

## Emerging bacterial pathogens in bottled water

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## Summary

Consumers believe that bottled water is a safe, natural product. However, scientists have shown that certain kinds of bottled water may cause infections especially in patients with compromised immune system. Under normal circumstances, the microbial population of bottled water is that which is indigenous to the source of the water (autochthonous flora). Such flora is considered to pose no risk to human health provided the European regulations governing the packaging of bottled water are adhered to. On the contrary, allochthonous bacteria in bottled water can cause water-borne infections in humans. Therefore, scientists in recent years have focused on identifying and characterizing these emerging allochthonous bacteria in bottled water. Using modern molecular techniques, scientists have identified a large number of emerging bacterial pathogens present in bottled water some of which exhibit resistance to many antibiotics. Examples of such bacteria include: The pathogenic *Escherichia coli* (*E. coli*); *Campylobacter jejuni* (*C. jejuni*); *Legionella pneumophila* (*L. pneumophila*), the non-tuberculous

or Environmental Mycobacteria (NTM), *Aeromonas hydrophila* and *Helicobacter pylori*. Guidelines governing the testing of bottled water for the presence of emerging bacterial pathogens do not exist. This, coupled with the absence as yet of specific surrogate indicators for these bacteria, makes the regular testing of bottled water practically non-existent. Therefore, the use of adequately sterilized water is strongly recommended for patients with extreme health issues leading to immune-compromised systems.



### Key words

Emerging bacteria, waterborne pathogens, bottled water safety

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## Introduction

In recent years there has been an explosive increase in consumption of bottled water worldwide. According to the World Watch Institute, consumption of bottled water during 1990-2005 quadrupled (50 billion liters per year).<sup>1</sup> This was attributed to the consumers' belief that bottled water was a safe, healthy and natural product. As a result of research outcomes indicating that certain bottled waters caused intestinal and gastrointestinal infections especially in immunocompromised patients, the industry of bottled water was forced to introduce tighter controls in the collection, treatment and packaging of such water.

At the international level there are three categories of bottled water: Natural mineral water, spring water, and table water from various sources.<sup>2</sup> The source of the natural mineral water is a protected underground source, presumably free of pathogens and, thus, not treated by either pasteurization, sterilization or other means in order to eliminate or reduce the number of microorganisms. On the contrary, spring water and table water from various sources are treated by ozonisation or UV light in order to comply with regulatory requirements when these exist.<sup>3</sup>

Present research studies are primarily focused on the identification of the presence of microorganisms in natural non-carbonated mineral water for two reasons: a) the low pH of carbonated water does not normally favour the growth of microorganisms and, b) natural mineral water is not subject to any degree of treatment prior to or during bottling as is the case with the other two types of bottled water. On the con-

trary, natural mineral water contains the autochthonous flora which for many years was considered to pose no risk to human health.

Emerging waterborne pathogens in bottled water are mainly of two types: The previously known bacteria which only recently were recognized as waterborne pathogens such as members of the *Legionella* spp., *Aeromonas* spp., *Mycobacteria* spp., and certain other bacteria that are enteric in origin;<sup>4</sup> and the recently identified waterborne microorganisms such as the *Escherichia coli* O157:H7.

A number of factors contribute to the recovery of waterborne pathogens from bottled water samples. These include, the development of new specific and more sensitive molecular, immunological, and immunomagnetic methods which allow for the correct identification of waterborne pathogens; the increasingly wide variety of identified microorganisms both autochthonous and allochthonous present in bottled water; the appearance of a wide variety of genes with different pathogenic activities and which are under the influence of environmental factors; the indiscriminate use of antibiotics leading to waterborne pathogens with increased resistance to antibiotics; changes in the immune system of humans as a result of exposure to pathogens in bottled water; and lastly, the low infectious dose of certain waterborne pathogens.<sup>5</sup>

## Emerging Bacterial Pathogens of Concern

### A. Infectious *E. coli*

*E. coli* is a gram-negative, facultative, anaerobic, rod-

shaped bacterium. Most strains of *E. coli* colonize the digestive tract of warm-blooded animals including humans. Usually are non pathogenic but certain strains possess virulence factors that are the cause of infections leading to diarrhea in humans.

Pathogenic *E. coli* are divided into six groups: enterohaemorrhagic (EHEC), enteroaggregative (EAEC), enterotoxinogenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and diffuse adherent (DAEC).<sup>6</sup> Among the pathogenic *E. coli*, the presence of *E. coli* O157:H7 is of particular significance in bottled water since it has been implicated in outbreaks of gastroenteritis following the consumption of municipal water,<sup>7</sup> and often causing symptoms of bloody diarrhea and hemolytic syndrome.<sup>8</sup> The severity of symptoms depends on the number of microorganisms ingested, the individual's health status and resistance to the toxins produced by the microorganisms.<sup>9</sup> The infectious dose of EHEC strains is very low (<100 cells). *E. coli* O157:H7 is known to survive in water at 15-18° C for 4-12 weeks.<sup>10,11</sup>

Known sources of water contamination with *E. coli* O157:H7 are primarily cattle and human excreta. Human excreta are also the source of other pathogenic *E. coli* strains.<sup>12</sup>

The majority of coliform bacteria isolated from bottled water, colonize the gastrointestinal tract without causing disease in healthy consumers. *E. coli* strains isolated from either tap or bottled water are not easily differentiated between pathogenic and non-pathogenic species. However, because of the severity of infections following the consumption of bottled drinking water containing pathogenic *E. coli*, various methods are proposed for the detection and characterization of *E. coli* O157:H7. Methods proposed by De Boer and Heuvelink<sup>13</sup> including the enzyme-linked immunosorbent assay (ELISA), colony immunoblot assays, the direct immunofluorescent filter technique and several immunocapture techniques for the detection of Shiga toxin of *E. coli* O157:H7 are most promising. The colorimetric method described by Su, Zhao, Qiao, Chen, Duan, Ai<sup>14</sup> in which functional Au@Pt nanoparticles as peroxidase mimetics are used, has also proven to be reliable. A study by Vasudaven,<sup>15</sup> observed that EAEC in bottled mineral water survive for quite a long time.

