

Surveying common bacterial contamination in bottled mineral water in Iran

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ABSTRACT

Background: Bottled mineral water is generally considered more pure than tap water in developing countries. Immunocompromised patients and those with co morbid conditions are often offered bottled mineral water since they are believed to be safer. The present study was designed to determine the risk of common bacterial contamination of commercially available bottled mineral water in Iran.

Materials and methods: For this descriptive study, 68 samples (35 brand named companies) of bottled mineral water were collected from different provinces of Iran. Initially 200 ml of bottled water was filtered with 0.45 micrometer pore (Sartorius label). The filter was divided by sterile pence and scissor to multiple pieces, suspension in nutrient broth and centrifuged. The culture was made by selective media: nutrient agar and broth, dextrose sodium azide agar, yersinia agar, and Clostridium selective agar. The bacterial contaminations were detected after incubation at 42°C, 35°C and 25°C for 24h, 48h and 7 days.

Results: Of 68 samples, 41 (60%) showed evidences of contamination with common bacterial, including 15 samples (36%) with Gram-positive spore-forming bacilli, 20 samples (49%) with Gram-positive non-spore-forming bacilli (diphtheroids) and 6 samples (15%) with Gram-positive cocci. The marker organisms, i.e. the classic fecal contamination indicators, were not detected in any of the samples.

Conclusion: Our results revealed that the use of bottled mineral water in immunocompetent individuals is safe, however, the use of this bottled mineral water in immunocompromised patients should be observed with meticulous precaution. Some of bacteria known as human commensals, contaminate water prior to bottling. It is recommended that bottling, packaging and distribution of mineral water to be carried out under safer processes.

Keywords: *Mineral water, Bacteria, Contamination, Commensals.*

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INTRODUCTION

Water, as the most important and highly consuming nutrition material, must be safe and healthy. It may be the source of different infectious diseases, therefore, periodic evaluation of drinking

water is of utmost importance. In Iran, drinking water is supplied from traditional sources including wells, springs and dams, while its quality is controlled periodically by Ministry of Health. Here, commercially available bottled mineral water is considered as healthy and standard drinking water. Now, there are more than 100 registered manufacturers providing and packaging drinking water in Iran, although, some are inactive. In Iran,

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14 Bottled mineral water contamination

during the recent years, using bottled mineral water has been increased as a predominant source of drinking water, particularly in urban population and travelers.

Subjects who are vulnerable to infections, such as elderly persons, infants, patients with HIV/AIDS, immunosuppressed individuals, those under chemotherapy, and organ transplanted patients require additional water, while health care providers recommend bottled mineral water for these groups (1).

It is mandatory for manufacturers to regularly check their products to ensure that FDA standards are met (2).

Prior investigators have postulated bacterial contamination of bottled mineral water. Klont et al reported a contamination rate of 40% among 68 bottled mineral water samples collected from 9 European and 7 non-European countries (3). In a study on 250 bottled mineral water in Bangal, 70 samples showed bacterial contamination (4). Fujikawa et al found 45 samples of 292 bottled water to be contaminated in Tokyo (5).

Unfortunately, there are scanty reports dealing with the contamination rate of commercially available bottled mineral water in Iran, thus, the present study was conducted to evaluate the frequency of bacterial contamination in bottled water provided and packaged in Iran.

PATIENTS and METHODS

In this cross-sectional study, we evaluated bacterial contamination of all registered bottled mineral water in Iran. The manufacturers were selected through the list provided by Ministry of Health. Two bottles (1.5 liter each) were gathered twice in a 3-month period from retailers of different parts of Iran. Intact bottles with untapped caps and valid expiration dates were selected. Samples were transported to microbiology laboratory of Tarbiat Modarres University.

Having mixed gently the contents of each bottle for 30 minutes for homogenous spreading of bacteria, a 200 ml portion was filtered through a membrane, pore size 0.45 μ m (Sartorius corp.) under sterile condition. The membrane was then placed on the nutrient agar plate and incubated at 35 \pm 0.5 $^{\circ}$ C for 24-48 hours. The number of colony was counted and identified. Another 200 ml of the same bottle was filtered through a membrane, pore size 0.45 μ m, then, the membrane was divided to multiple pieces and inoculated into nutrient broth. After mixing the broth for 15 min, it was centrifuged for 20 min (rpm 5000).

The precipitate was mixed with 1 ml nutrient broth and then placed on the surface of specialized media, including nutrient agar and broth, clostridium selective agar, yersinia agar, and dextrose sodium azide agar. After incubation at 25 $^{\circ}$ C, 35 $^{\circ}$ C and 42 $^{\circ}$ C for 24h, 48h and 1 week, colonies growing on or in agar were investigated. In case of positive samples, bacterial strains were identified by colony appearance, gram staining and differential tests.

RESULTS

Of a total of 68 water samples (35 brands), 41 (60%) were contaminated among which 15 (36%) were spore-forming gram positive bacilli (4 bacillus cereus, 7 bacillus subtilis, 4 other bacillus), 20 (49%) were non-spore-forming gram positive bacilli (diphtheroid) and 6 (15%) were gram positive cocci (coagulase negative staphylococci). Gram negative bacilli and coliforms were not identified.

Among positive samples, bacterial colonies were identified in 31 incubated at 25 $^{\circ}$ C, 1 at 35 $^{\circ}$ C and 9 at both 25 $^{\circ}$ C and 32 $^{\circ}$ C. Both samples of 16 and one sample of 9 manufacturers were revealed to be contaminated, however, samples collected from 9 manufacturers showed no bacterial contamination.

DISCUSSION

Of a total of 68 water samples, 41 (60%) were contaminated including 15 (36%) spore-forming gram positive bacilli, 20 (49%) non-spore-forming gram positive bacilli (diphtheroid) and 6 (15%) gram positive cocci. The following pathogens did not grow on tested media: coliform gram negative bacilli, *enterococcus fecalis*, *clostridium perfringens* and *yersinia enterocolitica*.

Klont et al. studied 68 bottled mineral water and found 21 (37%) contaminated samples including 8 coagulase negative staphylococcus, 10 non-fermenting gram negative strains and 9 gram negative bacilli (3). Their findings are in agreement with ours, except we didn't identify gram negative strains in our samples. In a study in Bangal on 250 bottled mineral water, 28% showed bacterial contamination with coliforms and other bacteria (4). On the other hand, Fujikawa et al. reported a 15% contamination rate mainly caused by gram positive and negative strains (5).

In our study, all isolated bacteria were gram positive, mostly human commensals. Therefore, deterioration of products with contaminated hands during the processes of providing and packaging could be postulated. No fecal coliform bacteria were found suggesting the absence of fecal contamination in selected bottled mineral water. Totally, bottled mineral water could be safely recommended for general population, namely, immunocompetent individuals, travelers and also outbreaks of water-borne diseases, nevertheless, it should be used cautiously by immunocompromised subjects such as infants, elderly persons, organ transplanted patients, HIV/AIDS and immunosuppressed patients.

Detection of gram positive and commensal bacteria suggests that contamination may be occurred before packaging by contaminated hands, therefore, precise surveillance system should be observed during the providing and packaging

processes. Meanwhile, automatically processes are strongly recommended in order to eliminate hand contacts. Moreover, providing, packaging and distribution system of bottled mineral water must be controlled precisely to achieve healthy and standard water (6-9).

Finally, manufacturers should be advised to check their products regularly under the supervision of Ministry of Health.

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