SHORT COMMUNICATION

Legionella pneumophila in commercial bottled mineral water

Rocus R. Klont[1,2], Antonius J.M. Rijs[1,2], Adilia Warris[1,2,3], Patrick D.J. Sturm[1,2], Willem J.G. Melchers[1,2] & Paul E. Verweij[1,2]

[1]Department of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [2]Department of Pediatrics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; and [3]Nijmegen University Center for Infectious Diseases, Nijmegen, The Netherlands

Correspondence: R.R. Klont, Department of Medical Microbiology, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: +31 24 3614356; fax: +31 24 3540216; e-mail: r.klont@mmb.umcn.nl

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Abstract

Sixty-eight commercial bottled mineral waters (64 brands, 68 different 'best-before dates') were tested for the presence of bacteria and fungi. Six samples were *Legionella* antigen positive and six were *Legionella pneumophila* PCR positive. Two samples were both *Legionella* antigen and *L. pneumophila* PCR positive. *Legionella* cultures were negative. Although the PCR might have detected only dead *Legionella* cells, the PCR has been described to detect specifically viable but not culturable (VBNC) *L. pneumophila* cells as well. Whether VBNC bacteria may be present in bottled mineral waters and the risk for infection this may pose for severely immunocompromised patients should be investigated.

Hospital water has been described as a potential transmission route for bacteria and fungi (e.g. *Aspergillus fumigatus*) (Warris *et al.*, 2003) and may therefore be a threat to immunocompromised patients. To prevent infection by tap water, severely immunocompromised patients, such as hematopoietic stem cell transplant (HSCT) recipients, may receive bottled mineral water under the assumption that this is microbiologically safer than tap water. We therefore investigated the presence of bacteria and fungi in 68 commercial mineral waters (64 brands) from nine European and six non-European countries (Table 1). All bottles had different 'best-before' dates and were therefore derived from different batches.

Coworkers from our department collected mainly locally available commercial bottled mineral waters during their holidays. All bottles were sealed properly, excluding the possibility of contamination after the production process. Water was filtered (0.2 μm filter) and the filter was cultured for moulds and bacteria, including *Legionella*. The presence of fungi and *Legionella* was determined by PCR. Ten tap water samples, randomly taken on a ward, served as controls for the *Legionella* PCR. All samples were also tested for the presence of the *Aspergillus* antigen galactomannan (Biotest AG, Dreieich, Germany) and *Legionella* antigen (BioRad, Marnes-la-Coquette, France).

Bacteria grew from 21 (30%) samples: coagulase negative staphylococci ($n = 8$), nonfermenters ($n = 10$) and gram-positive rods ($n = 9$) with bacterial counts of 60–180 CFU L^{-1}, 240–12 000 CFU L^{-1} and 40–12 000 CFU L^{-1}, respectively. Moulds were detected in three samples by culture (*Penicillium* spp., two samples) and by a pan-fungal PCR (nonspeciated, one sample), but galactomannan was not detected in any of the samples. *Legionella pneumophila*-specific DNA (Wellinghausen *et al.*, 2001) and *Legionella* antigen were each detected in six samples. The tap water controls were *Legionella* PCR negative. Two samples were both PCR- and antigen-positive. This suggests that the other four antigen-positive bottles contained a nonpneumophila species because the *Legionella* antigen test detects pneumophila as well as nonpneumophila spp. *Legionella* cultures remained all negative. This may be explained by the reduced sensitivity of culture compared to PCR and the presence of

Table 1. Results of culture, PCR and antigen detection of microorganisms in commercial bottled mineral water

Country of origin	No. of bottles	Bacterial culture no. positive	*Legionella* PCR no. positive	*Legionella* ELISA no. positive
Australia	1	0	0	0
Canada	2	0	0	0
Cuba	1	0	0	0
Germany	3	1	0	1
France	13	7	0	2
Greece	6	3	2	1
Hungary	3	1	0	0
India	4	1	0	0
Italy*	11	3	1	1
Mexico*	3	2	1	0
Norway†	12	2	1	1
Austria	3	1	0	0
Spain	1	0	0	0
Tanzania	1	0	0	0
Turkey	4	0	1	0

*Fungal culture positive with *Penicillium* spp.
†Pan-fungal PCR-positive.

nonfermenters, which are known to inhibit the growth of *Legionella* significantly (Toze *et al*., 1990). The presence of nonviable *Legionella* cannot be excluded. However, *Legionella* may be in a viable but not culturable state, especially when it is isolated from environmental waters (Leclerc & Moreau, 2002). Although the PCR may have detected only dead cells, the positive PCR reactions may have resulted specifically from amplification of DNA from viable but not culturable *Legionella* cells as well (Bej *et al*., 1991). This is an important consideration as viable but not culturable *Legionella* cells bacteria have been found to cause disease in animals (Roszak & Colwell, 1987) and reactivated viable but not culturable *Legionella* cells retain their virulence in human monocytes (Steinert *et al*., 1997), providing a basis for human infection and disease. In an outbreak of Pontiac fever a nonculturable *L. pneumophila* strain was responsible for the disease as it could be detected only by PCR and a direct fluorescent antibody test (Miller *et al*., 1993). Although inhalation from aerosols containing *Legionella* is the primary route of infection, pneumonia may follow from micro aspiration of stomach contents after the ingestion of water containing *Legionella* (Stout & Yu, 1997), especially in patients who have been bedridden, such as HSCT patients. Immunocompromised patients such as HSCT patients are especially susceptible to *Legionella* infections (Kool *et al*., 1998). Among bone marrow transplant recipients, pneumonia was caused by *Legionella* in 23% of the patients (Chow & Yu, 1998). There is some evidence that the incidence of nosocomial legionellosis is lower when sterile water is used by high risk patients (Marrie *et al*., 1991). Finally, an inoculum of $1\,\mathrm{CFU\,L^{-1}}$ may result in an infection in transplant patients (Mathys *et al*., 1999).

The general perception that bottled mineral water is safe may not be so true for severely immunocompromised patients as high levels of bacteria might be present. In addition, we found evidence for *L. pneumophila*, which has not been reported before. Recommendations provided by authorities such as the Centers for Disease Control and Prevention, the World Health Organization and the Food and Drug Administration do not include levels of *Legionella* in bottled mineral water. The water samples in our experiment were not sterile, showing that the decontamination methods used by the industry did not sterilize these waters. Because it is not common practice to sterilize bottled water used by severely immunocompromised patients, these patients may be at risk of becoming infected by microorganisms present in this water. We could not distinguish DNA originating from viable but not culturable *Legionella* cells from DNA originating from dead *Legionella* cells. However, these results should prompt further investigation for the presence of *Legionella* spp, especially *pneumophila*, in bottled mineral water and the risk of infection after oral intake.

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