### B. *Campylobacter jejuni*

*Campylobacter* species are generally recognized as the most common cause of bacterial gastroenteritis worldwide.<sup>16</sup> *Campylobacter* is a waterborne pathogen that can survive for extended periods in water in a viable but non-culturable (VBNC) stage.<sup>17</sup> *Campylobacter*, under the influence of environmental factors

shifts rapidly to VBNC forms and lose their ability to be cultivated. These tend to recover within 7 days following inoculation into embryonated eggs.

The survival of *C. jejuni* strains in bottled water was studied by Guillow *et al.*<sup>18</sup> after inoculation of this strain in bottled water with different mineral content and maintained at 4° C in the dark. It was shown that bacteria survived for 28-48 days and that their survival time increased with increasing levels of mineral in water and depended on the type of water, the strain studied and culture conditions.

Bottled water has been considered as a possible risk factor due to *Campylobacter* infection.<sup>18</sup> Based on the epidemiological observations reported by Evans *et al.*,<sup>20</sup> bottled drinking water is one of the five leading risk factors for sporadic *Campylobacter* infections along with the consumption of cooked chicken, a certain tomato-cucumber sauce and following contact with animals. The only case of documented outbreak due to *C. jejuni* has been reported by the Center of Disease Control (CDC) in United States of America (U.S.A) and involved 106 guardsmen of the Minesota Army National Guard during a session of training exercises in Greece. The source of the outbreak was bottled water produced in Greece.<sup>21</sup>

### C. *Legionella pneumophila*

*Legionella* species survive in the aquatic environment under various conditions of pH (0.5-8.5) and temperature range (0-63° C) with optimum growth at 20-50 C.<sup>22</sup> *Legionella* species develop symbiotic relationships with other bacteria such as *Flavobacteria*, *Acinetobacter* and *Alcaligenes*.<sup>23</sup> Protozoa such as Hartamella, Acanthamoeba etc., naturally found in the water, harbor *Legionella* spp. and thus creating for them suitable conditions for their propagation while protecting them from detrimental environmental conditions. A similar situation exists with their existence in biofilms.

The most frequently isolated *Legionella* species is *L. pneumophila*, the causative agent of pneumonia with high mortality rates especially in immuno-compromised patients. As a respiratory pathogen it is transmitted mainly by inhalation. It can also be transmitted by micro aspiration of the stomach contents following the ingestion of contaminated water.<sup>24</sup>

This is especially true for transplanted patients who are particularly sensitive to *Legionella* infections.<sup>25</sup> In these patients 23% of pneumonia cases are due to these waterborne pathogens. The infectious dose among immuno-compromised hosts is low. It has been reported that even a dose as low as one colony forming unit (cfu)/L is sufficient to cause infection in immuno-compromised patients.<sup>26</sup> On the contrary, in healthy individuals only a large dose of these bacteria

present in certain water systems such as showers, cooling towers or whirlpool baths all of which generate aerosols can become infective.

Guidelines for *Legionella* have been developed in a few countries. Such guidelines are related to the control of *Legionella* in cooling towers and piped water systems established outside municipal water distribution networks.<sup>27</sup> In addition guidelines exist for the heating, ventilation, and air conditioning industry. These aim at reducing the *Legionella* growth in such systems.<sup>28</sup> Guidelines for microbiological testing of bottled water issued by CDC, World Health Organization (WHO) and the Food and Drug Administration (FDA) of U.S.A. do not include testing for *Legionella* growth presumably because no cases of infection are linked to these bacteria although their presence has been reported lately by Klont, *et al.*<sup>29</sup> These investigators examined bottled mineral water originating from different countries by various techniques including PCR, ELISA and cultures. Of the 68 samples of bottled mineral water tested, six were positive for *Legionella* antigens and 6 of *L. pneumophila* in PCR. Two of these samples were positive for *Legionella* antigen and *L. pneumophila* PCR. The remaining four positive PCR have been reported to detect VBNC *L. pneumophila* or possibly dead *Legionella* cells. The presence of VBNC bacteria in bottled mineral water poses a risk for infection in immuno-compromised patients since reactivated VBNC of *Legionella* cells retain their virulence in human monocytes.<sup>30</sup> Miller, *et al.*<sup>31</sup> reported that an outbreak of Pontiac fever was due to a non-culturable *L. pneumophila* strain that was detected only by PCR and a direct fluorescent antibody test. Totaro *et al.*<sup>32</sup> also underlined the importance of using molecular methods instead of routine microbiological indicators. qPCR showed the presence of *Legionella* qPCR units in 24% of samples (from  $1.1 \times 10^2$  to  $5.8 \times 10^2$  qPCR units/L).

