards are additionally imposed on the production of bottled water.

Water for bottling is obtained from either natural sources (e.g., springs or wells) or from treated public water sources. Further processing is accomplished by a number of technologies to reduce chemical and microbiological contamination.

Currently, the presence or absence of coliform organisms found in bottled water samples is the determinant of microbiological safety. These levels are specified by the U.S. Environmental Protection Agency (EPA) for any potable water. Recently, the EPA made recommendations for monitoring Cryptosporidium spp., Giardia spp., and viruses (5).

A group of organisms, the nontuberculous mycobacteria (NTM) have gained much prominence in recent years, particularly in relation to AIDS patients (3, 18). One group of NTM, the Mycobacterium avium complex (MAC), infects between 40 and 50% of AIDS patients (12) and is the most common bacterial cause of disseminated disease in these patients. One investigator states that, second to the AIDS wasting syndrome, MAC is the most common cause of death in these patients (11). In addition, studies have reported NTM disease in patients with no evidence of predisposing conditions (13, 14).

The gastrointestinal tract appears to be the most common portal of entry for NTM (8). Since these organisms have been identified in tap water (7, 9, 10), concern exists that potable water, along with other environmental sources, may be a potential source of human infection. Molecular biology studies have now demonstrated that some strains of MAC in potable water are identical to strains found in both AIDS and non-AIDS patients (2, 16).

Our studies of Los Angeles water revealed that 45% of potable water samples collected from office buildings, hospitals, and dwellings contained NTM, a significant number of which were MAC (10). These findings suggest the possible use of bottled water as an alternative to the use of tap water for drinking. However, the use of bottled water must be viewed with caution. All bottled water is not processed by the same method and some could contain...
significant contaminants, particularly when used by the immunocompromised patient. A review article of the microbiological quality of global bottled water describes the presence of a large variety of microorganisms (17). Only one previous study (4) has addressed the subject of acid-fast organisms in bottled water. This investigation was done in Italy and revealed an insignificant number of NTM, none of which were MAC.

MATERIALS AND METHODS

Twenty brands of noncarbonated domestic and foreign bottled water purchased from grocery markets in the Los Angeles area were sampled. Seventeen were sampled one or two times. Of the three most frequently consumed bottled waters in Southern California, two were each sampled 13 times and one 14 times, using different lot samples for each run. An additional 8 carbonated samples were also examined. All samples were collected over a period of 22 months.

Two-liter samples of each brand were obtained. Three aliquots were decontaminated to eliminate background flora with at least one or more of the following decontaminants: 5% oxalic acid (OA), 1% sodium hydroxide (NaOH), and 0.04 or 0.004% cetylpyridinium chloride (CPC). One aliquot was not decontaminated.

One 500-ml aliquot was filtered through a sterile 0.45-μm pore size black grid HABG filter (Millipore, Bedford, MA) 47 mm in diameter, in a sterile funnel (either stainless steel or polysulfone). The filter was transferred to a petri dish (Falcon Plastics, Becton Dickinson Microbiological Systems, Cockeysville, MD) and decontaminated with 5% oxalic acid or 1% sodium hydroxide. After 15 min, the NaOH or OA was aspirated with a sterile pipette. The filters were rinsed in 20 ml of phosphate buffer (pH 6.8) for 5 min and transferred aseptically to selective Middlebrook 7H10 agar plates containing 500 μg of cycloheximide per ml (Becton Dickinson Microbiological Systems). The agar plates were sealed in gas-permeable polyethylene bags to prevent desiccation, incubated at 37°C, and examined at 3 and 8 weeks for acid-fast organisms using the Ziehl-Neelsen stain.

An additional 500-ml aliquot was wrapped in aluminum foil and exposed to 0.004% CPC (Aldrich Chemical Co., Milwaukee, WI) and/or 0.04% CPC for 30 min and 24 h, respectively, and subsequently filtered as above followed by 500 ml of sterile distilled water to remove residual CPC. Another 500-ml aliquot was filtered and received no decontamination. The filters were placed on Middlebrook 7H10 agar containing 500 μg of cycloheximide per ml, incubated as above, and examined at 3 and 8 weeks for the presence of acid-fast organisms. The Quality control involved in the above process for isolation of NTM from tap water is described in detail in our earlier paper (10), as is the effect of each of the above decontaminants on M. avium concentrations. In the present study we examined one brand of water in duplicate using samples seeded with Mycobacterium avium. Concentrations of 10², 10³, and 10⁴ CFU/500 ml were processed as above along with unseeded samples of this brand. After 8 weeks of incubation, no growth was found on the unseeded and appropriate growth was found on all seeded samples.

RESULTS AND DISCUSSION

Total plate counts of 69 untreated samples demonstrated contamination (1 CFU to too-numerous-to-count values) in 35 samples. Subsequent to decontamination only 2 samples had persistent contamination unaffected by any decontaminant. No acid-fast organisms were recovered from any of the samples tested.

The absence of acid-fast organisms in this study raises the question of advocating, for high-risk individuals, the use of bottled water to avoid NTM exposure. Such a recommendation is suggested by this statistically significant investigation. However, the presence of other microbial and chemical contamination must be considered before proposing the use of bottled water as an alternative to tap water for drinking. Aside from the known organisms found in water, potable water contains a variety of heterotrophic bacteria that grow optimally in nutrient-poor environments (15). Many of these organisms have not been adequately characterized; some strains do not fit described genera in any current taxonomic schema. Until further research is done, the possibility that some of these heterotrophs could be opportunistic pathogens cannot be ruled out.

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REFERENCES


