Environmental mycobacteria in bottled table waters in Greece

M. Papapetropoulou, A. Tsintzou, and A. Vantarakis

Abstract: A hundred and fifty samples of bottled table water sold by Greek factories were examined for the presence of environmental mycobacteria. Environmental mycobacteria were found in 23 (15.6%) of the 150 tested samples. Bacterial numbers of 1–100, 101–300, 301–1000, and $>10^3$ CFU/L were found in 8, 2, 1, and 4% of the samples, respectively. The identification of the environmental mycobacteria was performed by both polymerase chain reaction – restriction enzyme analysis (PCR–REA) and biochemical methods. The environmental mycobacteria found were 14 *Mycobacterium chelonae*, 3 *Mycobacterium phlei*, 4 *Mycobacterium gordonae*, and 2 *Mycobacterium flavescens*. The relatively high number of environmental mycobacteria in bottled table water leads us to believe that the search of these opportunistic microorganisms in bottled water could be a useful index of their hygienic quality when this water is to be consumed by immunologically compromised patients. No statistically significant correlation was found between the presence of mycobacteria and the bacteriological faecal indicators ($P < 0.005$).

Key words: environmental mycobacteria, bottled water, PCR–REA, Greece.

Résumé : Nous avons recherché la présence de mycobactéries environnementales dans 150 échantillons d'eau minérale naturelle embouteillée et distribuée par des producteurs grecs. Des mycobactéries atypiques ont été retrouvées dans 23 (15,6%) des 150 échantillons examinés. Des dénombrements de 1–100, 101–300, 301–1000 et de $>10^3$ CFU/L ont été observés dans 8, 2, 1 et 4% des échantillons respectivement. L'identification des mycobactéries atypiques a été effectuée par réaction en chaîne de la polymérase – analyse avec des enzymes de restriction (PCR–REA) et par des méthodes biochimiques. Parmi les mycobactéries atypiques retrouvées, on compte 14 *Mycobacterium chelonae*, 3 *Mycobacterium phlei*, 4 *Mycobacterium gordonae* et 2 *Mycobacterium flavescens*. Le nombre relativement élevé de mycobactéries environnementales dans l'eau embouteillée nous porte à croire que la recherche de ces microorganismes opportunistes dans l'eau embouteillée pourrait devenir un index utile de leur qualité hygiénique surtout lorsque cette eau est consommée par des personnes dont le système immunitaire est compromis. Aucune corrélation statistiquement significative ($P < 0,005$) n'a été établie entre le présence des mycobactéries et les indicateurs bactériologiques fécaux.

Mots clés : mycobactéries environnementales, eau minérale embouteillée, PCR–REA, Grèce.
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Opportunistic mycobacteria are ubiquitous in the environment (Schulze-Robbecke 1993) with some of them causing disease (Jenkins 1991). Environmental mycobacteria are increasingly implicated in human disease in both immunocompetent and immunocompromised patients (George and Yates 1986), particularly those suffering from acquired immunodeficiency syndrome (AIDS) (Hellyer et al. 1993; Jackson et al. 1992; Nachamkin et al. 1992).

There is increasing evidence to suggest that infection of human immunodeficiency virus (HIV) patients with environmental mycobacteria takes place via the gastrointestinal tract (Hellyer et al. 1993). In Greece, most hospitalized patients drink bottled water, assuming it is bacteriologically superior to tap water. The presence of sodium chloride and fluoride, and the possible contamination of underground water by toxic industrial wastes, further increases the consumption of bottled water by patients and healthy individuals (Tobin 1984). Since hospital patients drink bottled water, the presence of acid fast bacilli is a potential threat, particularly for those with a compromised immune response.

Two types of bottled water are produced in Greece: natural mineral water and table water. The Greek Public Health Act of 1979 determines the quality of the water, the conditions in the factory, the disinfection procedure, and the number of microbiological tests required according to the size of the factory. Evaluation of mycobacteria is not included in the bacteriological examinations prescribed for bottled table water.

The aim of the present study was to determine if bottled table water contains environmental mycobacteria and to correlate the presence of these acid fast bacilli with the presence of the routinely tested faecal indicators for bottled table waters.

Between December 1993 and June 1994, 150 samples of bottled table water, sold by 20 Greek factories, were examined. All water samples were tested for total plate count (PC) at 22°C and at 37°C, total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS), *Pseudomonas aeruginosa*, and *Clostridium perfringens*, according to International

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