#### D. Non-tuberculous or Environmental Mycobacteria

*Mycobacteria* belong to the family Mycobacteriaceae and the genus Mycobacterium. *Mycobacteria* are gram-negative, non-sporogenous obligatory aerobic, intracellular microorganisms, resistant to environmental conditions. *Mycobacteria* (other than those causing tuberculosis and leprosy) are divided into four groups based on growth rate and pigmentation properties.<sup>32</sup> They are further categorized into rapidly and slowly growing (> or <7 days) species. The majority of the mycobacteria are Non-Tuberculous – Mycobacteria (NTM) called also Environmental Mycobacteria and they are ubiquitous in the environment. Environmental mycobacteria are opportunistic pathogens with varying pathogenic effects.<sup>34</sup>

It has been shown that various types of environmental mycobacteria are present in drinking water particularly in municipal water supply networks and in bottled water.<sup>32</sup> These microorganisms colonize the surfaces of the tubes creating biofilms, both in the distribution system of municipal water supplies, and on the inner surfaces of PET bottles used in bottling water.<sup>35</sup> The production of the biofilm seems to depend on the mycobacterium strain, the existence of divalent ions such as  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{Zn}^{++}$  as well as the presence of trophic factors in the aquatic environment.<sup>36</sup> In addition, the existence of glucopeptidolipids located on the exterior of the cell envelope of mycobacteria helps their adhesion on abiotic surfaces creating biofilms, and thus increasing their survival time.

Opportunistic pathogenic environmental mycobacteria adapt to the oligotrophic water conditions and remain there for a long time because of their high resistance to the disinfectants used. Most environmental species including *M. gordonae*, *M. avium*, *M. flavescense*, *M. kansasii* and others isolated from drinking water are slow growing while *M. smegmatis*, *M. chelonae* and *M. phlei* are rapidly growing.<sup>37</sup> The infectious dose depends on the mycobacteria species, their concentration in the water the human body is exposed to, and to the condition of the human immune system. Normally, the concentration of these bacteria in mineral water is low, and non-infectious in healthy people. However, people with a weak immune system and particularly individuals with low CD4 cells should be offered bottled water with caution.<sup>38</sup> It has been estimated that in the U.S.A. 25-50% of individuals with human immunodeficiency virus (HIV) infections will develop environmental mycobacterial infections that affect the lungs, lymph nodes, skin and soft tissue.<sup>40</sup>

One of the most important opportunistic pathogens causing waterborne infections is *M. avium*. This conclusion was based on the observations that the DNA fingerprints of *M. avium* isolated from municipal drinking water in which patients with AIDS were exposed to, were the same as those isolated from clinical samples of these patients.<sup>37</sup> On the contrary, no such observations were reported in AIDS patients who consumed bottled water. Studies where the presence of environmental mycobacteria was controlled resulted in varying conclusions. Holtzan<sup>41</sup> reported negative results for mycobacteria when testing bottled water samples. Caroli *et al.*<sup>42</sup> screened 84 samples of bottled mineral water and found 7 positive samples (10.7%) for mycobacteria including 4 with *M. gordonae*, 1 with *M. phlei*, 1 with *M. sphagni*, and 1 with *M. flavescens*. The concentration of mycobacteria was low except for

*M. sphagni* for which the prevalence was much higher. Papapetropoulou *et al.*<sup>43</sup> reported the isolation of environmental mycobacteria in 23 of 150 bottled table water samples tested - a proportion of 15.6% at a concentration of 1-10<sup>3</sup> //L. Species detected in culture and identified by molecular methods such as PCR and REA included 14 samples with *M. chelonae*, three with *M. phlei*, four with *M. gordonae* and two with *M. flavescens*. Of the 150 bottled table water samples tested, 16 or 10.6% were not suitable for consumption according to Greek guidelines. The corresponding number found by Venieri *et al.*<sup>44</sup> was 13.95%. This proportion was even greater (21.2%) reported by Mavridou *et al.*<sup>45</sup> Donato *et al.*<sup>46</sup> isolated five species of environmental mycobacteria three of which were *M. chelonae*, one was *M. gordonae* and one belonging to Ryan group II from a total of 53 bottled mineral water samples an incidence of 10.6%. Data regarding the infectious dose of these bacteria in immuno-suppressed individuals consuming bottled drinking water is desirable.

### E. *Aeromonas* species

The potential environmental source for *Aeromonas* spp implicated as the causative agents in gastrointestinal disorders was first published by Holmerg *et al.*<sup>47</sup> Various species of the genus *Aeromonas* are ubiquitous in the aquatic environment and are a significant part of the autochthonous flora of the water. *Aeromonas* are mesophilic motile or psychrophilic non-motile Gram-negative bacteria. Many species of the genus *Aeromonas* are not pathogenic to humans. Reported pathogens for humans are *A. hydrophila*, *A. caviae* and *A. veronii* representing almost 85% of the bacteria that are isolated clinically. These bacteria produce severe infections mainly in immuno-compromised hosts, suffering from septicemia, peritonitis, wound infections, myonecrosis and hepatic cirrhosis.<sup>48,49</sup> In humans with a healthy immune system certain *Aeromonas* species may be responsible for traveler's diarrhea.<sup>50</sup> The *Aeromonas* species produce a wide range of extracellular enzymes including enterotoxins, proteases, phospholipases, haemolysins, and adhesions that may cause damage in the cells of the host.<sup>51</sup> *A. hydrophila* is considered the most dangerous pathogen of the *Aeromonas* species as it produces a large number of different enterotoxins<sup>52</sup> and it survives for a long time in bottled mineral water.<sup>53</sup> The infectious dose of *A. hydrophila* has not been established conclusively as yet. Certain studies reported this dose to be high with only a small number of the study participants suffering infection.<sup>54</sup>

Croci *et al.*<sup>55</sup> study the survival and growth of *A. hydrophila* isolates added to bottled mineral water with low, medium and high mineral content and maintai-

ned in temperatures at 10°C and 20°C. Analysis of samples was performed at several time intervals up to 60 days. The results of such experiments indicated that the growth of *A. hydrophila* depended on the mineral content of the water, the temperature, the length of storage and the type of container used. The growth of *A. hydrophila* was optimum at low mineral content of the water, and at a temperature of 10°C. The most robust proliferation was observed at 28 days. These findings are in agreement with the findings of Warburton *et al.*<sup>56</sup>

A few countries including those of the Netherlands, Canada and Italy suggested the introduction of guidelines pertaining to the detection of *Aeromonas* species in drinking water. As a result, in 1997 Italy established the maximum permissible levels for *Aeromonas* species in natural mineral waters. These were 10 cfu/100 ml of water at source and 100 cfu/100 ml after bottling.<sup>57</sup> However, at the end of 1998 these values were withdrawn and new guidelines were published. In Canada the Food and Drugs Act included criteria for *Aeromonas hydrophila* and standards were set for bottled water at 0 cfu/100 ml.<sup>56</sup> For Netherlands the corresponding levels were set at 200 cfu/100 ml of water.

In 1998 an assessment for the presence of *Aeromonas* species in a certain mineral water brand of bottled water in Italy was performed and all samples tested were found to be negative. However, a small number of *A. caviae* was constantly present without causing gastrointestinal disturbances in consumers.<sup>58</sup>

The routine identification of a virulent strain of *Aeromonas* species detected in water is often difficult as it requires the use of modern molecular techniques. The Biscardi *et al.*<sup>59</sup> results of a survey showed that six strains of *A. hydrophila* isolated from 61 samples of bottled mineral water were found to be cytotoxic. Molecular investigation revealed that these strains possessed an aerolysin gene.

The presence of *Aeromonas* species in bottled water has been documented by scientists who utilized various techniques including sequencing, 16SrDNA,<sup>60</sup> q PCR,<sup>61</sup> and macrorestriction analysis of genomic DNAs with x bal and PFGE.<sup>58</sup>

De Oliverra Scoaris *et al.*<sup>62</sup> recovered six *Aeromonas* in samples from 47 bottles of mineral water. Two of the six were *A. hydrophila*; one was *A. jandaei* while in three samples *Aeromonas* were not identifiable. Ahmed *et al.*<sup>61</sup> tested 16 brands of bottled water for microbiological content in Bangladesh using qPCR technology. Nine (56%) of these brands contained *A. hydrophila* lip.gene strains, responsible for causing septicemia and gastrointestinal disorders in children, elderly and immuno-compromised hosts. Hareesh *et*



*al*<sup>63</sup> using PCR assay, detected *Aeromonas* spp in 16% of the 25 samples of bottled water tested. While these results indicate that there is a need for constant identification and characterization of pathogens in water, the scientific community suggests that any controls for *Aeromonas* in water should be used as sanitary quality indicators only.

Todate, the link of *Aeromonas* species in bottled water with outbreaks of disease has not been established conclusively.<sup>58</sup> This is due partly to lack of guidelines in the various countries and the fact that most species of *Aeromonas* isolated from bottled water are avirulent. Furthermore, the routine identification and characterization of toxic agents of the various *Aeromonas* that may express pathogenic activity is a difficult task.

### F. *Helicobacter pylori*

Infections with *Helicobacter pylori* (*H. Pylori*) are very common worldwide. It is estimated that 50% of the population in developed countries is infected with this pathogen. An incidence of 90% is reported for developing countries and this incidence involves mainly children.<sup>64,65</sup>

*H. pylori* is a Gram-negative spiral bacterium, motile, that colonizes the gastric mucosa and causes severe stomach gastritis which may develop into a gastric cancer. Morphologically it occurs in two forms: the spiral and the coccoid. The spiral forms can be cultured and isolated with culture methods from clinical samples, while its coccoid form are recovered from the aquatic environment where *H. pylori* is subject to various environmental conditions.<sup>66</sup> These are not cultivable and their presence is confirmed only by molecular techniques such as PCR and fluorescent in situ DNA hybridization.<sup>67</sup>

*H. pylori* has been shown to survive for periods ranging from days up to weeks in water at temperatures of 4-25°C.<sup>68</sup>

The mode of transmission of *H. pylori* remains unclear. Different routes have been proposed including the oral-oral, gastric-oral and faecal-oral routes.<sup>69</sup>

Presently, existing controls for *H. pylori* in bottled water are inadequate and very limited. Bahrami *et al.*<sup>70</sup> was unable to detect *H. pylori* in bottled water when up-to-date molecular techniques were applied. Banjar *et al.*<sup>71</sup> using molecular techniques documented the presence of *H. pylori* in 8 of the 450 samples studied (1.77%). The level of *H. pylori* in the samples tested was higher in the month of July. The U.S. EPA lists *H. pylori* as an emerging waterborne pathogen but ranks it second for risk to humans. Therefore, it is not known whether *H. pylori* can be transmitted to humans via drinking of bottled water. Gesumaria<sup>72</sup> identified a

large number of microbial loads in water bottled water when cloning and sequencing of the 16SrRNA gene were used. Some of them including *Stenotrophomonas maltophilia* were thought to be dangerous opportunistic pathogens especially in patients with cystic fibrosis.<sup>73</sup> Also *Brevundomonas vesicularis* has been shown to cause pneumonia, meningitis, peritonitis and bacteraemia in immuno-compromised patients.<sup>74</sup> Furthermore, the bacteria *Bulkholderia cepacia*, *Sphingomonas paucimobilis*, *Raoultella terrigena*, *Ralstonia picketti*, and *Delfia acidovorans* are some of the bacteria that have been isolated from natural mineral waters.<sup>75</sup> Such bacteria, although not very common in bottled water, they are, nevertheless, infectious. Leclerc and da Costa.<sup>76</sup> Venieri *et al.*<sup>77</sup> isolated *Aeromonas* species, *Pasteurella*, *Citrobacter*, *Flavobacterium* and *Providencia* present in bottled non-carbonated water samples from various local brands in Greece.

Resistance to antibiotics in emerging bacterial pathogens present in bottled water is a major concern to health officials and to the public at large. Messi *et al.*<sup>78</sup> studied the antibiotic resistance of *Pseudomonas* spp, *Burkholderia* spp, *Agrobacterium/radiobacter* isolated from mineral waters. Strains with multi-antibiotic resistance represent 55% of the isolates and the most resistant organisms belonged to the genus *Pseudomonas*.

### Conclusions

To-date, the various types of bottled water available for human consumption in many countries are not regularly tested for the presence of emerging bacterial pathogens. There are several reasons for this and include a) the lack of guidelines imposing regular monitoring of bottled water, b) the absence of adequate surrogate indicators for the bacteria in question and, c) with the exemption of *E.coli* and *C. jejuni*, there are no reports for any serious outbreaks that can be conclusively attributed to the consumption of bottled water containing the emerging waterborne microorganism discussed above.<sup>21</sup> Nevertheless, the limited data available indicate that bottled water contaminated with emerging pathogens is a risk especially to humans who are immune-compromised. The incidence of people with weak immune systems is on the rise mainly due to increased lifespan, climate changes and unhealthy eating habits. These factors along with the exponential increase of bottled water manufacturers and its ever increasing consumption by the human population necessitate the formulation of strict guidelines across the countries. Such guidelines should include the use of surrogate indicators detec-

ted by molecular techniques and surrogate markers appropriate for detecting the presence of these emerging pathogens in bottled water. Furthermore, health care givers should be aware of the potentially harmful health effects bottled drinking water may have in patients with serious illness and whose immune system may be compromised and should suggest that sterile

water be consumed rather than bottled water. Resistance of emerging waterborne pathogens to antibiotics is of concern. In conclusion, the use of appropriate methodology is warranted for the identification and characterization of newly emerging waterborne bacteria especially the pathogenic *E. coli* in bottled water.



## Περίληψη

### Αναδυόμενα παθογόνα βακτήρια στο εμφιαλωμένο νερό

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Γενικά στους καταναλωτές υπάρχει η αντίληψη ότι τα εμφιαλωμένα νερά είναι ασφαλή, φυσικά προϊόντα. Εντούτοις μελέτες έχουν δείξει ότι μερικά από τα εμφιαλωμένα νερά μπορούν να προκαλέσουν λοιμώξεις, ιδίως σε ανοσοκατεσταλμένους ασθενείς. Σε κανονικές συνθήκες ο μικροβιακός πληθυσμός του εμφιαλωμένου νερού είναι αυτός της πηγής προέλευσής του (αυτόχθονος χλωρίδα). Ένας τέτοιος πληθυσμός δε θέτει σε κίνδυνο τη δημόσια υγεία, με την προϋπόθεση βέβαια ότι ακολουθούνται όλοι οι ευρωπαϊκοί κανονισμοί σχετικά με την εμφιαλωση του νερού. Αντίθετα, βακτήρια που δεν ανήκουν στην αυτόχθονη χλωρίδα του εμφιαλωμένου νερού ή αλλιώς «αλλόχθονα», μπορεί να προκαλέσουν υδατογενείς λοιμώξεις στους καταναλωτές. Για το λόγο αυτό τα τελευταία χρόνια μελετητές έχουν εστιάσει στην ανίχνευση και ταυτοποίηση τέτοιων παθογόνων στα εμφιαλωμένα νερά. Με τη βοήθεια των μοριακών τεχνικών έχει ανιχνευθεί ποικιλία βακτηρίων, πολλά από τα οποία μάλιστα παρουσιάζουν αντοχές στα αντιβιοτικά. Παραδείγματα αποτελούν: παθογόνα είδη *Escherichia coli*, *Campylobacter jejuni*, *Legionella pneumophila*, μη φυματιώδη ή περιβαλλοντικά μυκοβακτηρίδια (non-tuberculous Mycobacteria, NTM), *Aeromonas hydrophila* και *Helicobacter pylori*. Οδηγίες σχετικές με τον έλεγχο των εμφιαλωμένων νερών για την ύπαρξη των παραπάνω, αλλά και άλλων αναδυόμενων βακτηρίων, ουσιαστικά δεν υπάρχουν. Αυτό σε συνδυασμό με την απουσία ειδικών δεικτών ανίχνευσής τους έχει σαν αποτέλεσμα την απουσία ουσιαστικού ελέγχου ρουτίνας ύπαρξής τους εντός των εμφιαλωμένων νερών. Επομένως, η εφαρμογή αποτελεσματικών μεθόδων αποστείρωσης του εμφιαλωμένου νερού είναι επιβεβλημένη ειδικά στα άτομα με διαταραχή του ανοσοποιητικού τους συστήματος.



#### Λέξεις κλειδιά

αναδυόμενα βακτήρια, υδατογενή παθογόνα, ασφάλεια εμφιαλωμένου νερού

## References

1. Li Ling. Bottled Water Consumptions Jumps. World Watch Institute. November 8, 2007.
2. Dege N. Categories of bottled water. in Technology of bottled Water (3rd Edition). Wiley-Blackwell Publishing Ltd ., 2011.
3. Leclerc H , Moreau A. Microbiological safety of natural mineral water. *FEMS Microbiology Reviews* 2002; (26) 207-222.
4. WHO (World Health Organization) (2003). Emerging issues in water and infectious disease. ISSN 1728-2160, ISBN 9241590.
5. Szewzyk U, Szewzyk R, Manz W, Schleifer KH. Microbiological safety of drinking water. *An Rev Microbiol* 2000; 54:81-127.
6. Percival SL, Chalmers RL, Embrey M, Hunter PR, Sellwood J, Wyn-Jones P.(eds). Microbiology of Waterborne Diseases, First Edition. Elsevier Academic Press, San Diego, California, 2004 pages 480.
7. Clark WF, Sontrop JM, Macnab JJ, Salvadori M, Moist L, Suri R, *et al.* Long term risk for hypertension, renal impairment, and cardiovascular disease after gastroenteritis from drinking water contaminated with *Escherichia coli* O157:H7: a prospective cohort study. *BMJ* 2010; 341:c6020.
8. Riley LW. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983; 308(12):681-685.
9. Le Chevallier, MW. Committee report: Emerging pathogens - bacteria. *J Am Water* 1999; 91(9), 101-109.
10. Edberg SC, Rice EW, Karlin RJ, Allen MJ. *Escherichia coli*: the best biological drinking water indicator for public health protection. *Symp Ser Soc Appl Microbiol* 2000; 29:106S-116S.
11. Kerr M, Fitzgerald M, Sheridan JJ, McDowell DA, Blair IS. Survival of *Escherichia coli* O157:H7 in bottled natural mineral water. *J Appl Microbiol* 1999; 87(6):833-841.
12. AWWA (American Water Works Association). AWWA manual of water supply practices- M48 : Waterborne pathogens 2nd edition. American Water Works Association, Denver, Colorado, 2006.
13. De Boer E, Heuvelink AE. Methods for the detection and isolation of Shiga toxin-producing *Escherichia coli*. *Symp Ser Soc Appl Microbiol* 2000; (29):133S-143S.
14. Su H, Zhao H, Qiao F, Chen L, Duan R, Ai S. Colorimetric detection of *Escherichia coli* O157:H7 using functionalized Au@Pt nanoparticles as peroxidase mimetics. *Analyst* 2013; 138(10):3026-3033.
15. Vasudevan P, Annamalai T, Sartori L, Hoagland T, Venkitanarayanan K. Behavior of enteroaggregative *Escherichia coli* in bottled spring and mineral water. *J Food Prot* 2003; 66(3):497-500.
16. Allos BM. *Campylobacter jejuni* Infections: update on emerging issues and trends. *Clin Infect Dis* 2001; 32(8):1201-1206.
17. Rollins DM, and Colwell RR. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Appl Environ Microbiol* 1986; 52(3):531-538.
18. Guillou S, Leguerinel I, Garrec N, Renard MA, Cappelier JM, Federighi M. Survival of *Campylobacter Jejuni* mineral bottled water according to difference in mineral content: application of the Weibull model. *Water Res* 2008; 42(8-9):2213-2219.
19. Gillespie IA, O'Brien SJ, Frost JA, Adak GK Horby P, Swan AV, *et al.* A Case-Case Comparison of *Campylobacter coli* and *Campylobacter jejuni* Infection: A Tool for Generating Hypotheses. *Emerg Infect Dis* 2002; 8(9):937-942.
20. Evans MR, Ribeiro CD, Salmon RL. Hazards of healthy living: bottled water and salad vegetables as risk factors for *Campylobacter* infection. *Emerg Infect Dis* 2003; 9(10):1219-122
21. Osterholm M. Outbreak of *campylobacter jejuni* infection among Minnesota Army National Guard personnel returning from Greece 1997. Minnesota Department of Health Minneapolis, Minn., 1998.
2. Nguyen MH, Stout JE, Yu VL. Legionellosis. *Infect Dis Clin North Am.* 1991; 5(3):561-584.
23. Lin YS, Stout JE, Yu VL, Vidic RD. Disinfection of water distribution systems for Legionella. *Semin Respir Infect* 1998; 13(2):147-159.
24. Stout JE, Yu VL. Legionellosis. *N Engl J Med* 1997; 337(10):682-687.
25. Kool JL, Fiore AE, Kioski CM, Brown EW, Benson RF, Pruckler JM, *et al.* More than 10 years of unrecognized nosocomial transmission of legionnaires' disease among transplant patients. *Infect Control Hosp Epidemiol* 1998; 19(12):898-904.
26. Mathys W, Deng MC, Meyer J, Junge-Mathys E. Fatal nosocomial Legionnaires' disease after heart transplantation: clinical course, epidemiology and prevention strategies for the highly immuno-compromized host. *J Hosp Infect* 1997; 43(3):242-246.
27. Cunliffe D. Regulatory aspects in Legionella and the prevention of legionellosis. In Legionella and the prevention of legionellosis, Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S (eds). World Health Organization, Geneva Switzerland 2007; Chapter 10:161-174.
28. ASHRAE Guideline 12 (2000). Minimizing the Risk of Legionellosis Associated with Building Water Sys-

- tems American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc.
29. Klont RR, Rijs AJ, Warris A, Sturm PD, Melchers WJ, Verweij PE. *Legionella pneumophila* in commercial bottled mineral water. *FEMS Immunol Med Microbiol* 2006; 47(1):42-4.
  30. Steinert M, Emody L, Amann R, Hacker J. Resuscitation of viable but non culturable *Legionella philadelphia* JR32 by *Acanthamoeba castellanii*. *Appl Environ Microbiol* 1997; 63(5) 2047-2053.
  31. Miller LA, Beebe JL, Butler JC, Martin W, Benson R, Hoffman RE, et al. Use of polymerase chain reaction in an epidemiologic investigation of Pontiac fever. *J Infect Dis* 1993; 168(3):769-72.
  32. Totaro M., Casini B., Valentini P., Miccoli M., Lopalco PL., Baggiani A. Assessing natural mineral water microbiology quality in the absence of cultivable pathogen bacteria. *J Water Health* 2018; 16(3):425-434.
  33. Runyon EH. Anonymous mycobacteria in pulmonary disease. *Med Clin North Am* 1959; 43(1):273-290.
  34. Falkinham JO. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *J Appl Microbiol* 2009; 107(2):35-367.
  35. Tatchou-Nyamsi-König JA, Dailloux M, Block JC. Survival of *Mycobacterium avium* attached to polyethylene terephthalate (PET) water bottles. *J Appl Microbiol* 2009; 106(3):825-832.
  36. Carter G, Wu M, Drummond DC, Bermudez LE. Characterization of biofilm by clinical isolats of *Mycobacterium avium*. 2003;52:747-752.
  37. Won Reyn CF, Maslow JN, Barber TW, Falkinham JO, Arbeit RD. Persistent colonisation of potable water as a source of *Mycobacterium avium* infection in AIDS. *Lancet* 1994; 343:1137-1141.
  38. Karakousis PC, Moore RD, Chorisson RE. *Mycobacterium avium* complex in patients with HIV infection in the era of active antiretroviral therapy. *Lancet Infect Dis* 2004; 4:557-565.
  39. Takahashi M, Tsukamoto H, Kawamura T, Mochizuki Y, Ouchi M, Inoue S, et al. *Mycobacterium kansasii* pulmonary infection: CT findings in 29 cases. *Jpn J Radiol* 2012; 30(5):398-406.
  40. Talanow R, Vieweg H, Andersen R. Atypical Osteomyelitis Caused by *Mycobacterium chelonae* – A Multimodal Imaging Approach. *Case Rep Infect Dis*. 2013; 528-595.
  41. Holtzan AE. Examination of Bottled water for Nontuberculous Micobacteria. 1997; 60(2), 185-187.
  42. Caroli G, Levre E, Armani G, Biffi-Gentili S, Molinari G. Search for acid-fast bacilli in bottled mineral waters. *J Appl Bacteriol* 1985; 58(5):461-463.
  43. Papapetropoulou M, Tsintzou A, Vantarakis A. Environmental mycobacteria in bottled table waters in Greece. *Can J Microbiol* 1997; 43(5):499-502.
  44. Venieri D, Vantarakis A, Komninou G, Papapetropoulou M. Microbiological evaluation of bottled non-carbonated (still) water from domestic brands in Greece. *Int J Food Microbiology* 2006; 107 (1) 68-72.
  45. Mavridou A., Papapetropoulou M., Boufa P., Lambiri M. and Papadakis J.A. Microbiological quality of bottled water in Greece. *Letters in Applied Microbiology* 1994; 19 (4) 213–216.
  46. Donato R, Tortoli E, Simonetti MT, Ademollo B, Signorini LF. The search for nontuberculous mycobacteria in bottled mineral waters found in commerce. *Ann Ig.* 1994; 6(4-6):895-899.
  47. Holmberg SD, et al. *Aeromonas* intestinal infections in the United States. *Ann Intern Med* 1986; 105(5):683-689.
  48. Ko WC and Chuang YC. *Aeromonas* bacteremia: review of 59 episodes. *Clin Infect Dis.* 1995; 20(5):1298-1304.
  49. Martino R, Santamaría A, Pericas R, Sureda A, Brunet S. Acute rhabdomyolysis and myonecrosis complicating *aeromonas* bacteremia in neutropenic patients with hematologic malignancies: report of two cases. *Haematologica* 1997; 82(6):692-694.
  50. Vila J, Ruiz J, Gallardo F, Vargas M, Soler L, Figueras MJ, et al. *Aeromonas* spp. and traveler's diarrhea: clinical features and antimicrobial resistance. *Emerg Infect Dis* 2003; 9(5):552-555.
  51. Gavin R, Merino S, Tomas JM. Molecular Mechanisms of Bacterial Pathogenesis from an Emerging Pathogen: *Aeromonas* species. *Rec Res Dev Infec Immunol* 2003; 1(1):337-354.
  52. Schubert RH. *Aeromonads* and their significance as potential pathogens in water. *Soc Appl Bacteriol Symp Ser* 1991; 20:131S-135S.
  53. Messi P, Guerrieri E, Bondi M. Survival of an *Aeromonas hydrophila* in an artificial mineral water microcosm. *Water Res* 2002; 36(13):3410-5.
  54. Janda JM, Abbott SL. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clin Microbiol Rev* 2010; 23(1):35-73.
  55. Croci L, Di Pasquale S, Cozzi L, Toti L. Behavior of *Aeromonas hydrophila* in bottled mineral waters. *J Food Prot* 2001; 64(11):1836-1840.
  56. Warburton DW, McCormick JK, Bowen B. Survival and recovery of *Aeromonas hydrophila* in water: development of methodology for testing bottled water in Canada. *Can J Microbiol* 1994; 40(2):145-148.
  57. Italian Ministry of Health. Gazzetta Ufficiale della Repubblica Italiana, Serie generale, no. 170, 23 July 1997. Italian Ministry of Health, Rome, Italy.
  58. Villari P, Crispino M, Montuori P, Boccia S. Molecular typing of *Aeromonas* isolates in natural mineral waters. *Appl Environ Microbiol* 2003; 69(1):697-701.
  59. Biscardi D, Castaldo A, Gualillo O, de Fusco R. The occurrence of cytotoxic *Aeromonas hydrophila* strains in Italian mineral and thermal waters. *Sci Total Environ* 2002; 292(3):255-263.



60. Martinez-Murcia AJ, Benlloch S, Collins MD. Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridizations. *Int J Syst Bacteriol* 1992; 42(3):412-421.
61. Ahmed W, Yusuf R, Hasan I, Ashraf W, Goonetilleke A, Toze S, et al. Fecal indicators and bacterial pathogens in bottled water from Dhaka, Bangladesh. *Braz J Microbiol* 2013; 44(1):97-103.
62. De Oliveira Scoaris D, Bizerra FC, Yamada-Ogatta SF, Abreu Filho BA, Ueda-Nakamura T, Nakamura CV, et al. The Occurrence of *Aeromonas* spp. in the bottled mineral water, well water and tap water from the municipal supplies. *Braz Arch Biol Technol* 2008; 51: 1049-1055.
63. Didugu H., Thirtham M., Nelapati K., Reddy KK., Kumbhar BS., Poluru A., et al. *Vet World* 2015; 8(10): 1237-1242.
64. Bardhan PK. Epidemiological features of *Helicobacter pylori* infection in developing countries. *Clin Infect Dis* 1997; 25(5):973-978.
65. Farthing MJ. *Helicobacter pylori* infection: an overview. *Br Med Bull* 1998; 54(1):1-6.
66. Bode G, Mauch F, Malfertheiner P. The coccoid forms of *Helicobacter pylori*. Criteria for their viability. *Epidemiol Infect* 1993; 111(3):483-490.
67. Benson JA, Fode-Vaughan KA, Collins ML. Detection of *Helicobacter pylori* in water by direct PCR. *Lett Appl Microbiol* 2004; 39(3):221-225.
68. Azevedo NF, Almeida C, Fernandes I, Cerqueira L, Dias S, Keevil CW, et al. Survival of gastric and enterohepatic *Helicobacter* spp. in water: implications for transmission. *Appl Environ Microbiol* 2008; 74(6):1805-1811.
69. Percival SL and Thomas JG. Transmission of *Helicobacter pylori* and the role of water and biofilms. 2009; 7(3):469-477.
70. Bahrami AR, Rahimi E, Ghasemian Safaei H. Detection of *Helicobacter pylori* in city water, dental units' water, and bottled mineral water in Isfahan, Iran. *ScientificWorldJournal* 2013; ID:280510.
71. Ranjbar R., Khamesipour F., Jonaidi-Jafari N., Rahimi E. *Helicobacter pylori* in bottled mineral water: genotyping and antimicrobial resistance properties. *BMC Microbiol.* 2016; Mar 12;16:40. Doi: 10.1186/s12866-016-0647-1.
72. Gesumaria R. Microbiology of Bottled Water: A Molecular View. Thesis, University of Colorado at Boulder, 2011.
73. Brooke J.S. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin Microbiol Rev* 2012; 25(1):2-41.
74. Shih-Ta Shang, Sheng-Kang Chinb, Ming-ching chan, Ning-chi wang, Ya-Sung Yanbg, Jung-Chung Li et al. Invasive *Brevundomonas vesicularis* bacteremia: Two case reports and review of the literature. *J Micr Immunol Inf.* 2012; 45(6), 468-472.
75. Ryan MP., and Pembroke JT. *Brevundimonas* spp: Emergin global opportunistic pathogens. *Virulence*, 2018; 9(1): 480-493.
76. Leclerc H and da Costa MS. Microbiology of Natural Mineral Waters Technology of bottled water Third edition. Chapter 12. Nicolas Dege (ed). Blackwell Publishing Ltd. 2011.
77. Venieri D, Vantarakis A, Komninou G, Papapetropoulou M. Microbiological evaluation of bottled non-carbonated (still) water from domestic brands in Greece. *Int J Food Microbiology* 2006; 107 (1) 68-72.
78. Messi P, Guerrieri E, Bondi M. Antibiotic resistance and antibacterial activity in heterotrophic bacteria of mineral water origin. *Sci Total Environ* 2005; 346(1-3): 213-219.